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Genetic diversity and connectivity of the Irrawaddy dolphin in Southern Thailand: Emphasizing the last fourteen of the Songkhla dolphin status from a microsatellite perspective

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Abstract. *Budi T, Ninwat S, Sakornwimon W, Thongcham K, Phakphien R, Kalaya C, Phavaphutanon J. 2024. Genetic diversity and connectivity of the Irrawaddy dolphin in Southern Thailand: Emphasizing the last fourteen of the Songkhla dolphin status from a microsatellite perspective. Biodiversitas 25: 1729-1735.* Irrawaddy dolphin (*Orcaella brevirostris* Gray, 1866) can be found in diverse aquatic environments, ranging from coastal to brackish water, stretching from the West of Bengal in India to the central part of the Indonesian archipelago. Despite its widespread distribution and flexible habitat tolerance, Irrawaddy dolphins in Songkhla Lake, Southern Thailand, are hypothesized to be isolated and exhibit concerningly low population numbers, threatening their long-term survival. Limited knowledge exists regarding their population genetics status. This study analyzed 30 samples from Songkhla Lake (SKL) and the Lower Gulf of Thailand (LGT) to assess their genetic diversity, structure, and connectivity using 16 microsatellite loci. Extremely low genetic diversity was observed in SKL (H_o = 0.039±0.039; AR = 1.122±0.489) and LGT (H_o = 0.033±0.033; AR = 1.141±0.566) populations. Population differentiation (F_{ST} = 0.033, p>0.05) and clustering analyses based on both Principal Coordinate Analysis (PCoA) and Discriminant Analysis of Principal Component (DAPC) suggest limited genetic divergence and potential panmixia (single genetic pool) between both populations. These findings highlight critical conservation concerns due to low diversity despite the lack of isolation, emphasizing the need for immediate and targeted efforts to ensure the long-term viability of this iconic yet vulnerable population.

Keywords: Cetacean, conservation, panmixia, population differentiation, population structure

Abbreviations: LGT: Lower Gulf of Thailand, SKL: Songkhla Lake

INTRODUCTION

Despite their wide distribution and flexible habitat use in diverse aquatic environments such as riverine, coastal, and brackish water across the Bay of Bengal to the Indonesian archipelago, Irrawaddy dolphin (*Orcaella brevirostris* Gray, 1866) populations have experienced significant declines in both range and numbers (Minton et al. 2018). Some of the Irrawaddy dolphin populations, including the Mahakam River population in Indonesia and the brackish freshwater population in the Songkhla Lake of Thailand, have been isolated with concerning population numbers and categorized differently as Critically Endangered (CR) based on the International Union for Conservation of Nature's (IUCN) Red List of Threatened Species from its coastal relatives (Minton et al. 2018). Habitat loss, conversion, pollution, low birth rates, and high gillnet mortality threaten their long-term survival (Minton et al. 2018). Population estimates vary greatly, ranging from hundreds in the eastern Upper Gulf of Thailand to less than 30 in Songkhla Lake (Jutapruet 2013; Hines et al. 2015). Alarmingly, the latest report by the Department of Marine and Coastal Resources (DMCR) reveals only 14 Irrawaddy dolphins remain in Songkhla Lake (DMCR 2020). This dwindling number has served as a focal point for conservation efforts, with the designation of "last fourteen" acting as a powerful tool to raise public awareness regarding this population.

Southern Thailand, situated on the Malay Peninsula and bordered by the Kra Isthmus, harbors significant biodiversity and serves as a critical habitat for threatened species (Chutipong et al. 2019; Tantipisanuh and Gale 2022). However, this region faces substantial development pressures due to its key role in Thailand's tourism industry and its strategic position as a major shipping hub within China's Maritime Silk Road initiative (Ng et al. 2020). These extreme anthropogenic activities can lead to overfishing, aquatic ecosystems, coral damage, pollution, and the spread of invasive species (Häder et al. 2020; Priya et al. 2023). Coastal ecosystems experience similar threats from land reclamation, dredging, pollution, sedimentation, and overexploitation (Yaakub et al. 2014), with cascading effects potentially impacting endangered species like the Irrawaddy dolphin in Songkhla Lake.

