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**Abstract**

The Irrawaddy dolphin (*Orcaella brevirostris*) is an endangered cetacean that ranges throughout much of Southeast Asia and lives in coastal, estuarine, and riverine habitats including three river systems: Ayeyarwady, Mekong, and Mahakam. Many populations face risks from human interference, but overall rangewide diversity and connectivity is not well-understood. Here we sequenced 77 complete mitogenomes from across the range of the Irrawaddy dolphin including all obligate riverine populations; eighteen of these were sequenced from historical museum specimens. Phylogenetic analysis showed haplotypes from each riverine population formed separate clades nested within the wider species implying each river system was separately invaded only once. All Irrawaddy dolphin mitogenomes were dated to a last common ancestor  $\sim 764$  kya. Most lineages appeared after inundation cycles of the Sunda Shelf were initiated  $\sim 400$  kya. Despite the lack of monophyly among many haplotypes from the same population, no population shared any haplotypes. Rangewide nucleotide diversity was average compared to other odontocetes, but riverine populations were especially low. Differentiation was significant among all populations analyzed with the most divergence occurring between isolated riverine populations. These analyses add more evidence for the necessity of conservation efforts directed towards riverine and other isolated populations of the Irrawaddy dolphin.

**KEYWORDS**

cetaceans, mitochondrion, museomics, *Orcaella*, phylogeography, Sundaland

**1 | INTRODUCTION**

Both shallow coastal and riverine environments remain some of most threatened aquatic biomes due to their proximity to human activities and escalating risk from climate change and global sea level rise (Crain et al., 2009; Reid et al., 2019). Coastal Southeast Asia represents an area with increasing intensity of potential stressors and is particularly at risk for biodiversity loss (O'Hara et al., 2021). Indeed, small cetaceans in vulnerable riverine, estuarine, and shallow coastal environments represent some of the most threatened marine mammal species and their numbers continue to drastically decline globally (Braulik et al. 2023; Brownell et al., 2019).

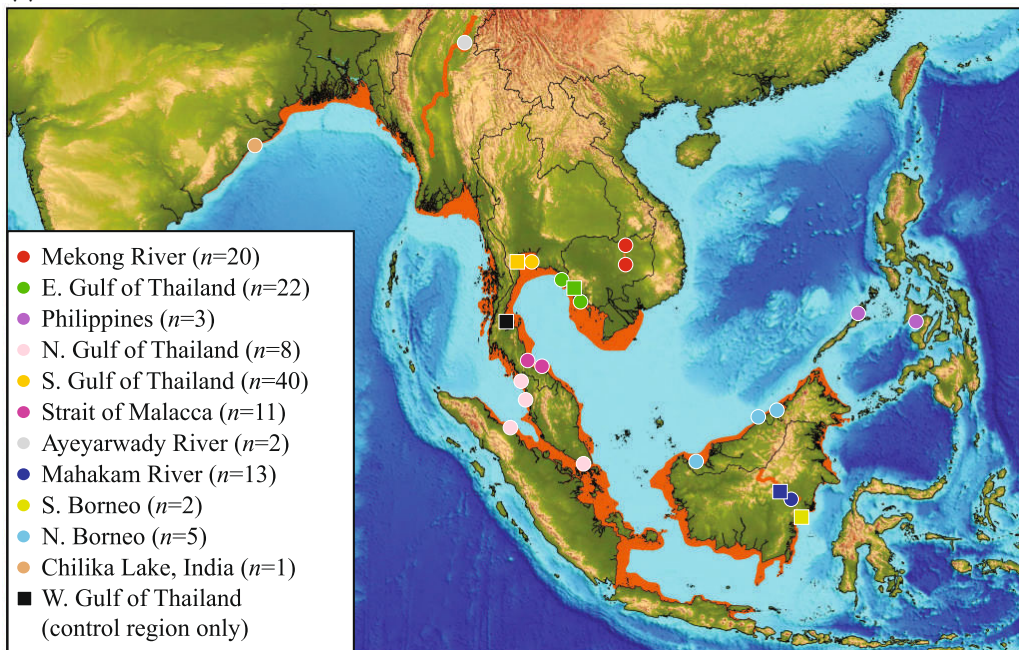
The Irrawaddy dolphin (*Orcaella brevirostris*) is a small coastal, estuarine, and riverine dolphin species of the family Delphinidae (true dolphins) that ranges throughout Southeast Asia from NE India to Vietnam and south to Sumatra, Java, and Borneo as well as east to the Philippines (Beasley et al., 2005; Minton et al., 2017; Stacey & Arnold, 1999; Figure 1). In some areas such as Chilika Lake in India or Songkhla Lake in Thailand, the Irrawaddy dolphin inhabits brackish lagoons, and has colonized at least three separate river systems (Ayeyarwady [Irrawaddy], Mekong, Mahakam) where these populations are obligately freshwater and found up to 1,300 km upstream (Stacey & Arnold, 1999). In coastal areas, Irrawaddy dolphins preferentially inhabit areas that are <50 m deep and in some instances are associated with freshwater outputs and estuaries (Hines et al., 2015; Kuit et al., 2019; Minton et al., 2017; Peter et al., 2016; Smith, 2018). The IUCN Red List identifies the Irrawaddy dolphin as endangered, but freshwater, some shallow lagoon, and Philippines populations are considered critically endangered (Dolar et al., 2018; Jefferson et al., 2008; Minton et al., 2017; Smith, 2004; Smith & Beasley, 2004a,b). Many populations face risks from a myriad of sources, including bycatch via gill nets, electrofishing, prey reduction due to overfishing, ship strikes, dams, pollution, and habitat loss through development (Brownell et al., 2019; Minton et al., 2017; Smith, 2018; Sonne et al., 2022; Temple et al., 2021). Population estimates of obligate river populations hover around 70–90 individuals and are declining; in all three river systems, they inhabit a fraction of their original distribution (Beasley et al., 2013; Brownell et al., 2019; Smith, 2018).

Essential to addressing conservation priorities for this species is the knowledge of its history and population structure. Perhaps because the Irrawaddy dolphin occurs over a large range which is geopolitically complex, its evolutionary history and population-level differentiation is only beginning to be understood. Previous analyses using mitochondrial control region DNA data have shown weakly supported phylogenetic structure; however, most of these studies supported an early diverging Mekong River clade with low diversity and some showed significant differentiation among other populations (Beasley et al., 2005; Budi et al., 2022; Caballero et al., 2019; Dai et al., 2021; Krützen et al., 2018). A recent study using mitochondrial genomes from Thailand and Borneo provided some context to evolutionary relationships among mitochondrial lineages (Budi et al., 2022), but a complete analysis across most of the range of the Irrawaddy dolphin has proved elusive and genetic samples from some regions can be difficult to access (e.g., Myanmar).

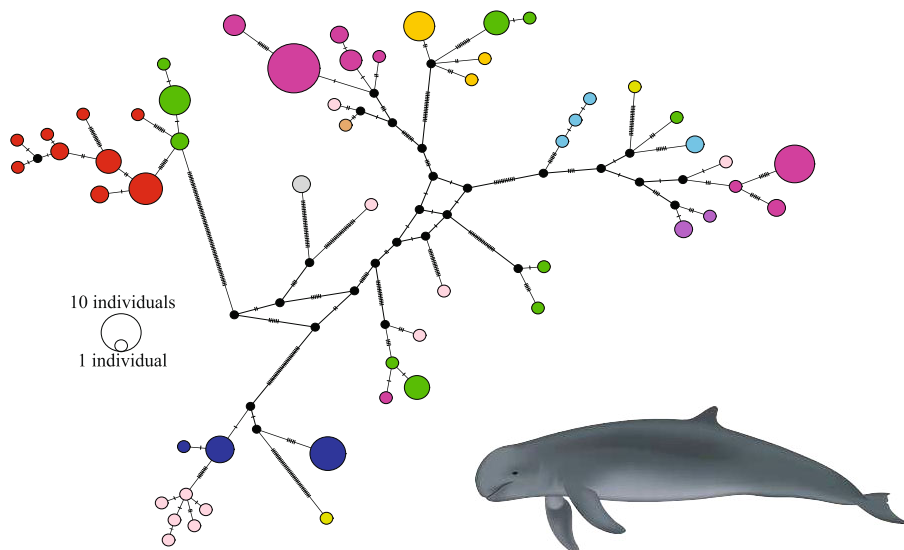
Osteological specimens from museums offer a unique opportunity to fill in these gaps. For example, two individuals collected from the Ayeyarwady River in the 1870–1880s are present at natural history museums in London and Genoa (Andersen, 1879; Fea, 1897) and to our knowledge represent the only specimens from this river system outside Myanmar. “Museomics,” or extracting and analyzing DNA from historical museum specimens <200 years old, has been expanding in recent years as sequencing technologies such as Illumina and approaches such as target capture make it easier to sequence and analyze fragmented DNA (Lalueza-Fox, 2022; Raxworthy & Smith, 2021). Historical specimens from natural history collections have been used recently in studies of cetacean population genomics and phylogenomics to confirm a hybridization event, provide data on populations that are not well-represented, and to confirm the status of holotype specimens (Carroll et al., 2021; Louis et al., 2020; Skorvind et al., 2019). In this study, sequencing museum specimens has allowed us to expand our investigation to areas that were not represented in any previous analysis (Myanmar, Sumatra, northern Borneo, western peninsular Malaysia), as well as expand representation among other populations (Mekong, Mahakam).

Here we sequenced 75 complete mitochondrial genomes from across the range of the Irrawaddy dolphin (Brunei, Cambodia, India, Indonesia, Laos, Malaysia, Myanmar, Philippines, Singapore, Thailand) including all three obligate riverine populations (Ayeyarwady, Mekong, Mahakam). We also added two mitogenomes from its sister species, the Australian snubfin dolphin (*Orcaella heinsohni*) for a total of 77 mitogenomes. Eighteen of these mitogenomes were sequenced from historical museum specimens collected between 1875 and 1972. We use these mitogenomes to reconstruct evolutionary relationships within the Irrawaddy dolphin and date its invasion of the riverine environment, examine its diversity within and among populations, and address the potential for evolutionary significant units for further conservation action.

