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Determining the Matrilineal Origin of Indonesian Kerinci Duck Breed (*Anas platyrhynchos*) Based on MT-ND2 Gene Diversity

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ABSTRACT

The Kerinci duck (*Anas platyrhynchos*), hailing from Jambi Province, represents one of the local duck breeds of Indonesia. This study aimed to ascertain the matrilineal origin of Kerinci ducks through analysis of the mitochondrial NADH Dehydrogenase subunit 2 (MT-ND2) gene. In this pursuit, forward sequences of the MT-ND2 gene (490 bp) were extracted from unsexed Kerinci ducks, totalling forty-eight (48) sequences, procured from blood samples. The findings demonstrated the identification of fifteen (15) distinct haplotypes within the MT-ND2 gene, with corresponding haplotype diversity (Hd) and nucleotide diversity (pi) values of 0.74 and 0.003, respectively. The resulting phylogenetic tree unveiled that Kerinci ducks exhibit two matrilineal origins: an Asian and an independent Kerinci lineage. Moreover, most Kerinci ducks were categorised within the H4 group (24 birds) of the Asian lineage. Nevertheless, this study also revealed the existence of an independent Kerinci lineage comprising eight (8) duck haplotypes. In conclusion, the analysis of the MT-ND2 gene underscored the genetic introgression of *A. zonorhyncha* and *A. poecilorhyncha* in Kerinci ducks.

1. Introduction

The Kerinci duck (*Anas platyrhynchos*), originating from the Kerinci Regency in Jambi Province, Indonesia, has been officially recognised as an Indonesian duck breed since 2012 through the Minister of Agriculture's Decree No. 2834/Kpts/LB.430/8/2012 (Hartatik 2019). This duck is reared for both meat and egg production. Khanza *et al.* (2021) revealed that the average weights of Kerinci duck eggs and Day-Old Ducklings (DOD) were 71.80±1.27 g (males), 67.20±1.92 g (females), and 54.28±4.07 g (males) and 44.80±3.94 g (females), respectively. Additionally, Salsabila *et al.* (2022) indicated that at three months of age, Kerinci ducks have an average body weight of approximately 1,673.91±47.25 g (males) and 1,441.18±41.94 g (females). However, data from the Badan Pusat Statistik or Indonesian Statistics (2023) shows a decline in the local duck population in Kerinci District from 110,045 individuals in 2020 to 52,209 in 2021. Therefore, implementing breeding and genetic improvement programs is essential to boost the future population and economic significance of Kerinci ducks.

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Genetic analysis is vital in preserving germplasm and characterising breeds (Glowatzki-Mullis *et al.* 2006). This process establishes breed standards for safeguarding genetic diversity across breeds, particularly within pure breeding programs. The use of mitochondrial DNA (mtDNA) for genetic analysis in livestock breeds gains prominence due to its higher mutation site density compared to other genetic materials, as demonstrated by Natonek-Wisniewska *et al.* (2021) and Jia *et al.* (2023). As a result, the genetic structure and inheritance patterns within mtDNA can be harnessed to trace breed lineages and differentiate duck breeds. The configuration of mtDNA in *Anas platyrhynchos* exhibits a circular double-helix DNA structure, approximately 16,741 base pairs long in Bengal ducks (Pal *et al.* 2022) and 16,597 base pairs in Chaohu ducks (Jia *et al.* 2023), containing 13 protein-coding genes, two rRNA genes (12S rRNA and 16S rRNA), 22 tRNA genes, and a single control region or D-loop.