Restricted dispersal due to human disturbances and low population numbers make Irrawaddy dolphins in Songkhla Lake and the Lower Gulf of Thailand particularly vulnerable to demographic stochasticity, genetic drift, and inbreeding (Budi et al. 2022), leading to genetic diversity loss, reduced population fitness, and increased risk of local extinction (Ruiz-García et al. 2018; Vachon et al. 2018). Conversely, enhanced dispersal and gene flow among remnant populations within spatially structured metapopulations can potentially mitigate these negative effects. This mitigation is achieved through increased migration and colonization events, ultimately promoting the restoration of evolutionary adaptation and the persistence of these populations (Charmouh et al. 2022). However, the information on the genetic connectivity and gene flow among Irrawaddy dolphin populations was poorly explored.

While the information on gene flow's role in mitigating these negative impacts is lacking, integrating genetic data into existing demographic monitoring is crucial for accurate extinction risk assessment and effective conservation efforts (Hohenlohe et al. 2020; Willi et al. 2021). This approach can inform the delineation of Management Units (MUs; Harrisson et al. 2014), the establishment of important marine mammal areas, and, ultimately, promoting species survival.

Previous studies on the Irrawaddy dolphin population genetics primarily focused on mitochondrial DNA (mtDNA) D-loop analysis, revealing population-level diversity and structure across the Mekong River, Gulf of Thailand, and Indonesia (Krützen et al. 2018; Caballero et al. 2019; Dai et al. 2021; Budi et al. 2022). While these mtDNA D-loop-based studies suggest population differentiation with close genetic affinity among Thai populations from Songkhla Lake, the Upper Gulf of Thailand, the Lower Gulf of Thailand, and the Andaman Sea (Krützen et al. 2018; Caballero et al. 2019; Budi et al. 2022), microsatellite analysis indicates a single gene pool with potential gene flow between neighboring populations (Dai et al. 2021). Notably, despite the critically endangered status of the Songkhla population, no microsatellite-based genetic assessment has been conducted. Thus, a comprehensive investigation of the genetic connectivity and comparative genetic diversity in Southern Thailand is deemed necessary to evaluate their long-term survivability and formulate appropriate conservation strategies to enhance their adaptive potential toward rapid environmental changes.

Following the problem statement described, this study utilizes 17 microsatellite markers to investigate genetic diversity, structure, and connectivity among Brackish freshwater and coastal Irrawaddy dolphin populations in southern Thailand. This approach aims to elucidate population genetic diversity and connectivity of the Irrawaddy dolphin in Southern Thailand and inform critical conservation decisions necessary to ensure the long-term persistence of this endangered species.

MATERIALS AND METHODS

Specimen collection and DNA extraction

Tissue (muscle or skin) specimens were collected from stranded dolphin in Songkhla Lake (SKL; N = 9) and the Lower Gulf of Thailand (LGT; N = 21). Tissue specimens were collected by the local veterinary team during necropsies conducted between 2007- 2014. Samples were provided by the Department of Marine and Coastal Resources of Thailand from the stranded Irrawaddy dolphin. Specimens' acquisitions were approved by the Department of Fisheries, Ministry of Agriculture and Cooperatives, Thailand (approved on 10 March 2021, Memo No. 0407/425). This study followed the ethical guidelines set forth by Kasetsart University's Animal Experiment Committee (Approval numbers: ACKU64-VET-031) and the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines (https://arriveguidelines.org/). Therefore, following the manufacturer's instructions, total genomic DNA from muscle or skin samples was extracted using the PureLinkTM Genomic DNA Mini Kit (Thermo Fisher; USA). DNA quality and quantity were evaluated as described by Budi et al. (2022).

Polymerase Chain Reactions (PCR) for microsatellite loci amplification

PCR analysis was performed in a 25 µL reaction that contained a 100 ng template, and each sample was adjusted to 50 ng/µL. A set of seventeen microsatellite markers was used for the analysis (MK6, MK9 (Krützen et al. 2002), Sco11, Sco28, Sco65, Sco66 (Mirimin et al. 2006), Tur4-66, Tur4-80, Tur4-91, Tur4-98, Tur4-111, Tur4-117, Tur4-128, Tur4-138, Tur4-141, Tur4-153 and Tur4-E12 (Nater et al. 2009) and selected based on PIC value. The PCR products were checked on a 1.0% agarose gel electrophoresis, and an analysis of DNA fragments was conducted using QIAxcel capillary electrophoresis (Qiagen; USA), and allelic sizes were determined in QIAxcel ScreenGel Software (Qiagen; USA).