(a)



(b)



**FIGURE 1** (a) Map of the Indo-Pacific region including Southeast Asia. The orange shading represents the known range of the Irrawaddy dolphin. Polygons on the map represent sample localities. Each polygon is symbolized by a color representing a population or group of populations labeled in the key in the lower left. Circles represent locales of individuals new to this study while squares represent individuals from Budi et al. (2022). Numbers of individuals from each population are indicated. The population from western Gulf of Thailand includes control region data only, derived from Dai et al. (2021). (b) A median-joining network of mitogenomes from 127 individuals. Each circle represents a specific haplotype with the relative size signifying the number of individuals with that haplotype. Colors of circles correspond to the populations listed in Figure 1a. Hashmarks between each circle represent number of steps between haplotypes. Image of Irrawaddy dolphin by Uko Gorter.

## 2 | METHODS

### 2.1 | Sample information and DNA extraction

Tissue samples were obtained from several sources including the Southwest Fisheries Science Center's (SWFSC) Marine Mammal and Turtle Molecular Research Tissue Sample Collection (MMASTR) in La Jolla, California ( $n = 29$ ), the Marine and Coastal Resources Research and Development Center (DMCR) in Klaeng, Rayong, Thailand ( $n = 11$ ), and The MareCet Research Organization (MareCet) in Selangor, Malaysia ( $n = 6$ ; with permission from the Department of Fisheries Malaysia). We also obtained teeth and bone samples from recently stranded individuals from DMCR ( $n = 11$ ) and MareCet ( $n = 2$ ). Table S1 includes a full list of individual samples with locality and associated metadata. All samples from DMCR in Thailand and MareCet were the result of strandings. This is also the case for most SWFSC samples, but two samples (Z-107757, Z-114880) were a result of biopsies collected in the Mekong River in 2012–2013 (see SWFSC for details). DNA from the tissue, bone, and tooth samples mentioned above were extracted using a Qiagen Dneasy Blood and Tissue kit, and in the case of tissues from MMASTR, sodium chloride protein precipitation (Miller et al., 1988). DNA from the Malaysian tooth and bone samples were extracted in the historical DNA clean room at the Smithsonian NMNH Laboratories of Analytical Biology (LAB) using the protocol in Dabney and Meyer (2019).

In addition to these, we sampled osteocrusts, bone, or teeth from 16 osteological specimens of *Orcaella brevirostris* and two bone samples from *Orcaella heinsohni* stored in eight museum collections in the United States and Europe, originally collected between 1875 and 1972 (Table S1). Many of these represented regions for which we did not have modern samples including Sumatra, Ayeyarwady River in Myanmar, Chilika Lake in India, and the northern coast of Borneo. We extracted DNA from these specimens in the historical DNA clean room of LAB using the protocols of Dabney and Meyer (2019). Initially we also obtained tooth or osteocrust samples from seven other museum specimens collected between 1880 and 1892, including one from the Hugli River near Kolkata, India, one from the Baram River, Sarawak, Malaysia and six specimens from the Mekong River in Cambodia (Table S1). The populations from the Baram and Hughli Rivers represent populations adapted to brackish estuaries and are not obligate riverine individuals. We were either unable to extract DNA from these specimens or unable to generate enough reads for further analysis.

### 2.2 | Library preparation, hybridization, and sequencing

For all 77 samples, we sheared DNA (if necessary) using Covaris ME220 (Covaris, Woburn, MA) to 200 bp and constructed genomic libraries using New England Biolabs (Ipswich, MA) NEBNext Ultra II DNA Library Prep kits. We enlisted the services of Daicel Arbor Biosciences (Ann Arbor, MI) to construct a panel of 100 base pair (bp) RNA-biotinylated baits (myBaits) that consisted of the complete mitochondrial (mt) reference genome of the Irrawaddy dolphin (Genbank NC\_019590; Vilstrup et al., 2011). We hybridized these baits to our libraries using the protocol outlined in the myBaits manual version 5.01 with a hybridization temperature of 60°C. Samples were grouped into three pools and sequenced separately (one lane each, paired-end 150-bp, Illumina HiSeq 4000) by Admera Health (South Plainfield, NJ).

After demultiplexing, Illumina paired-end reads were cleaned using Trimmomatic v0.33 (Bolger et al., 2014) and aligned to the Irrawaddy dolphin mt reference genome (NC\_019590) using default parameters in Geneious Prime (Biomatters Ltd., Auckland, New Zealand). For each sample, a consensus sequence of mapped reads was generated using default parameters in Geneious Prime, which uses the sum quality of contributing bases minus noncontributing bases to generate a consensus. Complete mitogenomes for each sample were deposited in NCBI Genbank (Accession numbers: PP828961-PP829035, PP829132, PP829133).

## 2.3 | Additional sequences and alignment

We downloaded 25 additional mitogenome sequences from Genbank, including eleven *O. brevirostris* from Thailand and Indonesia (11 haplotypes representing 52 individuals), one *O. heinsohni*, and sequences from 13 outgroup delphinids (Table S1). We created two data sets for downstream analyses: one consisting of all 102 mitogenomes and one containing just the 86 Irrawaddy dolphin mitogenome sequences (representing 127 individuals). Both data sets were aligned using default parameters in the “Geneious Alignment” feature of Geneious Prime. Alignments were inspected by eye and gaps were modified to conform to boundaries of protein-coding genes.

To supplement our analyses on the complete mitogenome, we also downloaded 44 available Irrawaddy dolphin control region sequences from NCBI Genbank (Table S1) derived from Jayasankar et al. (2011) and Dai et al. (2021). We then aligned these control region sequences to the complete mitogenomes using default parameters in the “Geneious Alignment” feature of Geneious Prime. This alignment was trimmed to 461 bp to exclude all missing data.

## 2.4 | Phylogenetic analysis and divergence dating

We partitioned our data set consisting of 102 mitogenomes of Irrawaddy dolphins and outgroups. We included individual partitions for each coding position of each protein-coding gene as well as individual partitions for each rRNA and tRNA genes and the control region for a total of 64 partitions. For individual sites that are part of two genes or regions, we included these sites in the larger partition only. We used ModelFinder (Kalyaanamoorthy et al., 2017) in IQTREE v2.1.2 (Minh et al., 2020) to find the best partition scheme. We then conducted a maximum likelihood (ML) analysis with IQTREE v2.1.2 using our best partition scheme from ModelFinder ( $n = 8$ ; Table S2) and 1,000 Ultrafast bootstrap replicates (Hoang et al., 2018). These analyses were conducted using the CIPRES Science Gateway v3.3 (Miller et al., 2010).

For divergence dating analyses, we followed Morin et al. (2015) and Louis et al. (2020) in which we pared down our set of 102 mitogenomes to 17, keeping only two of the most divergent Irrawaddy dolphin mitogenomes as determined from our phylogenetic analysis (Obre\_Mahakam\_Indonesia\_74700; Obre\_Mekong\_Cambodia\_107765) and two *O. heinsohni* mitogenomes (Ohei\_Queensland\_NC\_019591; Ohei\_NT\_USNM\_284429). We used ModelFinder to recalculate the best partition scheme ( $n = 9$ ; Table S2). We then generated divergence dates in BEAST v.2.7.1 (Bouckaert et al., 2019) using this scheme, two node calibrations within Delphinidae (Table S2; Bianucci, 2013; Murakami et al., 2014), an optimized relaxed clock, and a Yule prior. BEAST was run twice for 200 million generations for each independent run with results output every 1,000 generations and the initial 10% discarded as burn-in. Results were visualized in Tracer 1.72 (Rambaut et al., 2018) and most ESS values were assessed to be  $>200$ ; for some parameters, 14 out of 143 ESS values were between 100 and 200. We then used the output of the timing of the most recent common ancestor (MRCA) of the Irrawaddy dolphin as the calibration range for the root node (see parameters used in Table S2) in the tree consisting of only the 86 Irrawaddy dolphin mitogenomes using a partition scheme determined by ModelFinder ( $n = 5$ ; Table S2) and a constant size coalescent model with a strict clock. For this analysis, BEAST was run twice for 100 million generations for each independent run with results output every 1,000 generations and the initial 10% discarded as burn-in. Results were visualized in Tracer 1.72 and all ESS values were assessed to be greater than 200.

## 2.5 | Haplotype networks, diversity indices, and population differentiation

We used the 86 Irrawaddy dolphin mitogenomes representing 127 individuals to construct a haplotype median-joining network in PopArt v. 1.7 (Leigh & Bryant, 2015). Individuals were grouped into 11 localities (Figure 1a): Straits of Malacca ( $n = 11$ ; consisting of samples from the west coast of Peninsular Malaysia, Singapore, and the east coast of Sumatra); coastal southern Borneo ( $n = 2$ ); Mekong River ( $n = 20$ ); eastern Gulf of Thailand ( $n = 22$ ; consisting of samples from Trat, Thailand and coastal Cambodia); Philippines ( $n = 3$ ); Northern Gulf of Thailand ( $n = 8$ );



Southern Gulf of Thailand ( $n = 40$ ; consisting of samples from Songkhla Lake, Phattalung, and Patani, Thailand); Ayeyarwady River, Myanmar ( $n = 2$ ), Mahakam River, Borneo, Indonesia ( $n = 13$ ); coastal northern Borneo, Malaysia, and Brunei ( $n = 5$ ); Chilika Lake, India ( $n = 1$ ). The brackish Songkhla Lake population was grouped with coastal southern Gulf of Thailand samples as Budi et al. (2022) showed low differentiation between these populations likely due to high historical connectivity (<100 years).