Various partial mtDNA regions, including Cyt-b, D-loop, COI, and 18s rRNA, have been employed to characterise native Indonesian duck breeds (Hitosugi *et al.* 2007; Susanti *et al.* 2017, 2018; Kusumaningrum *et al.* 2018). As a result, each region can characterise Indonesia's duck breeds. Beyond breed characterisation, these partial mtDNA regions

have also been used to trace maternal ancestry among duck breeds from East Asia, China, and the Andaman region (Hitosugi *et al.* 2007; He *et al.* 2008; De *et al.* 2021). Within these mtDNA gene regions, NADH Dehydrogenase subunit 2 (ND2) is a reliable genetic marker for various diving duck species, especially those of the Aythya genus (Luttrell *et al.* 2020). That study revealed that a total of twenty-two (22) segregation sites of the MT-ND2 gene were found between *A. collaris* and *A. americana*. The MT-ND2 region has also been utilised as a molecular marker for species within the Osteoglossidae fish family (Mu *et al.* 2012) and Hemiphyllodactylus lizards (Sung *et al.* 2018). In addition, the MT-ND2 gene can identify bovine and porcine in meat products (Barido *et al.* 2023). Interestingly, a genetic mutation in the MT-ND2 gene is associated with high-altitude adaptability in yak (*Bos mutus*), as Shi *et al.* (2017) reported. Kerinci ducks live in the highland area of Kerinci Regency and may have a specific MT-ND2 gene diversity. Nevertheless, the utility of the MT-ND2 gene to characterise duck breeds remains absent from the scientific literature. Thus, the present study aims to delve into the partial MT-ND2 gene in Kerinci ducks, seeking to unveil their matrilineal origins. The results of this investigation hold promise as a potential genetic marker for conserving Kerinci duck germplasm.

2. Materials and Methods

2.1. Ethics Approval

The Faculty of Animal Science, Jambi University Ref's ethics committee has issued ethics approval or ethical clearance. 02/UN21.7/ECC/2023, Date, May 2nd, 2023.

2.2. Duck Samples and Research Site

Eight hundred fertilised eggs were bought from a duck farmer at Kerinci Regency. Therefore, 635 DOD were produced with hatchery machines at the Teaching Farm of the Faculty of Animal Science, Jambi University of Indonesia. After four months of rearing at a teaching farm, 185 ducks were deemed Kerinci breeds based on their qualitative characteristics, as described by Supriawan *et al.* (2023). In contrast, the remains are different duck breeds, such as Alabio, Mojosari and Tegal. In the scope of the present investigation, 48 unsexed native Kerinci ducks (*Anas platyrhynchos*), each at the age of three months, were utilised. These ducks were procured from local farmers in the Kerinci Regency of Jambi Province, Indonesia. The geographical coordinates of this region encompass altitudes ranging from approximately 500 to 1,500 meters above sea level, positioned within a latitude range of 01°40'24" to 02°26'54" S and a longitude range spanning from 04°59'31" to 05°40'40" E. Additionally, the prevailing climate in this area features air temperatures varying from 16 to 20°C, coupled with an average annual rainfall ranging between 800 to 3,000 mm and a relative humidity level of 82%.

2.3. DNA Extraction

Blood samples of approximately 2 ml were collected from each duck using a syringe and a vacutainer tube containing EDTA via the wing axillary vein. Subsequently, these collected blood samples were preserved by storing them in a freezer maintained at a temperature of -20°C, awaiting subsequent analysis. The DNA extraction process was executed using the Genomic DNA Extraction Kit (manufactured by Geneaid, Taiwan) following the manufacturer's prescribed procedures.

2.4. PCR and Sequencing Analyses

PCR analysis was conducted within a total volume of 30 µL, comprising 3 µL of DNA template, 0.60 µL of each primer (10 pmol), 10.8 µL of nuclease-free water, and 15 µL of the KAPA 2G HotStart Ready Mix Kit (manufactured by Sigma-Aldrich, USA). The primer pairs Forward: 5'- TGC AAC CCC AGT CCT AGT C -3' and Reverse: 5'- GAA GGC TAG GAT TTT GCG TGT -3', as outlined by Henrik *et al.* (2018), were employed in this study to amplify the Anas platyrhynchos MT-ND2 gene (GenBank: MK770342), spanning a length of 532 bp.