Microsatellite data analysis

Due to the low amplification rate, locus Sco11 was excluded for further analysis. Genetic diversity within subpopulations and across the total population was assessed using Arlequin (Excoffier and Lischer 2010). This included measures of observed Heterozygosity (H_o), expected Heterozygosity (H_e), Number of alleles (N_a), and Wright's F-statistics (F_{IS} and F_{ST}). Polymorphic Information Content (PIC) was calculated using the Excel Microsatellite Toolkit (Park 2001), while Allelic Richness (AR) was determined using FSTAT (Goudet 1995). Population structure was investigated using a combination of methods, including Analysis of Molecular Variance (AMOVA) performed in Arlequin version 3.5.2.2 (Excoffier and Lischer 2010), Principal Coordinate Analysis (PCoA) performed in GenAlEx version 6.5 (Peakall and Smouse 2012), and Discriminant Analysis of Principal Component (DAPC) performed using the package ADEGENET 2.0 (Jombart 2008) in R 4.1.2 (R Core Team 2022).

Population structure was further identified using the model-based program STRUCTURE version 2.3.4 (Pritchard et al. 2000). After a burn-in period of 100,000 generations, the run length was adjusted to 100,000 Markov Chain Monte Carlo (MCMC) repeats utilizing correlated allelic frequencies under a straight mixing model. The number of clusters (K) was run from 1 to 4, with 25 replicates for each value of K. The most appropriate K number was determined based on Evanno's ΔK and the mean ln P(K) value (Pritchard et al. 2000). The ΔK strategy was also applied using Structure Harvester (Earl and vonHoldt 2011). Deviations from Hardy-Weinberg equilibrium within each locus were evaluated by a MCMC approach in the "genepop" function (R Core Team 2022). Significant differences and unequal variances in H_o and H_e between subpopulations were assessed using Welch's t-test and Bartlett's test, respectively (R Core Team 2022).

RESULTS AND DISCUSSION

Genetic diversity of the Irrawaddy dolphin populations in Southern Thailand

Genetic analyses revealed low diversity in southern Thailand's Irrawaddy dolphin populations. Amplification of 16 microsatellite loci in 30 samples (Table 1) yielded an average observed Heterozygosity (H_o) of 0.036±0.025 and an expected Heterozygosity (H_e) of 0.041±0.028. The mean number of alleles per locus was 1.156±0.111, with 19 alleles observed. Polymorphic Information Content (PIC) was 0.040 for Songkhla Lake (SKL) and 0.033 for Lower Gulf of Thailand (LGT) populations. The average Allelic Richness (AR) was 1.122±0.489 (SKL) and 1.141±0.566 (LGT), while Wright's F-statistic (F) was 0.101±0.046 (SKL) and 0.143±0.039 (LGT). There is no significant difference between H_o and H_e (Welch's t-test, p = 0.946). The mean F_{IS} values in both populations were considered low, however, individual loci F_{IS} can not be determined due to monomorphic loci. The M ratio of both populations were <0.68, reflecting a historical population decline (Garza and Williamson 2001).

Genetic structure of the Irrawaddy dolphin populations in Southern Thailand

Pairwise F_{ST} value between SKL and LGT was low and insignificant ($F_{ST} = 0.033$, $p>0.05$). Analysis of Molecular Variance (AMOVA) revealed high within-population variation (94%) and low between-population variation (6%). The PCoA and DAPC plots (Figure 1) displayed no population clustering between two populations. Bayesian clustering using STRUCTURE indicated a single genetic pool (panmixia) across various K-values (K = 2-4) (Figure 2). The K = 2 was the best K value based on both Evanno's ΔK and the mean ln P(K) value.