We calculated number of variable sites ( $S$ ), number of unique haplotypes ( $N_h$ ), haplotype diversity ( $h$ ), and nucleotide diversity ( $\pi$ ) for all Irrawaddy dolphins and each population using DnaSP v. 6.12.03 (Rozas et al., 2003). As DnaSP cannot process sequences with ambiguity codes, we reduced our complete mitogenome data set to 81 (eliminating three sequences from the Mekong, one from the Eastern Gulf of Thailand, and the sole sequence from Chilika Lake), which represented 121 individuals (due to some mitogenomes representing haplotypes). This was repeated for the control region data set which included all control region sequences from the complete mitogenomes as well as additional sequences from Genbank ( $n = 171$  individuals). These Genbank sequences added individuals to two populations not included in the complete mitogenome data set (western Gulf of Thailand [Figure 1] and Chilika Lake, India) as well as northern Gulf of Thailand, southern Gulf of Thailand, and eastern Gulf of Thailand (Table S1, Figure S1). Genbank control region sequences from Kien Gang, Vietnam were included in the Eastern Gulf of Thailand population and sequences from the Andaman Sea of Thailand were included with the Strait of Malacca population (Figure S1). We also compared range wide nucleotide diversity ( $\pi$ ) for the Irrawaddy dolphin with mitogenomes from 16 other odontocete species compiled from different sources (Table S4; Albertson et al., 2022; Alexander et al., 2013; Carroll et al., 2021; Leslie et al., 2019; Louis et al., 2020; Morin et al., 2010; Van Cise et al., 2019; Westbury et al., 2021).

For both the mitogenome and control region data sets, we estimated differentiation among populations with at least eight individuals by computing pairwise  $\Phi_{ST}$  in Arlequin v.3.5.2.2 (Excoffier & Lischer, 2010) using the Tamura-Nei substitution model (Tamura & Nei, 1993). Populations with less than eight individuals were excluded due to potential bias due to low sample size. Values were regarded as significant if  $p < .05$ .

To examine differentiation at a coarser geographical resolution, we grouped all complete mitogenomes into four larger strata: Borneo/Philippines ( $n = 23$ ), northern and eastern Gulf of Thailand/Mekong ( $n = 50$ ), southern Gulf of Thailand ( $n = 40$ ), and Strait of Malacca ( $n = 11$ ). Mitogenomes from Ayeyarwady River and India were not included in this assessment. For each pair of strata, we computed Nei's measure of net nucleotide divergence ( $d_A$ ; Nei, 1987) with the "strataG" package (Archer et al., 2016) using the Tamura-Nei 93 substitution model as implemented in the R package "ape" (Paradis & Schliep, 2019). To assess diagnosability, we fit a Random Forest model to predict strata based on mitogenome variable sites following Archer et al. (2017). Each tree in the model was constructed using a random five samples from each stratum, with the remainder reserved as out-of-bag (OOB) for testing the model. A total of 15,000 trees were grown. Model stability was assessed by examining the OOB error trace and inbag distribution using the R package "rfPermute" (Archer, 2023).

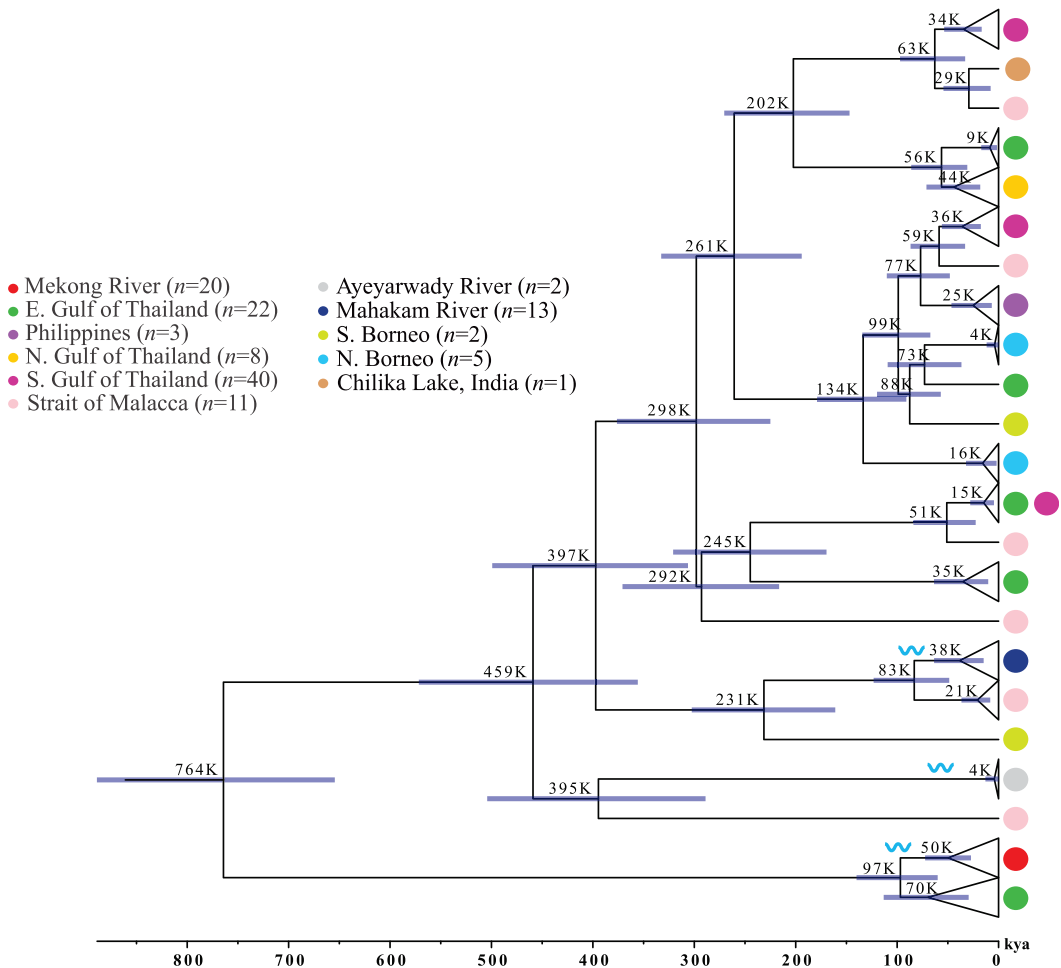
### 3 | RESULTS

For modern tissue samples, the number of total reads which mapped to the mitochondrial genome ranged from 107,766 (SWFSC Z107762, Mekong River, Cambodia) to 60,055,158 (MareCet 44, Perlis, Malaysia) which resulted in 1,557.11X to 564,381.69X coverage (Table S3). Historical samples ranged from a total of 12,797 (MNHN ZM-AC-1888-388, Mekong River, Cambodia) to 10,540,221 reads (USNM 486170, Sumatra, Indonesia) ranging in coverage from 116.98X to 97,773.88X (Table S3). There is statistically significant correlation between year of collection and number of reads mapped among historical samples  $r = 0.51$ ,  $p = .036$ . We found ambiguous bases in only six sequenced genomes, including 10 Ns in MNHN ZM-AC-1888-388, 2Ns in ZMA-MAM-7050, and 1 N each in the remaining four.

Phylogenetic analysis of Irrawaddy dolphin and outgroups produced a tree with 100% bootstrap support for the monophyly of the genus *Orcaella*. In addition, all 86 Irrawaddy dolphin mitogenome sequences were

strongly supported as monophyletic (Figure S2). Mekong and Mahakam River populations were each supported as monophyletic (although with bootstrap values <95%), with both Ayeyarwady River individuals collected in the 1880s having the same haplotype. Riverine populations as a whole did not form a distinct clade. Among nonriverine populations, only the Philippines and the northern Gulf of Thailand were monophyletic. Individuals from the southern and eastern Gulf of Thailand as well as the Strait of Malacca are distributed across the tree in three or more separate lineages. In some cases, individuals from disparate localities have similar haplotypes, such as the Mahakam River population and Strait of Malacca (Figure S2). Our only individual from India groups with an individual from Sumatra and most of the individuals from the Southern Gulf of Thailand population (Figure S2).

Divergence dating using BEAST showed a mean age of 6.822 Mya for the split between the Irrawaddy dolphin and its sister species, the Australian snubfin dolphin (*Orcaella heinsohni*) (Figure S3). BEAST recovered the mean age of the most recent common ancestor of all known Irrawaddy dolphin mitogenome lineages as 764 kya (889–655 kya 95% credible interval [CI]) (Figure 2). The earliest-diverging lineage only includes dolphins from the Mekong River and eastern Gulf of Thailand with an estimation of the later invasion of the Mekong River between 140 and 27 Kya



**FIGURE 2** Time-tree of mitochondrial lineages with time is in thousands of years (kya). Mean divergence time with 95% credible interval as a bar is shown at each node. Some relationships are collapsed for convenience. Circles at the tips of the tree correspond to geographically defined populations in Figure 1A. Branches where colonization of rivers occurred are demarcated with a blue river icon.



(95% CI). All other Irrawaddy dolphins diversified after a mean of 459 kya, 95% CI[571, 356] with the invasion of the Ayeyarwady River potentially occurring as early as 504 kya. Invasion of the Mahakam River dates from between 123 and 14.7 kya (95% CI) and dispersal to the Philippines occurred anywhere from 110 to 67 kya (95% CI).

Using all 86 complete mitogenome sequences (127 individuals), we generated a haplotype median-joining network in PopArt (Figure 1B). After reducing our data set to include only complete mitogenome sequences without ambiguous sites (81 mitogenomes, 121 individuals), we found 54 unique haplotypes across the range of the Irrawaddy dolphin with a total of 398 polymorphic sites (Table 1). No population shared haplotypes and as in our phylogenetic analysis, we do see dense clusters of closely related haplotypes for both the Mekong and Mahakam rivers; however, neither are monophyletic in this analysis (Figure 1B). For most populations in which we had representatives from both recent and historical eras (Mahakam River, 1900, 1914; Mekong River, 1888; Penang, Malaysia, 1964), historical samples grouped strongly with recent members of their respective populations. However, the mitogenome sequence from the 1883 Singapore specimen is distantly related to that of a recent sample from neighboring Johor, Malaysia (Figure S1).