The targeted sequence of this specific primer pair is located within the ND2 region. Amplification of the MT-ND2 gene was carried out using a thermocycler machine manufactured by Eppendorf, involving a PCR reaction encompassing 35 cycles. The process commenced with an initial pre-denaturation step at 95°C for 5 minutes, followed by denaturation at 95°C for 15 seconds, annealing at 60°C for 15 seconds, extension at 72°C for 20 seconds, and a final extension at 72°C for 3 minutes. Subsequently, DNA visualisation was achieved using a 2% agarose gel for electrophoresis analysis, conducted at 100 Volts for 25 minutes. Visualisation was facilitated using a Gel Documentation System from Syngene in the UK and the SYBR® DNA staining reagent provided by Invitrogen in the USA. Forward sequencing was performed by First Base Laboratory Service in Malaysia, utilising the Sanger sequencing machine ABI Prism 3730xl Genetic Analyzer manufactured by Thermo Scientific in the USA.

2.5. Bioinformatic Analysis

For the bioinformatic analysis conducted in this research, three (3) molecular software applications were employed, including BioEdit (Hall 2011), MEGA (Hall 2013), and DNAsp (Librado *et al.* 2009). BioEdit was specifically utilised for the alignment of sequences. MEGA was employed to calculate pairwise genetic distances and construct a phylogenetic tree using the Maximum Likelihood (ML) method, incorporating 1,000 bootstrap replications. The DNAsp was performed to estimate the number of mutation sites, haplotypes, haplotype diversity (Hd), nucleotide diversity (pi), Tajima's D test and Fu's Fs statistics values. For comparative purposes with the MT-ND2 gene of Kerinci ducks, many reference sequences of MT-ND2 gene from *A. platyrhynchos* (KJ883269; MH744426; MN720361), *A. zonorrhyncha* (MZ593724), *A. poecilorhynca* (KC466567), A. acuta (NC024631), *A. crecca* (KC771255), mallard duck (EU755253) and many Asian native duck breeds (Table 1) were sourced from the GenBank database (https://www.ncbi.nlm.nih.gov).

Table 1. Reference sequences of MT-ND2 in Asian native duck breeds used in the present study

Breed	Origin	GenBank
Chaohu	China	MZ922472
JiAn red	China	MW354666
Jianchang	China	FJ167857
Jinding	China	MF069248
Jingxi	China	KJ689447
Liancheng white	China	MF069249
Linwu	China	KJ637997
Longsheng	China	KJ739616; MZ895120
Pekin	China	EU009397; EU755252; NC009684
Putian black	China	MF069250
Quanzhou	China	MZ895121
Rongshui	China	KJ833587
Shan partridge	China	MF069251
Shaoxing	China	HM010684
Shennjin lake	China	MK770342
Sichuan	China	KX592536
Xilin	China	KJ833586
You xian	China	KJ778676
Zongyang medium	China	MZ962673
West Bengal	China	MN011574

3. Results

3.1. Diversity of MT-ND2 Gene

A segment of 532 bp from the partial MT-ND2 gene in Kerinci ducks was effectively amplified and visualised in a 2% agarose gel (Figure 1). Consequently, 490 bp from the MT-ND2 gene sequences of Kerinci ducks were analysed to calculate genetic diversity parameters, as outlined in Table 2. Therefore, the sequence alignment of the MT-ND2 gene of birds under study was compared with the *Anas platyrhynchos* MT-ND2 gene (MK770342) to detect their mutation sites, as outlined in Table 3.