Discussion

This study documented critically low genetic diversity within the Irrawaddy dolphin population inhabiting Southern Thailand. This finding aligns with the "last fourteen dolphins" label highlighting Songkhla Lake dolphins' critically low population size. Previous estimation suggests less than 30 individuals (Jutapruet 2013) in Songkhla Lake, consistent with established theories linking small, isolated populations to lower genetic diversity (Ralls et al. 2020). As predicted, the results demonstrate the lowest genetic diversity reported for Irrawaddy dolphins elsewhere (Krützen et al. 2018; Dai et al. 2021). Notably, the lack of significant difference between H_o and H_e ($p = 0.946$) suggests no inbreeding signatures in these populations. Similarly, no evidence for inbreeding despite low genetic diversity was observed in cetaceans elsewhere (Parra et al. 2018; Westbury et al. 2019; de Greef et al. 2022). The observed low diversity is unlikely attributable to inbreeding (Westbury et al. 2019). The interplay between geographic range, demographic dynamics, and life history shapes a species' genetic diversity and population structure (Romiguier et al. 2014; Ellegren and Galtier 2016). This is particularly evident in species with restricted distributions, low population sizes, high longevity, and historical declines, such as the Irrawaddy dolphin, which often exhibits low genetic diversity (Parra et al. 2018). This further confirmed by M ratio test result that indicating likelihood of bottleneck event. Moreover, reduced genetic diversity often translates to diminished population fitness and heightened local extinction risk (Nordstrom et al. 2023). This underscores the urgency of implementing immediate conservation interventions to secure the survival of the critically small, fourteen-strong Songkhla Irrawaddy dolphin population.

Table 1. Genetic diversity of the Irrawaddy dolphin in Southern Thailand based on 16 microsatellite loci

Population		N_a	AR	N_{ea}	I	H_o	H_e	PIC	F	F_{IS}	M ratio
SKL	Mean	1.188	1.122	1.143	0.078	0.039	0.043	0.0397	0.101	0.048	0.042
	SE	0.188	0.489	0.143	0.078	0.039	0.043	0.1589	0.046	-	-
LGT	Mean	1.125	1.141	1.098	0.063	0.033	0.038	0.0331	0.143	0.167	0.062
	SE	0.125	0.566	0.098	0.063	0.033	0.038	0.1323	0.039	-	-
Overall	Mean	1.156	1.132	1.120	0.070	0.036	0.041	0.0364	0.122	0.134	0.050
	SE	0.111	0.527	0.085	0.049	0.025	0.028	0.1456	0.005	-	-

Note: N_a: Number of alleles, AR: Allelic Richness, N_{ea}: Number of effective alleles, I: Shannon’s information index, H_o: Observed heterozygosity, H_e: Expected heterozygosity, PIC: Polymorphic Information Content, F: Fixation index, F_{IS}: Inbreeding coefficient, N_a/allelic range: M ratio

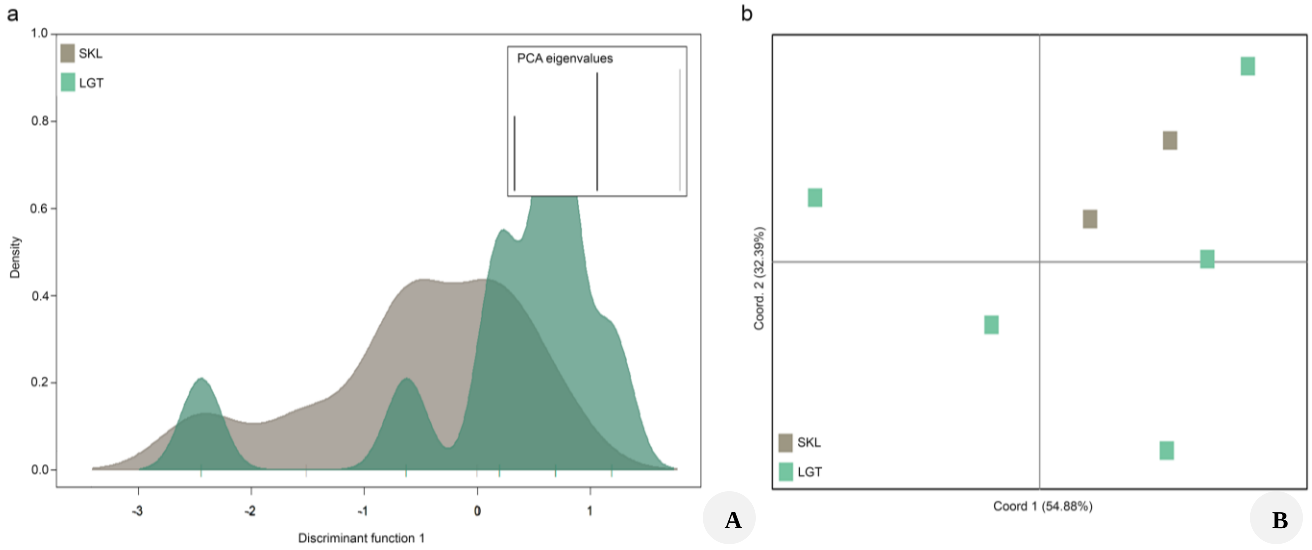

Figure 1. The result of Discriminant Analysis of Principle Component (DAPC). A. Principal Coordinate Analysis (PCoA), B. Irrawaddy dolphin in Southern Thailand based on 16 microsatellite loci