Range-wide nucleotide diversity ( $\pi$ ) was  $4.21 \times 10^{-3}$  which positions this species as about average when compared to 16 other odontocete species (mean  $\pi = 4.61 \times 10^{-3}$ ), and higher than odontocetes such as the sperm whale (*Physeter macrocephalus*), short-finned pilot whale (*Globicephala macrorhynchus*), and orca (*Orcinus orca*) that have much larger ranges (Alexander et al., 2013; Louis et al., 2020; Morin et al., 2010; Van Cise et al., 2019; Table S4). In examining mitogenomes, nucleotide diversity was highest in the eastern Gulf of Thailand population ( $4.29 \times 10^{-3}$ ) and lowest in two river populations, Mekong ( $2.70 \times 10^{-4}$ ) and Mahakam (0.00022), as well as the northern Gulf of Thailand ( $1.4 \times 10^{-4}$ ) (Table 1). However, when examining more individuals with the control region, the northern Gulf of Thailand increases in diversity ( $5.05 \times 10^{-3}$ ), while diversity in the Mekong and Mahakam Rivers remain comparatively low (Table 1).

All  $\Phi_{ST}$  values were significant across tested populations ( $n \geq 8$ ) whether examining complete mitogenomes or control region sequences, establishing some degree of fixation among populations examined (Table 2). The three population pairs with the highest fixation indices in the mitogenome data included Mekong River v. Northern Gulf of Thailand ( $\Phi_{ST} = 0.965$ ), Mekong River v. Mahakam River ( $\Phi_{ST} = 0.958$ ) and Mahakam River v. Northern Gulf of Thailand ( $\Phi_{ST} = 0.936$ ). All three populations were each monophyletic in the phylogenetic tree and shared no haplotypes (Figure 1B; Figure S2). When examining data from the control region, Mekong River v. Mahakam River was still high ( $\Phi_{ST} = 0.978$ ), but Mahakam River v. India ( $\Phi_{ST} = 0.984$ ) and Mekong River v. India ( $\Phi_{ST} = 0.956$ ) were now in the top three. The population pair with the least amount of differentiation in the mitogenome data was Strait

**TABLE 1** Within-population and rangewide statistics generated in DNAsp: total population ( $n$ ), number of variable sites ( $S$ ), number of unique haplotypes ( $N_h$ ), haplotype diversity ( $h$ ), and nucleotide diversity ( $\pi$ ). Statistics are shown for complete mitogenomes on the left and control region data on the right. Gulf of Thailand is abbreviated as GoT. Mitogenome data from more than one individual was not available for comparison for western Gulf of Thailand and India. Only populations with eight or more individuals were included.

Population	$n$	$S$	$N_h$	$h$	$\pi$	$n$	$S$	$N_h$	$h$	$\pi$
Mekong	17	23	9	0.868	$2.70 \times 10^{-4}$	20	3	4	0.363	$1.02 \times 10^{-4}$
E. GoT	21	182	11	0.905	$4.29 \times 10^{-3}$	33	10	6	0.748	$9.39 \times 10^{-3}$
Malacca	11	150	11	1	$2.72 \times 10^{-3}$	13	13	9	0.923	$1.10 \times 10^{-2}$
N. GoT	8	8	3	0.464	$1.40 \times 10^{-4}$	20	9	6	0.726	$5.05 \times 10^{-3}$
S. GoT	40	80	9	0.758	$1.41 \times 10^{-3}$	40	3	3	0.483	$1.19 \times 10^{-3}$
Mahakam	13	7	2	0.513	$2.20 \times 10^{-4}$	14	0	1	0	0
W. GoT	—	—	—	—	—	8	12	4	0.786	$1.05 \times 10^{-2}$
India	—	—	—	—	—	12	2	3	0.318	$7.2 \times 10^{-4}$
Rangewide	121	398	54	0.962	$4.21 \times 10^{-3}$	171	27	32	0.93	$1.16 \times 10^{-2}$

**TABLE 2**  $\Phi_{ST}$  values for population comparisons using complete mitochondrial genomes (above diagonal midline) and control region data (below diagonal midline). All values are significant ( $p \geq .05$ ). Gulf of Thailand is abbreviated as GoT. Mitogenome data from more than one individual was not available for comparison for western Gulf of Thailand and India. Only populations with eight or more individuals were included.

	Malacca	S. GoT	N. GoT	Mekong	E. GoT	India	W. GoT	Mahakam
Malacca	0	0.460	0.569	0.823	0.269	NA	NA	0.392
S. GoT	0.603	0	0.563	0.858	0.424	NA	NA	0.741
N. GoT	0.415	0.570	0	0.965	0.361	NA	NA	0.936
Mekong	0.777	0.934	0.865	0	0.470	NA	NA	0.958
E. GoT	0.340	0.419	0.391	0.489	0	NA	NA	0.530
India	0.523	0.686	0.600	0.956	0.469	0	NA	NA
W. GoT	0.243	0.494	0.258	0.780	0.171	0.331	0	NA
Mahakam	0.401	0.952	0.837	0.978	0.690	0.984	0.791	0

of Malacca versus Eastern Gulf of Thailand ( $\Phi_{ST} = 0.269$ ), populations which are not adjacent to one another but contain the most diversity in haplotypes. The population pair with the least amount of differentiation in the control region data was western Gulf of Thailand versus eastern Gulf of Thailand ( $2(\Phi_{ST}) = 0.171$ ).

Nei's  $d_A$  for comparisons among four broader geographic regions ranged from 0.0002 to 0.0024 (Table S5). All pairwise comparisons with Gulf of Thailand/Mekong had the greatest values of  $d_A$  ( $>0.002$ ). Conversely, differentiation between Borneo/Philippines and the Strait of Malacca was an order of magnitude lower at 0.0002. Approximately 90% of all samples were correctly classified in the Random Forest analysis (Table S6; Figure S4). Borneo/Philippines had the greatest diagnosability (100%), followed by southern Gulf of Thailand (95%), Gulf of Thailand/Mekong (84%). The Strait of Malacca was the least diagnosable at 73%, with a fairly large confidence interval (39%–94%) reflecting the relatively low sample size. Assignments in the Random Forest model were relatively robust as indicated by the distribution of votes in the model (Figure S5). Median assignment probability was  $>90\%$  for all strata except for Borneo/Philippines where it was 71%.

## 4 | DISCUSSION

“Museomics” allowed for the expansion of knowledge of the genetic landscape of the Irrawaddy dolphin by inclusion of areas that were not represented in previous analyses, such as Myanmar, Sumatra, northern Borneo, and western peninsular Malaysia. The addition of these new samples allowed for a broader view of *Orcaella* history by discovering new haplotypes, establishing the distinctiveness of both Ayeyarwady and Philippines populations which have not been assessed before, as well as surveying diversity in geographically transitional areas such as the Strait of Malacca and Northern Borneo. Without the addition of historical specimens, diversity in these areas would remain unknown and knowledge concerning potential loss of diversity in the near past could not be assessed.

Our results indicated that the Irrawaddy dolphin lineage split from the Australian snubfin dolphin lineage (*Orcaella heinsohni*) in the Late Miocene (mean 6.82 mya; Figure S3). A more recent divergence between these two lineages (Pliocene) was noted in previous studies using both mitochondrial (Budi et al., 2022 [mean 3.072 mya]; Viilstrup et al., 2011 [mean ~4.8 mya]) and nuclear data (McGowen et al., 2020 [mean ~3.2 mya]). Discrepancies in these dates are likely due to type of data (mt vs. nuclear), type of analysis, and use of substitution rates and/or calibration dates, all of which differ between these analyses. The Australian snubfin dolphin inhabits the coast of southern New Guinea and northern Australia and is separated from the Irrawaddy dolphin by Wallace's Line (Parra et al., 2017). As both species are coastal and estuarine specialists, movement between the Sunda and Sahul shelves

would have been difficult for their common ancestor. Although theoretically movement across great distances is presumably easy for a cetacean, both Irrawaddy and Australian snubfin dolphins have rarely been sighted more than a few kilometers offshore and both prefer shallow waters with low salinity (Wang et al., 2014). It is likely that the greater extent of shallow seas across Wallacea in the Late Miocene-Early Pliocene may have lessened the distance between Sundaland and Australia (Hall, 2013). The Late Miocene also coincides with divergence between Sundaland and Wallacea in some terrestrial mammals including *Crocidura* shrews (Hinckley et al., 2022).

We estimate the oldest mitochondrial lineage in the Irrawaddy dolphin to have diverged  $\sim 764$  kya at a time when the Sunda Shelf was completely above sea level (Figure 2). As most individuals in this basal lineage are from the Mekong River, it is possible this represents an early relict lineage that invaded the ancient Mekong drainage with later dispersal to the Eastern Gulf of Thailand. More likely, the invasion of the Mekong River took place after  $\sim 97$  kya (Figure 2). Although it is assumed that riverine populations do not move between coastal and riverine environments, the comparative physiological limits of riverine versus coastal individuals have not been tested. Budi et al. (2022) retrieved a younger date for the age of all modern lineages of the Irrawaddy dolphin at 501.2 kya, but the study did not include mitogenomes from the Mekong Delta. The overall age of Irrawaddy dolphin mitogenome lineages is similar to that of orca mitogenomes ( $\sim 700$  kya; Morin et al., 2010), a much more widely dispersed cetacean.

There is little correlation between phylogeny and location with exception of riverine lineages and the Philippines, leaving some difficulty in interpreting splits in relation to geography (Figure 1). Around 400 kya, patterns of climate oscillation due to glacial cycles created intermittent episodes of marine inundation across shallow areas of Sundaland up through the Holocene (Husson et al., 2020). This pattern may have created windows for Irrawaddy dolphins to disperse to new areas via recently opened coastal habitat. This scenario could explain the relatively high degree of geographic structuring across the range of the species while retaining multiple haplotype lineages in some populations located in more open or transitional areas, such as the Strait of Malacca or Gulf of Thailand (Figure 1).