As indicated in Table 3, the MT-ND2 gene of Kerinci ducks exhibited twenty-five (25) mutation sites. These mutation sites allowed the classification of the MT-ND2 gene into fifteen (15) distinct haplogroups, denoted as H1 to H15. Consequently, the analysis revealed a relatively high haplotype diversity (Hd) of 0.74 and a comparatively low nucleotide diversity (pi) of 0.003 in the MT-ND2 gene of Kerinci ducks. Fu's Fs and Tajima's D tests' statistical values were negative. Predominantly, Kerinci ducks were clustered within H4 (24 ducks), followed by H3 (5 birds), H5/H12 (3 ducks), H1/H9 (2 ducks), and a single bird for an alternate haplotype, as outlined in Table 2. Notably, Haplotype 14 (H14) displayed a higher count of mutation sites than the other haplotypes. This study's calculated pairwise genetic distance ranged from 0.000 to 0.038, as depicted in Table 4. Consequently, Haplotype 14 (H14) exhibited

Table 2. Genetic diversity in partial MT-ND2 gene of Kerinci ducks (*Anas platyrhynchos*)

Parameter	Value
Number of the observed sequence	48.00
Number of observed site	490
Number of the mutation site	25
Number of haplotypes	15
Haplotype diversity (Hd)	0.74
Nucleotide diversity (pi)	0.003
Tajima's D test	-2.44
Fu's Fs statistics	-2.03

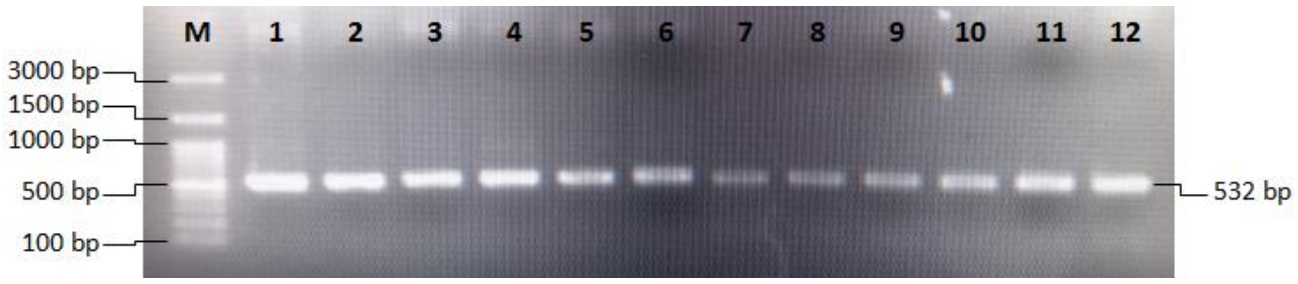

Figure 1. The amplicons of Kerinci MT-ND2 gene along 532 bp on 2% agarose gel. M: DNA ladder 100 bp; line 1-12: DNA sample

Table 3. Mutation sites in the partial MT-ND2 gene of Kerinci ducks (*Anas platyrhynchos*)

Sequence	N	5104	5110	5120	5128	5217	5392	5487	5502	5515	5521	5532	5533	5538	5544	5550	5551	5552	5553	5556	5557	5568	5584	5586/5587	5587	5588	5589
MK770342		A	A	A	G	G	A	T	A	G	G	A	G	A	A	A	A	T	A	A	C	A	C	C	G	G	C
Haplotype 1	2	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	T	.	.	.
Haplotype 2	1	.	.	-	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Haplotype 3	5	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Haplotype 4	24	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Haplotype 5	3	.	.	.	.	A	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Haplotype 6	1	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	T	.	.	.
Haplotype 7	1	-	.	.	A	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Haplotype 8	1	.	.	.	A	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Haplotype 9	2	.	-	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Haplotype 10	1	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.
Haplotype 11	1	.	.	.	A	A	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Haplotype 12	3	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Haplotype 13	1	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Haplotype 14	1	..	.	.	.	.	T	A	G	C	C	C	C	T	G	G	T	G	.	G	T	G	.	.	C	C	T
Haplotype 15	1	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	..