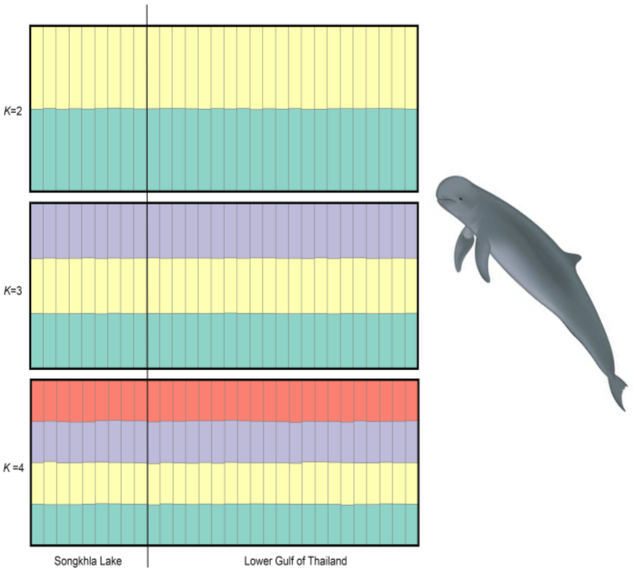

Figure 2. The population structure of the Irrawaddy dolphin in Southern Thailand is based on 16 microsatellite loci. Black vertical lines indicate population boundaries. The y-axis in each genetic cluster represents the proportion of membership (posterior probability), while each vertical bar on the x-axis represents an individual

Population differentiation analyses revealed limited genetic divergence ($F_{ST} = 0.033$, $p>0.05$) between coastal and lake populations of Irrawaddy dolphins. Similarly, AMOVA revealed high within-population variation compared to between-population variation. PCoA and DAPC plots displayed no population clustering, further supporting this finding. Bayesian clustering using STRUCTURE indicated a single genetic pool (panmixia) across various K-values (Figure 2).

Despite ecological isolation and habitat modifications, suggesting limited connectivity between lake and coastal populations, genetic data indicated gene pool sharing. This aligns with previous assessments using microsatellite loci (Dai et al. 2021) and mitochondrial DNA (Budi et al. 2022), suggesting historical or ongoing gene flow. However, a single gene pool does not necessitate single-unit management. Local factors can influence population dynamics and lead to isolation despite shared ancestry (Durante et al. 2022). Further research and local-level monitoring are crucial to assess potential metapopulation structure and inform appropriate management strategies.

The apparent absence of genetic structure suggesting a panmictic population could be attributed to the Irrawaddy dolphin's extended generation time and lifespan (Vachon et al. 2018; Louis et al. 2021). These factors may impede allele fixation or loss within populations (Pfennig and Lachance 2022), thereby delaying genetic differentiation. This hypothesis finds support in recent estimates suggesting relatively recent divergence times despite significant but low levels of population differentiation based on mtDNA analysis (Budi et al. 2022). The single gene pool observed in this study might reflect the low sample number. Sample size limitations warrant consideration. While current estimates suggest sufficient coverage of the lake population (9 individuals sampled, representing >50%), data on the coastal population along Songkhla-Phatthalung province is lacking. However, the sample number used for the UGT population was 21 individuals, reflecting sufficient numbers for most population-level studies (Grünwald et al. 2017).

The number of loci used for this study was considered appropriate (Arthofer et al. 2018); however, the loci used to assess the population genetic diversity are likely to be monomorphic, and the probabilities of identity PID and PID-sibs value for those loci used were considered high ($PID>10^{-8}$ and $PID\text{-}sib>10^{-4}$) indicating that this result is prone to be bias due to close siblings which could lead to an underestimation of other genetic parameters including heterozygosity, relatedness, and structure (Gomes et al.