Invasion of the Ayeyarwady drainage potentially occurred at some point after the initiation of inundation cycles (mean  $\sim 395$  kya), while the invasion of the Mahakam drainage in Borneo likely occurred after  $\sim 83$  kya. In comparison, Budi et al. (2022) found a much older date for the separation of the Mahakam lineage (mean 303 kya). Mixing of haplotypes during periods of gradual sea level change could have created the phenomenon in which the Mahakam River population is most closely related to a clade of mitogenomes from northwestern Malaya, a 2,000 km distance, although increased sampling is needed across more of the range of the Irrawaddy dolphin especially across Java where the Irrawaddy dolphin is known to occur (Khalifa et al., 2014; Rudolph et al., 1997) and South and West Kalimantan where second-hand reports have suggested their occurrence (Perrin et al., 1996; Rudolph et al., 1997). Dispersal to the Philippines occurred at some point after the separation of this lineage  $\sim 77$  kya. Although there is no evidence of a land connection between Palawan and Sundaland or Palawan and other major Philippine islands after this time, waters may have been shallow enough to allow for dispersal (Robles et al., 2015). Presently Irrawaddy dolphins occur in two populations in Palawan (Malampaya Sound and coastal Quezon Province), one population off Negros, Panay, and Guimaras Islands in the Visayan region (De La Paz et al. 2020; Dolar et al. 2002, 2018; Smith et al. 2004), and a newly discovered population in San Miguel Bay off Bicol on Luzon (<https://science.upd.edu.ph/survey-confirms-disappearing-population-of-critically-endangered-irrawaddy-dolphins-in-bicol/>).

Although we do not find reciprocal monophyly among any populations, we note that some populations are distinctive and form their own clades, including Mekong, Mahakam, Ayeyarwady, Northern Gulf of Thailand, and the Philippines. However, further analysis of control region sequences from the northern Gulf of Thailand show that this population does not form a monophyletic group and is incredibly diverse (Budi et al., 2022; Dai et al., 2021). These results should be interpreted with caution due to the greater effects of saturation when using control region sequences (Morin et al., 2023). In the case of the Mekong and Mahakam Rivers, both with large sample sizes compared to current population estimates ( $n = 80$  in 2015 and  $n = 77$  in 2016, respectively; Minton et al., 2017; Phan et al. 2015), it is unlikely that increasing sample sizes would find any new haplotypes. Abundance estimates of populations in the Philippines (Malampaya Sound,  $n = 35$  in 2016; Iloilo-Guimaras,  $n = 6$ –13 in 2015–2016) and

Ayeyarwady River ( $n = 58\text{--}72$  in 2007) are similarly low (Dolar et al., 2018; Smith & Tun, 2007; Whitty, 2016). Brownell et al. (2019) estimate that almost 60% of the former range of the Ayeyarwady River population has been lost since observations by Anderson (1879). Although our sample sizes were low for each of these populations, it is quite possible that the number of haplotypes in each is not much greater.

The overall nucleotide diversity of the Irrawaddy dolphin mitogenome is average compared to 17 other toothed whales examined at  $\pi = 0.00421$  (Table S4). Comparatively, nucleotide diversity within riverine populations such as Mekong and Mahakam were very low (0.00027 and 0.00022, respectively). This confirms earlier analyses of control region data in the Mekong River, which found very low nucleotide diversity among four closely related haplotypes (Caballero et al., 2019; Krützen et al. 2018). We found no new haplotypes in the Mekong among control region segments of our mitogenomes including both historical samples collected in 1888. Krützen et al. (2018) found no structure using both mitochondrial and microsatellite data among sampling sites within the Mekong and discovered evidence of a recent exponential decline in the population. Both Mekong and Mahakam River populations show evidence of being very isolated from other populations with (mostly) higher  $\Phi_{ST}$  values approaching 1.0 (Table 2). Although numbers have declined in both rivers (Beasley et al., 2013; Brownell et al., 2019; Minton et al., 2017), diversity estimates likely reflect long-term isolation coupled with low effective population sizes.

Coastal populations generally have higher diversity and greater connectivity with more potential opportunities for movement. The Gulf of Thailand and the Strait of Malacca are particularly diverse with haplotypes from a multitude of lineages (Figure 1) as well as generally lower  $\Phi_{ST}$  values when compared to other populations (Table 2). In addition, estimates of population size in these areas are much greater than riverine environments (423 in Trat Province, Thailand; Hines et al., 2015; 763 off Matang, Perak, Malaysia; Kuit et al., 2021). However, because no haplotypes were shared between populations, it is likely that much of the dispersal between populations took place in the past and that ongoing dispersal is rare. A recent microsatellite analysis of Gulf of Thailand populations as well as representatives from the Andaman Sea across the Isthmus of Kra show statistically significant differentiation across these populations (Dai et al., 2021) and stable isotope values suggest that Irrawaddy dolphin individuals do not move among populations within the Gulf of Thailand or Andaman Sea during their lifetimes (Jackson-Ricketts et al., 2019).

We saw evidence of deeper evolutionary differentiation among several larger geographic regions. From a comparison of recognized pairs of cetacean subspecies and species, Morin et al. (2023) proposed a threshold of greater than 95% diagnosability and  $d_A > 0.0006$  for identifying subspecies based on mitogenome data alone. To delimit species, they propose  $d_A > 0.008$ . In our study, all comparisons except for that between Borneo/Philippines and Strait of Malacca met the  $d_A$  threshold for subspecies, but only Borneo/Philippines and southern Gulf of Thailand were sufficiently diagnosable for subspecies. While these results are not conclusive with respect to taxonomic subdivision for some regions, they do suggest that *Orcaella* in the south and east of their range may be on separate evolutionary pathways than those in the north.

The relatively high nucleotide diversity gives some hope to the genetic health of the Irrawaddy dolphin as a whole, but the low genetic diversity in several populations, particularly the riverine populations of Mekong, Mahakam, and Ayeyarwady, as well as the isolated populations in the Philippines give cause for great concern. In addition, for the Mekong population, analyses of genetic diversity undertaken for individuals from different generations (adults and calves) evidenced a decline in intergenerational genetic diversity (Dove, 2022). Brownell et al. (2019) singled out all these populations in a recent paper on the most critically endangered cetaceans and all have populations of  $n < 100$  individuals each. Recent evidence indicates that Irrawaddy dolphins have been extirpated from the Laotian portion of the Mekong River (Sonne et al., 2022). Riverine and estuarine populations are especially under threat from a variety of dangers including bycatch via gill nets, electrofishing, ship strikes, habitat loss, and dam construction (Brownell et al., 2019; Sonne et al., 2022; Thomas et al., 2019).

Two major concerns of conservation genetics are the preservation of genetic diversity through the identification of conservation units as well as the recognition of intraspecific variation and its contribution to human populations (Coates et al., 2018; Des Roches et al., 2021; Funk et al., 2012; Moritz, 1994).

Indeed, riverine dolphins in the Ayeyarwady River hold significant cultural significance where they are known to assist fishers by herding catches into nets (Smith et al., 2009). The IUCN Red List individually assessed six populations as critically endangered, including all riverine and both Philippine populations (Dolar et al., 2018; Jefferson et al., 2008; Minton et al., 2017; Smith, 2004; Smith & Beasley, 2004a,b). The spotlight on these populations agrees with our results here, which highlight the genetic distinctiveness of the separate riverine populations as well as the Philippines populations even though they are nested within other mitochondrial lineages. Indeed, some populations that we examined above (Table 2) could essentially be identified as “management units (MUs),” which were defined by Moritz (1994) as “...populations with significant divergence of allele frequencies at nuclear or mitochondrial loci, regardless of the phylogenetic distinctiveness of the alleles....” Use of nuclear markers or whole genomes to examine diversity across populations are needed to confirm our results here and provide more information on inbreeding and migration (Coates et al., 2018). Further sampling in the western range (India, Bangladesh, coastal Myanmar) especially, as well as coastal Cambodia, Vietnam, eastern Peninsular Malaysia, Sabah, and other parts of Indonesia is needed. Bangladesh has perhaps the largest population of Irrawaddy dolphins at >5,000 individuals (Smith et al., 2008) and some populations in these regions remain understudied.

## 4.1 | Conclusion

We sequenced the largest number of complete Irrawaddy dolphin mitogenomes to date from across their range and from areas that have not been sampled previously, such as Myanmar, Malaysian Borneo, Peninsular Malaysia, Sumatra, and the Philippines. These included 18 samples derived from bone, teeth, or dried tissue from museum specimens originally collected between 1875 and 1972. Here we have shown that sequencing historical osteological specimens can aid in understanding the evolutionary history, phylogeography, and population genetics of an endangered species. We dated the most recent common ancestor of the Irrawaddy dolphin mitogenome to ~764 kya and identified that all populations examined showed significant differentiation from one another with riverine populations the most isolated. Populations were highly structured with evidence of intermittent reconnection between regions driven by the rise and fall of Sundaland during glacial cycles. Future integration of nuclear markers and genomic data will serve to confirm these patterns.