N: Number of duck

Table 4. Pairwise genetic distance among haplotype of MT-ND2 gene in Kerinci ducks (*Anas platyrhynchos*)

Haplotype	H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12	H13	H14	H15
H1	-														
H2	0.002	-													
H3	0.002	0.000	-												
H4	0.002	0.000	0.000	-											
H5	0.004	0.002	0.002	0.002	-										
H6	0.000	0.002	0.002	0.002	0.004	-									
H7	0.004	0.002	0.002	0.002	0.004	0.004	-								
H8	0.004	0.002	0.002	0.002	0.004	0.004	0.000	-							
H9	0.002	0.000	0.000	0.000	0.002	0.002	0.002	0.002	-						
H10	0.004	0.002	0.002	0.002	0.004	0.004	0.004	0.004	0.002	-					
H11	0.006	0.004	0.004	0.004	0.002	0.006	0.002	0.002	0.004	0.006	-				
H12	0.002	0.000	0.000	0.000	0.002	0.002	0.002	0.002	0.000	0.002	0.004	-			
H13	0.002	0.000	0.000	0.000	0.002	0.002	0.002	0.002	0.000	0.002	0.004	0.000	-		
H14	0.038	0.036	0.036	0.036	0.038	0.038	0.038	0.038	0.036	0.038	0.040	0.036	0.036	-	
H15	0.002	0.000	0.000	0.000	0.002	0.002	0.002	0.002	0.000	0.002	0.004	0.000	0.000	0.036	-

the highest genetic distance, ranging from 0.036 to 0.038, compared to the other haplotypes.

3.2. Phylogenetic Tree of Kerinci Duck

The phylogenetic tree among haplotype MT-ND2 gene in Kerinci ducks and twenty-four (24) Asian duck breeds is illustrated in Figure 2.

Based on the diversity observed in the MT-ND2 gene, the categorisation of Kerinci ducks can be delineated into two distinct lineages: the Asian lineage encompassing H2, H3, H4, H9, H12, H13, and H15 and the independent Kerinci lineage including H1, H5, H6, H7, H8, H10, H11, and H14. According to Figure 2, seven haplotypes of MT-ND2 were classified into similar clusters with native Asian duck breeds and mentioned as Asian lineage. In contrast, the other haplotypes of the Kerinci duck were classified into separate clusters. The phylogenetic of the Kerinci duck with ML method reveals the close genetic relationship among *A. poecilorhyncha, A. zonorhyncha* and Asian duck breeds, including Kerinci duck. Consequently, *A. acuta* and *A. crecca* were identified as the outgroup species concerning *A. platyrhynchos*.