2024). Therefore, the monomorphic loci might mask the real genetic diversity and structure within both populations. Microsatellite markers employed in this study were selected from previously established panels for other species, prioritizing polymorphic loci. However, it is important to note that loci exhibiting polymorphism in other species might not necessarily be polymorphic in Irrawaddy dolphins (Cunha et al. 2021). Alternatively, the observed result may be attributed solely to the inherently low population size, increasing the likelihood of relatedness within a population (Sethuraman 2018). Further studies are necessary to elucidate the genetic status of the remaining fourteen Songkhla Irrawaddy dolphins. Employing a broader panel of microsatellite loci or Single Nucleotide Polymorphisms (SNPs) could enhance our understanding of population structure and potential isolation mechanisms.

Irrawaddy dolphin populations in Southern Thailand face dire conservation challenges, with the SKL population classified as Critically Endangered (CR) and the LGT population as Endangered (EN) (Minton et al. 2018). This necessitates immediate intervention to ensure their long-term survival. A crucial question in conservation management is determining if distinct groups of individuals should be considered separate populations for management purposes. While this study indicates panmixia in the Southern Thailand Irrawaddy dolphins, aligning with the common treatment of metapopulations as single units, the absence of observed differentiation does not definitively negate its existence (Waples and Lindley 2018). Notably, relying solely on genetic data for management decisions can be misleading.

Waples and Lindley (2018) emphasize integrating genetic data with other ecological information for effective population management. If ecological assessments suggest a metapopulation structure, separate management approaches might be necessary to mitigate local disturbances that could disrupt population dynamics and lead to isolation (Durante et al. 2022). Enhancing the long-term persistence of these populations requires strategies geared toward increasing or maintaining genetic diversity, effective population size, and connectivity. Implementing genetic monitoring is crucial to assess the ongoing maintenance of genetic variation and map diversity across their range (e.g., within and between populations). Seascape genetics approaches can also elucidate how habitat heterogeneity influences genetic structuring and dispersal (Nielsen et al. 2020).

The alarmingly low population numbers of the Irrawaddy dolphin reflect their limited effective population size, emphasizing the detrimental consequences of minimal human-caused mortality, such as by-catch incidents due to high fisheries activity in both SKL and LGT (Benson et al. 2023). Given their already low genetic diversity, conservation efforts must prioritize mitigating direct human-related threats. Restrictions on gillnet use, coupled with the promotion of non-entangling and more selective fishing gear, have been identified as potential strategies to mitigate entanglement mortality in Irrawaddy dolphins (RASI 2024, pers.com. (personal communication). The implementation of electronic pingers on gillnets represents an alternative approach. While positive results have been documented for their use with Mahakam River Irrawaddy dolphins (RASI 2024, pers.com. (personal communication). Further research is necessary to determine their broader efficacy across various populations and ecological contexts. Alternatively, habitat management through the implementation of protection acts, stricter fisheries regulations, and enforcement of protected area regulations represents another potential conservation strategy for Irrawaddy dolphins. The success of this approach is exemplified by the stable population size observed in the Mahakam River dolphins (RASI 2024, pers.com. (personal communication).

Genetic analysis of Irrawaddy dolphin populations in Southern Thailand revealed low diversity, particularly in the critically endangered Songkhla Lake population. Despite this diversity deficit, no evidence of inbreeding depression was detected. Additionally, no significant genetic differentiation was observed between the Songkhla Lake and Lower Gulf of Thailand populations, suggesting potential panmixia. However, relying solely on genetic data for management decisions is cautioned against, and even with panmictic tendencies, considering each population as separate units for management may be advisable. This study provides valuable insights into the population genetics and connectivity of Irrawaddy dolphins in Southern Thailand, informing crucial conservation efforts. Interpretation of these results should be undertaken with caution due to potential biases arising from the limited sample size and the utilization of monomorphic loci. The opportunistic nature of sample collection also raises concerns regarding generalizability. While the findings may be consistent with existing data, their representativeness of true population patterns remains undetermined. Further studies employing a larger sample size and enhanced genomic resolution through a SNPs approach are warranted to definitively assess the genetic consequences within this low population of Irrawaddy dolphins.

This study revealed critically low genetic diversity, based on microsatellite loci analysis, within the Irrawaddy dolphin population inhabiting southern Thailand, particularly in the endangered Songkhla Lake population. This finding suggests potential limitations in their adaptive capacity to respond to environmental changes and anthropogenic pressures. This result further emphasizes the urgency of mitigating human-caused threats, to prevent further declines and enhance the long-term persistence of these critically endangered dolphins.

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