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## REFERENCES

- Albertson, G. R., Alexander, A., Archer, F. I., Caballero, S., Martien, K. K., Hemery, L. G., Baird, R. W., Oremus, M., Poole, M. M., Duffield, D. A., Brownell, R. L., Jr., Kerem, D., Mignucci-Giannoni, A. A., & Baker, C. S. (2022). Worldwide phylogeography of rough-toothed dolphins (*Steno bredanensis*) provides evidence for subspecies delimitation. *Marine Mammal Science*, 38(4), 1371–1397. <https://doi.org/10.1111/mms.12933>
- Alexander, A., Steel, D., Silkas, B., Hoekzema, K., Carraher, C., Parks, M., Cronn, R., & Baker, C. S. (2013). Low diversity in the mitogenome of sperm whales revealed by next-generation sequencing. *Genome Biology and Evolution*, 5(1), 113–129. <https://doi.org/10.1093/gbe/evs126>
- Anderson, J. (1879). *Anatomical and zoological researches: Comprising an account of the zoological results of the two expedition to western Yunan in 1868 and 1875; and a monograph of the two cetacean genera, Platanista and Orcella*. Bernard Quaritch.
- Archer, F. I. (2023). *rfPermute: Estimate permutation p-values for Random Forest importance metrics* (R package version 2.5.2) [Computer software]. <https://CRAN.R-project.org/package=rfPermute>
- Archer, F. I., Adams, P. E., & Schneiders, B. B. (2016). STRATAG: An R package for manipulating, summarizing and analysing population genetic data. *Molecular Ecology Resources*, 17(1), 5–11. <https://doi.org/10.1111/1755-0998.12559>
- Archer, F. I., Martien, K. K., & Taylor, B. L. (2017). Diagnosability of mtDNA with Random Forests: Using sequence data to delimit subspecies. *Marine Mammal Science*, 33(S1), 101–131. <https://doi.org/10.1111/mms.12414>
- Beasley, I., Arnold, P., & Heinsohn, G. (2002). Geographical variation in skull morphology of the Irrawaddy dolphin, *Orcaella brevirostris* (Owen in Gray, 1866). *Raffles Bulletin of Zoology, Supplement* 10, 15–34.
- Beasley, I., Pollock, K., Jefferson, T. A., Arnold, P., Morse, L., Yim, S., Kim, S. L., & Marsh, H. (2013). Likely future extirpation of another Asian river dolphin: The critically endangered population of the Irrawaddy dolphin in the Mekong River is small and declining. *Marine Mammal Science*, 29(3), E226–E252. <https://doi.org/10.1111/j.1748-7692.2012.00614.x>
- Beasley, I., Robertson, K. M., & Arnold, P. (2005). Description of a new dolphin, the Australian snubfin dolphin *Orcaella heinsohni* sp. N. (Cetacea, Delphinidae). *Marine Mammal Science*, 21(3), 365–400. <https://doi.org/10.1111/j.1748-7692.2005.tb01239.x>



- Bianucci, G. (2013). *Septidelphis morii*, n. gen. et sp., from the Pliocene of Italy: new evidence of the explosive radiation of true dolphins (Odontoceti, Delphinidae). *Journal of Vertebrate Paleontology*, 33(3), 722–740. <https://doi.org/10.1080/02724634.2013.744757>
- Bolger, A. M., Lohse, M., & Usadel, B. (2014). Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics*, 30(15), 2114–2120. <https://doi.org/10.1093/bioinformatics/btu170>
- Bouckaert, R., Vaughan, T. G., Barido-Sottani, J., Duchêne, S., Fourment, M., Gavryushkina, A., Heled, J., Jones, G., Kühnert, D., De Maio, N., Matschiner, M., Mendes, F. K., Müller, N. F., Ogilvie, H. A., du Plessis, L., Poppinga, A., Rambaut, A., Rasmussen, D., Siveroni, I. ... Dummond, A. J. (2019). BEAST 2.5: an advanced software platform for Bayesian evolutionary analysis. *PLoS Computational Biology*, 15(4), e1006650. <https://doi.org/10.1371/journal.pcbi.1006650>
- Braulik, G. T., Taylor, B. L., Minton, G., Notarbartolo di Sciarra, G., Collins, T., Rojas-Bracho, L., Crespo, E. A., Ponnampalam, L. S., Double, M. C. & Reeves, R. R. (2023). Red-list status and extinction risk of the world's whales, dolphins, and porpoises. *Conservation Biology*, 37(5), e14090. <https://doi.org/10.1111/cobi.14090>
- Brownell, R. L., Jr., Reeves, R. R., Read A. J., Smith, B. D., Thomas, P. O., Ralls, K., Amano, M., Berggren, P., Chit, A. M., Collins, T., Currey, R., Dolar, M. L. L., Genov, T., Hobbs, R. C., Krebs, D., Marsh, H., Mei Z., Perrin, W. F., Phay, S., ... Wang, J. Y. (2019). Bycatch in gillnet fisheries threatens Critically Endangered small cetaceans and other aquatic megafauna. *Endangered Species Reserach*, 40, 285–296. <https://doi.org/10.3354/esr00994>
- Budi, T., Piyapattanakorn, S., Krebs, D., Yuda, P., Ninwat, S., Hardwises, P., Prachamkhai, P., Senanan, W., Thongsukdee, S., Phavaphutanon, J., & Klinsawat, W. (2022). Mitogenomes provide insight into complex evolutionary history of freshwater and coastal Irrawaddy dolphin (*Orcaella brevirostris* Gray, 1966) in Thailand and Indonesia. *Agriculture and Natural Resources*, 56(3), 591–606. <https://doi.org/10.34044/j.anres.2022.56.3.15>
- Caballero, S., Dove, V., Jackson-Ricketts, J., Junchompoo, C., Cohen, S., & Hines, E. (2019). Mitochondrial DNA diversity and population structure in the Irrawaddy dolphin (*Orcaella brevirostris*) from the Gulf of Thailand and the Mekong River. *Marine Mammal Science*, 35(1), 300–310. <https://doi.org/10.1111/mms.12518>
- Carroll, E. L., McGowen, M. R., McCarthy, M. L., Marx, F. G., Aguilar, N., Dalebout, M. L., Dreyer, S., Gaggiotti, O. E., Hansen, S. S., van Helden, A., Onoufriou, A. B., Baird, R. W., Baker, C. S., Berrow, S., Cholewiak, D., Claridge, D., Constantine, R., Davison, N. J., Eira, C., ... Olsen, M. T. (2021). Speciation in the deep: genomics and morphology reveal a new species of beaked whale *Mesoplodon eueu*. *Proceedings of the Royal Society B: Biological Sciences*, 288(1961), Article 20211213. <https://doi.org/10.1098/rspb.2021.1213>
- Coates, D. J., Byrne, M., & Moritz, C. (2018). Genetic diversity and conservation units: dealing with the species-population continuum in the age of genomics. *Frontiers in Ecology and Evolution*, 6, Article 165. <https://doi.org/10.3389/fevo.2018.00165>
- Crain, C. M., Halpern, B. S., Beck, M. W., & Kappel, C. V. (2009). Understanding and managing human threats to the coastal marine environment. *Annals of the New York Academy of Science*, 1162(1), 39–62. <https://doi.org/10.1111/j.1749-6632.2009.04496.x>
- Dabney, J., & Meyer, M. (2019). Extraction of highly degraded DNA from ancient bones and teeth. In B. Shapiro, A. Barlow, P. Heintzman, M. Hofreiter, J. Pajmians, & A. Soares (Eds.), *Ancient DNA: Methods and protocols* (2nd ed., pp. 25–29). Humana Press. [https://doi.org/10.1007/978-1-4939-9176-1\\_4](https://doi.org/10.1007/978-1-4939-9176-1_4)
- Dai, Y., Chantra, R., Kittiwattanawong, K., Zhao, L., Sakornwimon, W., Aierken, R., Wu, F., & Wang, X. (2021). Genetic structure of the endangered Irrawaddy dolphin (*Orcaella brevirostris*) in the Gulf of Thailand. *Genetics and Molecular Biology*, 44(2), e20200365. <https://doi.org/10.1590/1678-4685-GMB-2020-0365>
- De La Paz, M. E., Palomar-Abesamis, N., Sabater, E., Señorón, J. A., & Dolar, M. L. (2020). Habitat use and site fidelity of Irrawaddy dolphins (*Orcaella brevirostris*) in the coastal waters of Bago-Pulupandan, Negros Occidental, Philippines. *Raffles Bulletin of Zoology*, 68, 562–573. <https://doi.org/10.26107/RBZ-2020-0072>
- Des Roches, S., Pendleton, L. H., Shapiro, B., & Palkovacs, E. P. (2021). Conserving intraspecific variation for nature's contribution to people. *Nature Ecology and Evolution*, 5, 574–582. <https://doi.org/10.1038/s41559-021-01403-5>
- Dolar, M. L. L., de la Paz, M. E. L., & Sabater, E. (2018). *Orcaella brevirostris* (Iloilo-Guimaras subpopulation). *IUCN Red List of Threatened Species*, e.T123095978A123095988. <https://doi.org/10.2305/IUCN.UK.2018-2.RLTS.T123095978A123095988.en>
- Dolar, M. L. L., Perrin, W. F., Gaudiano, J. P., Yaptinchay, A. A. S. P., & Tan, J. M. L. (2002). Preliminary report on a small estuarine population of Irrawaddy dolphins *Orcaella brevirostris* in the Philippines. *Raffles Bulletin of Zoology*, 10, 155–160.
- Dove, V. (2022). *The impact of health and environmental factors on population of Mekong River Irrawaddy dolphins (Orcaella brevirostris) in Cambodia* [Doctoral dissertation]. Murdoch University.
- Excoffier, L., & Lischer, H. E. L. (2010). Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources*, 10(3), 564–567. <https://doi.org/10.1111/j.1755-0998.2010.02847.x>
- Fea, L. (1897). Viaggio di Leonardo Fea in Birmania e regioni vicine. LXXVI. Riassunto generale dei risultati zoologici [Leonardo Fea's journey to Burma and nearby regions. LXXVI. General summary of zoological results]. *Annali del Museo civico di Storia naturale di Genova*, 37, 385–658.