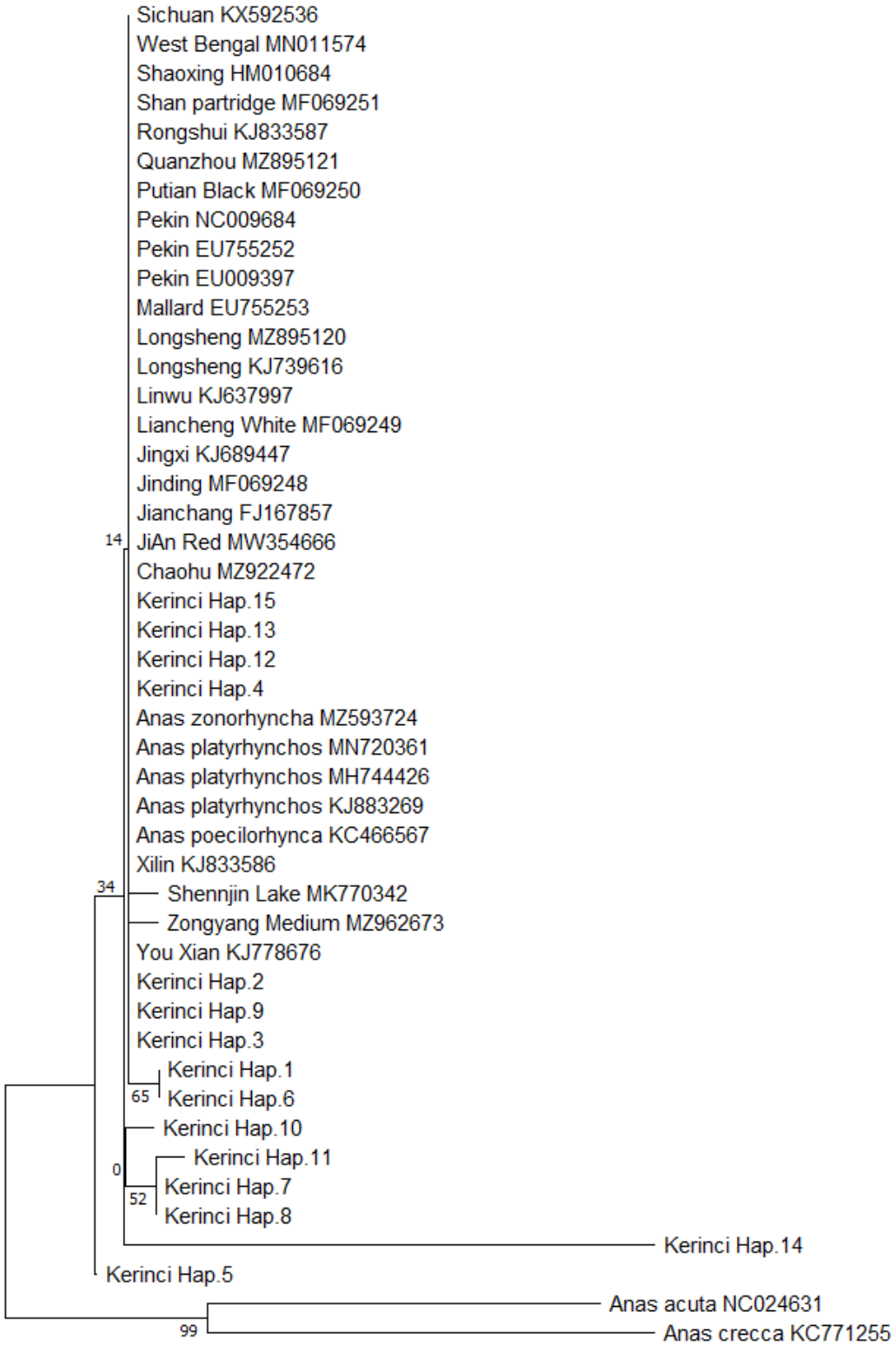

Figure 2. The dendrogram of Kerinci and Asian duck breeds based on the MT-ND2 gene (490 bp) calculated with Maximum Likelihood (ML) 1,000 bootstrap replication method

4. Discussion

The MT-ND2 gene diversity analysis of Kerinci ducks demonstrated polymorphism with an Hd value exceeding 0.50, indicating a high level of diversity. This trend was also observed in various mtDNA gene regions, such as Cytb and D-loop, among Chinese native duck breeds (He *et al.* 2008; Li *et al.* 2010). Similarly, Nigerian and Iraqi native ducks showcased Hd values of 0.38 and 0.67, respectively, in their mitochondrial D-loop genes (Adebambo *et al.* 2017; Abdulkareem 2020). However, the relatively low pi value of 0.003 in the MT-ND2 gene of Kerinci ducks suggested limited allelic variation likely due to selection, a phenomenon elucidated (VanBuren *et al.* 2016). Nei and Kumar (2000) categorised Hd values below and above 0.50 as low and high, respectively, while pi values were classified into three categories: low (0.01-0.04), moderate (0.05-0.07), and high (0.08-0.10). Low population size, inbreeding and crosssbreding may affect the genetic diversity in Kerinci ducks. Despite this, farmers prefer to select Kerinci ducks that produce a blue eggshell rather than a white eggshell. Nurdin and Prayogi (2018) reported that the fertility and hatchability rates in blue eggshell colour were higher than in white eggshells. Hence, the selection of eggshell colour can influence the genetic diversity of Kerinci ducks.