- Funk, W. C., McKay, J. K., Hohenlohe, P. A., & Allendorf, F. W. (2012). Harnessing genomics for delineating conservation units. *Trends in Ecology and Evolution*, 27, 489–496. <https://doi.org/10.1016/j.tree.2012.05.012>
- Hall, R. (2013). The palaeogeography of Sundaland and Wallacea since the Late Jurassic. *Journal of Limnology*, 72(S2), e1. <https://doi.org/10.4081/jlimnol.2013.s2.e1>
- Hinckley, A., Camacho-Sanchez, M., Ruedi, M., Hawkins, M. T. R., Mullon, M., Cornellas, A., Yuh, F. T. Y., & Leonard, J. A. (2022). Evolutionary history of Sundaland shrews (Eulipotyphla: Soricidae: Crocidura) with a focus on Borneo. *Zoological Journal of the Linnean Society*, 194(2), 478–501. <https://doi.org/10.1093/zoolinnean/zlab045>
- Hines, E. M., Strindberg, S., Junchumpoo, C., Ponnampalam, L. S., Ilangakoon, A. D., Jackson-Ricketts, J., & Monanunsap, S. (2015). Line transect estimates of Irrawaddy dolphin abundance along the eastern Gulf Coast of Thailand. *Frontiers in Marine Science*, 2, Article 63. <https://doi.org/10.3389/fmars.2015.00063>
- Hoang, D. T., Chernomor, O., von Haeseler, A., Minh, B. Q., & Vinh, L. S. (2018). UFBoot2: improving the ultrafast bootstrap approximation. *Molecular Biology and Evolution*, 35, 518–522. <https://doi.org/10.1093/molbev/msx281>
- Husson, L., Boucher, F. C., Sarr, A.-C., Sepulchre, P., & Cahyarini, S. Y. (2020). Evidence of Sundaland's subsidence requires revisiting its biogeography. *Journal of Biogeography*, 47(4), 843–853. <https://doi.org/10.1111/jbi.13762>
- Jackson-Ricketts, J., Ruiz-Cooley, R. I., Junchumpoo, C., Thongsukdee, S., Intongkham, A., Ninwat, S., Kittiwattanawong, K., Hines, E. M., & Costa, D. P. (2019). Ontogenetic variation in diet and habitat of Irrawaddy dolphins (*Orcaella brevirostris*) in the Gulf of Thailand and Andaman Sea. *Marine Mammal Science*, 35(2), 492–521. 1: [doi.org/10.1111/mms.12547](https://doi.org/10.1111/mms.12547)
- Jayasankar, P., Patel, A., Khan, M., Das, P., & Panda, S. (2011). Mitochondrial DNA diversity and PCR-based sex determination of Irrawaddy dolphin (*Orcaella brevirostris*) from Chilika Lagoon, India. *Molecular Biology Reports*, 38(3), 1661–1668. <https://doi.org/10.1007/s11033-010-0277-x>
- Jefferson, T. A., Karczmarski, L., Krebs, D., Laidre, K., O'Corry-Crowe, G., Reeves, R., Rojas-Bracho, L., Secchi, E., Slooten, E., Smith, B. D., Wang, J. Y., & Zhou, K. (2008). *Orcaella brevirostris* (Mahakam River subpopulation) (errata version published in 2016). *IUCN Red List of Threatened Species*, e.T39428A98842174. <https://doi.org/10.2305/IUCN.UK.2008.RLTS.T39428A10237530.en>
- Kalyaanamoorthy, S., Minh, B. Q., Wong, T. K. F., von Haeseler, A., & Jeremiin, L. S. (2017). ModelFinder: fast model selection for accurate phylogenetic estimates. *Nature Methods*, 14, 587–589. <https://doi.org/10.1038/nmeth.4285>
- Khalifa, M. A., Kamal, M. M., Adiwilaga, E. M., & Sunuddin, A. (2014). Preliminary study on the distribution of Irrawaddy dolphin, *Orcaella brevirostris*, in Banten Bay. *Open Journal of Marine Science*, 4(4), 338–343. <https://doi.org/10.4236/ojms.2014.44030>
- Krützen, M., Beasley, I., Ackermann, C. Y., Lieckfeldt, D., Ludwig, A., Ryan, G. E., Bejder, L., Parra, G. J., Wolfensberger, R., & Spencer, P. B. S. (2018). Demographic collapse and low genetic diversity of the Irrawaddy dolphin population inhabiting the Mekong River. *PLoS ONE*, 13(1), e0189200. <https://doi.org/10.1371/journal.pone.0189200>
- Kuit, S. H., Ponnampalam, L. S., Hammond, P. S., Chong, V. C., & Then, A. Y.-H. (2021). Abundance estimates of three cetacean species in the coastal waters of Matang, Perak, Peninsular Malaysia. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 31(11), 3120–3132. <https://doi.org/10.1002/aqc.3699>
- Kuit, S. H., Ponnampalam, L. S., Ng, J. E., Chong, V. C., & Then, A. Y.-H. (2019). Distribution and habitat characteristics of three sympatric cetacean species in the coastal waters of Matang, Perak, Peninsular Malaysia. *Aquatic Conservation*, 29(10), 1681–1696. <https://doi.org/10.1002/aqc.3121>
- Laluzza-Fox, C. (2022). Museomics. *Current Biology*, 32(21), R1214–R1215. <https://doi.org/10.1016/j.cub.2022.09.019>
- Leigh, J. W., & Bryant, D. (2015). POPART: full-feature software for haplotype network construction. *Methods in Ecology and Evolution*, 6, 1110–1116. <https://doi.org/10.1111/2041-210X.12410>
- Leslie, M. S., Archer, F. I., & Morin, P. A. (2019). Mitogenomic differentiation in spinner (*Stenella longirostris*) and pantropical spotted dolphins (*S. attenuata*) from the eastern tropical Pacific Ocean. *Marine Mammal Science*, 35(2), 522–551. <https://doi.org/10.1111/mms.12545>
- Louis, M., Skorvind, M., Castruita, J. A. S., Garilao, C., Kaschner, K., Gopalakrishnan, S., Haile, J. S., Lydersen, C., Kovacs, K. M., Garde, E., Heide-Jørgensen, M. P., Postma, L., Ferguson, S. F., Willerslev, E., & Lorenzen, E. D. (2020). Influence of past climate change on phylogeography and demographic history of narwhals, *Monodon monoceros*. *Proceedings of the Royal Society B: Biological Sciences*, 287(1925), Article 20192964. <https://doi.org/10.1098/rspb.2019.2964>
- McGowen, M. R., Tsagkogeorga, G., Álvarez-Carretero, S., dos Reis, M., Struebig, M., Deaville, R., Jepson, P. D., Jarman, S., Polanowski, A., Morin, P. A., & Rossiter, S. J. (2020). Phylogenomic resolution of the cetacean tree of life using target sequence capture. *Systematic Biology*, 69(3), 479–501. <https://doi.org/10.1093/sysbio/sy0268>
- Miller, S. A., Dykes, D. D., & Polesky, H. F. (1988). A simple salting out protocol for extracting DNA from human nucleated cells. *Nucleic Acid Research*, 16(3), Article 1215. <https://doi.org/10.1093/nar/16.3.1215>
- Miller, M. A., Pfeiffer, W., & Schwartz, T. (2010, November 14). Creating the CIPRES Science Gateway for inference of large phylogenetic trees. *Proceedings of the Gateway Computing Environments Workshop (GCE)*, New Orleans, LA. <https://doi.org/10.1109/GCE.2010.5676129>
- Minh, B. Q., Schmidt, H. A., Chernomor, O., Schrempf, D., Woodhams, M. D., von Haeseler, A., Lanfear, R. (2020). IQ-TREE 2: New models and efficient methods for phylogenetic inference in the genomic era. *Molecular Biology and Evolution*, 37(5), 1530–1534. <https://doi.org/10.1093/molbev/msaa015>

- Minton, G., Smith, B. D., Braulik, G. T., Krebs, D., Sutaria, D., & Reeves, R. (2017). *Orcaella brevirostris* (errata version published in 2018). *IUCN Red List of Threatened Species*, e.T15419A123790805. <https://doi.org/10.2305/IUCN.UK.2017-3.RLTS.T15419A50367860.en>
- Morin, P. A., Archer, F. I., Foote, A. D., Vilstrup, J., Allen, E. E., Wade, P., Durban, J., Parsons, K., Pitman, R., Li, L., Bouffard, P., Nielsen, S. C. A., Rasmussen, M., Willerslev, E., Gilbert, M. T. P., & Harkins, T. (2010). Complete mitochondrial genome phylogenetic analysis of killer whales (*Orcinus orca*) indicates multiple species. *Genome Research*, 20, 908–916. <https://doi.org/10.1101/gr.102954.109>
- Morin, P. A., Martien, K. K., Lang, A. R., Hancock-Hanser, B. L., Pease, V. L., Roberston, K. M., Sattler, M., Slikas, E., Rosel, P. E., Baker, C. S., Taylor, B. L., & Archer, F. I. (2023). Guidelines and quantitative standards for improved cetacean taxonomy using full mitochondrial genomes. *Journal of Heredity*, 114(6), 612–624. <https://doi.org/10.1093/jhered/esad049>
- Morin, P. A., Parsons, K. M., Archer, F. I., Ávila-Arcos, M. C., Barrett-Lennard, L. G., Dalla Rosa, L., Duchêne, S., Durban, J. W., Ellis, G. M., Ferguson, S. H., Ford, J. K., Ford, M. J., Garilao, C., Gilbert, M. T. P., Kaschner, K., Matkin, C. O., Petersen, S. D., Robertson, K. M., Visser, I. N., ... Foote, A. D. (2015). Geographic and temporal dynamics of a global radiation and diversification in the killer whale. *Molecular Ecology*, 24, 3964–3979. <https://doi.org/10.1111/mec.13284>
- Moritz, C. (1994). Defining 'evolutionary significant units' for conservation. *Trends in Ecology and Evolution*, 9(10), 373–375. [https://doi.org/10.1016/0169-5347\(94\)90057-4](https://doi.org/10.1016/0169-5347(94)90057-4)
- Murakami, M., Shimada, C., Hikida, Y., Soeda, Y., & Hirano, H. (2014). *Eodelphis kabatensis*, a new name for the oldest true dolphin *Stenella kabatensis* Horikawa, 1977 (Cetacea, Odontoceti, Delphinidae), from the upper Miocene of Japan, and the phylogeny and paleobiogeography of Delphinoidea. *Journal of Vertebrate Paleontology*, 34(3), 491–511. <https://doi.org/10.1080/02724634.2013.816720>
- Nei, M. (1987). *Molecular evolutionary genetics*. Columbia University Press.
- O'Hara, C. C., Frazier, M., & Halpern, B. S. (2021). At-risk marine biodiversity faces extensive, expanding, and intensifying human impacts. *Science*, 372(6537), 84–87. <https://doi.org/10.1126/science.abe6731>
- Paradis, E., & Schliep, K. (2019). ape 5.0: an environment for modern phylogenetics and evolutionary analyses in R. *Bioinformatics*, 35(3), 526–528. <https://doi.org/10.1093/bioinformatics/bty633>
- Parra, G., Cagnazzi, D., & Beasley, I. (2017). *Orcaella heinsohni* (errata version published in 2018). *IUCN Red List of Threatened Species*, e.T136315A123793740. <https://doi.org/10.2305/IUCN.UK.2017-3.RLTS.T136315A50385982.en>
- Perrin, W. F., Dolar, M. L. L., & Alava, M. N. R. (1996). *Report of the workshop on the biology and conservation of small cetaceans and dugongs of Southeast Asia, Dumagette, 27–30 June 1995*. UNEP, Bangkok.
- Peter, C., Zulkifli Poh, A. N., Ngeian, J., Tuen, A. A., & Minton, G. (2016). Identifying habitat characteristics and critical areas for Irrawaddy dolphin, *Orcaella brevirostris*: implications for conservation. In I. Das & A. A. Tuen (Eds.), *Naturalists, explorers and field scientists in South-East Asia and Australasia* (pp. 225–238). Springer.
- Phan, C., Hang, S., Tan, S., & Lor, K. (2015). *Population monitoring of the critically endangered Mekong dolphin based on mark-resight models*. WWF-Cambodia Technical Report.
- Rambaut, A., Drummond, A. J., Xie, D., Baele, G., & Suchard, M.A. (2018). Posterior summarization in Bayesian phylogenetics using Tracer 1.7. *Systematic Biology*, 67(5), 901–904. <https://doi.org/10.1093/sysbio/syy032>
- Raxworthy, C. J., & Smith, B. T. (2021). Mining museums for historical DNA: advances and challenges in museumomics. *Trends in Ecology and Evolution*, 36(11), 1049–1060. <https://doi.org/10.1016/j.tree.2021.07.009>
- Reid, A. J., Carlson, A. K., Creed, I. F., Eliason, E. J., Gell, P. A., Johnson, P. T. J., Kidd, K. A., MacCormack, T. J., Olden, J. D., Ormerod, S. J., Smol, J. P., Taylor, W. W., Tockner, K., Vermaire, J. C., Dudgeon, D., & Cooke, S. J. (2019). Emerging threats and persistent conservation challenges for freshwater biodiversity. *Biological Reviews*, 94(3), 849–873. <https://doi.org/10.1111/brv.12480>
- Robles, E., Piper, P., Ochoa, J., Lewis, H., Paz, V., & Ronquillo, W. (2015). Late Quaternary sea-level changes and the palaeohistory of Palawan Island, Philippines. *Journal of Island and Coastal Archaeology*, 10(1), 76–96. <https://doi.org/10.1080/15564894.2014.880758>
- Rozas, J., Sánchez-DelBarrio, J. C., Messeguer, X., & Rozas, R. (2003). DnaSP, DNA polymorphism analyses by the coalescent and other methods. *Bioinformatics*, 19(18), 2496–2497. <https://doi.org/10.1093/bioinformatics/btg359>
- Rudolph, P., Smeenk, C., & Leatherwood, S. (1997). Preliminary checklist of Cetacea in the Indonesian Archipelago and adjacent waters. *Zoologische Verhandlungen*, 312, 1–48.
- Skovrind, M., Samaniego Castruita, J. A., Haile, J., Treadway, E. C., Golpalakrishnan, S., Westbury, M. V., Heide-Jørgensen, M. P., Szpak, P., & Lorenzen, E. D. (2019). Hybridization between two high Arctic cetaceans confirmed by genomic analysis. *Scientific Reports*, 9, Article 7729. <https://doi.org/10.1038/s41598-019-44038-0>
- Smith, B. D. (2004). *Orcaella brevirostris* (Ayeyarwady River subpopulation). *IUCN Red List of Threatened Species*, e.T44556A10919593. <https://doi.org/10.2305/IUCN.UK.2004.RLTS.T44556A10919593.en>
- Smith, B. D. (2018). Irrawaddy dolphin. In B. Würsig, J. G. M. Thewissen, & K. M. Kovacs (Eds.) *Encyclopedia of marine mammals* (Third ed., pp. 525–529). Academic Press.
- Smith, B. D., Ahmed, B., Mowgli, R. M., Strindberg, S. (2008). Species occurrence and distributional ecology of nearshore cetaceans in the Bay of Bengal, Bangladesh, with abundance estimates for Irrawaddy dolphins *Orcaella brevirostris*

- and finless porpoises *Neophocaena phocaenoides*. *Journal of Cetacean Research and Management*, 10(1), 45–58. <https://doi.org/10.47536/jcrm.v10i1.659>
- Smith, B. D., & Beasley, I. (2004a). *Orcaella brevirostris* (Mekong River subpopulation). *IUCN Red List of Threatened Species*, e.T44555A10919444. <https://doi.org/10.2305/IUCN.UK.2004.RLTS.T44555A10919444.en>
- Smith, B. D., & Beasley, I. (2004b). *Orcaella brevirostris* (Malampaya Sound subpopulation). *IUCN Red List of Threatened Species*, e.T44187A10858619. <https://doi.org/10.2305/IUCN.UK.2004.RLTS.T44187A10858619.en>
- Smith, B. D., Beasley, I., Buccat, M., Calderon, V., Evina, R., de Valle, J. L., Cadigal, A., Tura, E., & Visitacion, Z. (2004). Status, ecology and conservation of Irrawaddy dolphins (*Orcaella brevirostris*) in Malampaya Sound, Palawan, Philippines. *Journal of Cetacean Research and Management*, 6(1), 41–52. <https://doi.org/10.47536/jcrm.v6i1.789>
- Smith, B. D. & Tun, M. T. (2007). Review of the status and conservation of Irrawaddy dolphins *Orcaella brevirostris* in the Ayeyarwady River of Myanmar. In B. D. Smith, R. G. Shore, & A. Lopez (Eds.), *Status and conservation of freshwater populations of Irrawaddy dolphins* (pp. 21–40). Wildlife Conservation Society.
- Smith, B. D., Tun, M. T., Chut, A. M., Win, H., & Moe, T. (2009). Catch composition and conservation management of a human-dolphin cooperative cast-net fishery in the Ayeyarwady River, Myanmar. *Biological Conservation*, 142(5), 1042–1049. <https://doi.org/10.1016/j.biocon.2009.01.015>
- Sonne, C., Xia, S., & Lam, S. S. (2022). Irrawaddy dolphins continue to decline. *Science*, 376(6595), 810. <https://doi.org/10.1126/science.abq5774>
- Stacey, P. J., & Arnold, P. W. (1999). *Orcaella brevirostris*. *Mammalian Species*, 616, 1–8. <https://doi.org/10.2307/3504387>
- Tamura, K., & Nei, M. (1993). Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution*, 10(3), 512–526. <https://doi.org/10.1093/oxfordjournals.molbev.a040023>
- Temple, A. J., Westmerland, E. & Berggren, P. (2021). By-catch risk for toothed whales in global small-scale fisheries. *Fish and Fisheries*, 22(6), 1155–1159. <https://doi.org/10.1111/faf.12581>
- Thomas, P. O., Gulland, F. M. D., Reeves, R. R., Krebs, D., Ding, W., Smith, B., Malik, M. I., Ryan, G. E., & Phay, S. (2019). Electrofishing as a potential threat to freshwater cetaceans. *Endangered Species Research*, 39, 207–220. <https://doi.org/10.3354/esr00962>
- Van Cise, A. M., Baird, R. W., Baker, C. S., Cerchio, S., Claridge, D., Fielding, R., Hancock-Hanser, B., Marrero, J., Martien, K. K., Mignucci-Giannoni, A. A., Oleson, E. M., Oremus, M., Poole, M. M., Rosel, P. E., Taylor, B. L., & Morin, P. A. (2019). Oceanographic barriers, divergence, and admixture: phylogeography and taxonomy of two putative subspecies of short-finned pilot whale. *Molecular Ecology*, 28(11), 2886–2902. <https://doi.org/10.1111/mec.15107>
- Vilstrup, J. T., Ho, S. Y., Foote, A. D., Morin, P. A., Krebs, D., Krützen, M., Parra, G. J., Robertson, K. M., de Stephanis, R., Verborgh, P., Willerslev, E., Orlando, L., & Gilbert, M. T. (2011). Mitogenomic phylogenetic analyses of the Delphinidae with an emphasis on the Globicephalinae. *BMC Evolutionary Biology*, 11, Article 65. <https://doi.org/10.1186/1471-2148-11-65>
- Wang, J. Y., Riehl, K. N., & Dungan, S. Z. (2014). Family Delphinidae (ocean dolphins). In D. E. Wilson & R. A. Mittermeier (Eds.), *Handbook of the mammals of the world, Volume 4: Sea mammals* (pp. 410–527). Lynx Edicions.
- Westbury, M. V., Thompson, K. F., Louis, M., Cabrera, A. A., Skovrind, M., Castruita, J. A. S., Constantine, R., Stevens, J. R., & Lorenzen, E. D. (2021). Ocean-wide genomic variation in Gray's beaked whales, *Mesoplodon grayi*. *Royal Society Open Science*, 8(3), Article 201788. <https://doi.org/10.1098/rsos.201788>
- Whitty, T. S. (2016). Multi-methods approach to characterizing the magnitude, impact, and spatial risk of Irrawaddy dolphin (*Orcaella brevirostris*) bycatch in small-scale fisheries in Malampaya Sound, Philippines. *Marine Mammal Science*, 32(3), 1022–1043. <https://doi.org/10.1111/mms.12322>

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