The negative results of the neutrality test in Kerinci ducks pointed toward species expansion and inbreeding. This negative outcome also implied an overabundance of alleles, possibly due to the dominance of selection constraints. Compared to Asian native ducks, Kerinci ducks were primarily clustered within the Asian lineage. Hitosugi *et al.* (2007) proposed that the domestication of ducks took place in China about 3,000 years ago, originating from wild Mallards (*A. platyrhynchos*) and Eastern spot-billed ducks (*A. zonorhyncha*). Based on mitochondrial Cyt-b gene analysis, most Indonesian native duck breeds were classified within the Southeast Asia lineage, distinct from the Mallard lineage (Hitosugi *et al.* 2007). A similar conclusion was reached by De *et al.* in 2021, identifying two major duck lineages globally: Euro-Asia and North America.

Moreover, Chinese native duck breeds exhibited two matrilineal origins, i.e., *A. platyrhynchos* and *A. zonorhyncha* lineages, based on the mitochondrial D-loop gene (Li *et al.* 2010). Consequently, the genetic introgression of both *Anatidae* species was established in Indonesian duck breeds, including Kerinci ducks, based on the mitochondrial D-loop gene (Susanti *et al.* 2017). The present study reveals similar findings to Susanti *et al.* (2017), who reported two genetic introgression from the Indian spot-billed duck (*A. poecilorhyncha*) and Eastern spot-billed duck (*A. zonorhyncha*) in Kerinci ducks based on MT-ND2 gene diversity. Thus, the hybridisation among Anatidae species belonging to mallard duck (*A. platyrhynchos, A. poecilorhyncha* and *A. zonorhyncha*, are the main factors that contribute to the genetic composition of native Asian duck breeds. In the future, the ND2 gene may have the potency to discriminate Indonesian duck breeds.

This investigation established the independent Kerinci lineage through MT-ND2 gene analysis, a finding parallel to the classification of Andaman local ducks into independent Andaman lineages based on the mitochondrial D-loop gene (De *et al.* 2021). As the highland duck type, the Kerinci MT-ND2 gene diversity may have been associated with high-altitute adaptability traits, as in yak (Shi *et al.* 2017). Hence, many Kerinci ducks have different MT-ND2 gene sequences classified into independent clusters. The presence of the Asian gene pool in Kerinci duck haplotypes could be attributed to historical commercial exchanges between the Jambi kingdoms and Asian trading centres between 1480 and 1834, during which poultry species were likely transported. The migration of mallards, the sole ancestor of ducks, from mainland Asia to Southeast Asia, including the Kerinci Regency on Sumatra Island, has been ongoing for hundreds of years. The independent Kerinci lineage indicates local microenvironment-driven evolution shaped by inbreeding, crossbreeding, and environmental influences. Inbreeding could impact MT-ND2 diversity in Kerinci ducks due to population decline, while crossbreeding might alter diversity by introducing genetic variation from other Indonesian duck breeds. Additionally, the geographical separation of Sumatra from mainland Asia could lead to unique adaptations driven by natural selection or genetic drift, leading to genetic differentiation in Kerinci ducks. De *et al.* (2021) reported that geographical and environmental effects influenced the independent cluster of Andaman local ducks.

In conclusion, the MT-ND2 gene diversity analysis suggested that Kerinci ducks predominantly share a matrilineal origin within the Asian lineage. The

genetic introgression of *A. zonorhyncha* and *A. poecilorhyncha* was evidenced through MT-ND2 gene analysis. This study also uncovered an independent Kerinci lineage marked by substantial MT-ND2 diversity. In future studies, conducting whole mitochondrial DNA (mitogenome) sequence analyses between Kerinci ducks and other Indonesian duck breeds could yield further insights into their accurate matrilineal origin.

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