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Distinct Island Lineages of Binturong (*Arctictis binturong*) from Indonesia and Its Conservation Implications

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ABSTRACT

Binturong (*Arctictis binturong*) is a threatened carnivore that inhabits the forests of South and Southeast Asia. Despite its wide range, binturong is relatively scarce across its habitat distribution and is currently under the threat of poaching and illegal trade. Captive breeding has unfortunately been conducted rather haphazardly with a lack of origin record maintained, implicating potential risks to the management such as inbreeding or genetic swamping. This study thus aims to characterise the phylogenetic relationship of Indonesian binturong within the context of Southeast Asian binturong and further probe the distinctness of lineages originating from Java, Sumatra, Indonesian Borneo, and Bangka using Cytochrome B (*Cyt*B) and Cytochrome C Oxidase Subunit 1 (CO1). Genetic distance, phylogram topology, and haplotype analysis of both encoding genes further corroborate the distinctness of Java, Borneo, and Bangka binturong from other binturong from Indochinese regions such as India, Laos, and Myanmar. Search for prospective single nucleotide polymorphism markers to discriminate island lineages consistently found that each Java, Bangka, and Bornean binturong be distinct from each other and other lineages, especially when assessed using haplotype-based clustering. Assigning binturong originated from Sumatra is nonetheless more complicated, suggesting the possibility. Our findings substantiated the much-needed systematic research of Southeast Asian binturong as *ex-situ* insurance population management grows in Indonesia and other parts of the world to protect the diversity of binturong lineages and their corresponding unique evolutionary history.

1. Introduction

Human activities have affected the abundance and distribution of biodiversity, especially on species extensively hunted and distributed as pets. This includes the binturong (*Arctictis binturong*), also known as "bearcat", a member of the Viverridae family which mostly lives in the dense forest canopy and is active during the night (Semiadi *et al.* 2016). It is also among the few carnivores in the world with a prehensile tail, an important adaptation to arboreality (Youlatos 2003). Binturong consumes fig fruits (Nakabayashi and Ahmad 2018) and speeds up the germination rate of the strangler fig's seed (Colon and Campos-Arceiz 2013), consequently influencing the availability of one of the most important tropical trees where they live. Binturong is listed as Vulnerable according to the IUCN Red List (Wilcox *et al.* 2016) and is currently still commonly found in captivity and around the chain of illegal wildlife trade (McGrath 2016).

In Indonesia, the threat has been coming predominantly from wildlife trade, promoting further legal protection by the national law (P.106/MENLHK/SETJEN/KUM.1/12/2018). Nonetheless, authorities have frequently confiscated binturong from either the poachers, wildlife traders, or private owners and then transferred them to captive facilities such as zoos and/or rescue centres belonging to either government or private institutions to receive further care (authors' personal

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experience). Such binturongs usually do not have clear information about their geographical origin and/or the source of procurement. With the rise in the number of commercial breeders permitted by the government, the population provenance of binturong in Indonesia will become even murkier.

This is presumably due to binturong being treated as one species in conservation status assessment by default (Wilcox *et al.* 2016). This treatment neglects the potentially different evolutionary significant units coming from different islands previously described from colour and cranial character variation (Pocock 1933) and, recently, genetics (Veron *et al.* 2020). Due to the presumably lack of study, the potential binturong subspecies described by Pocock (1933) have been treated as synonyms (Wozencraft 2005). Clarifying the degree of intraspecific variation within binturong hence has been recognised as one of the priority projects for binturong conservation according to the IUCN/SSC Small Carnivores Specialist Group (Schreiber *et al.* 1989). The available studies on the genetic relationship within different binturong individuals from different geographic origins support the existence of two major clades from the Indochina and Sundaic regions (Cosson *et al.* 2007; Veron *et al.* 2020). While managing captive-bred binturongs might suffice when considering these two clades at minimum (Cosson *et al.* 2007), we might lose important lineages yet to be discovered as we mix individuals from different areas or islands within each clade (Veron *et al.* 2020).

Clarifying the degree of distinctness of population origin of binturong is especially important in archipelago countries such as Indonesia, as inter-island mixing can further confound population management. As an increasing number of official commercial breeders started their breeding program using confiscated individuals without any origin records or prior genetic information, the existence of island-specific lineages is compromised. The pool of captive-bred binturongs may already have lost some level of genetic diversity due to the inbreeding of closely related individuals (Keller and Waller 2002) or losing local adaptation from unintentionally mixed breeding or misplaced reintroduction (Adavoudi and Pilot 2022)

Putative binturong origin is currently determined by the coat colour in practice, i.e. the white-dominant coated, grizzled individuals come from Java, whilst the black-dominant coated individuals might be from Sumatra or Borneo following Pocock (1933). This method is, unfortunately, lacking in precision and vulnerable to misidentification of binturong origin as the morphological study of binturong is lacking, and consistent colouration marks are absent. When we direct our effort to use genetic markers, however, there is a gap in the genetic basis of binturong subspecies, especially the ones that inhabit Indonesia, such as Sumatera, Bangka, Java, and Indonesian Borneo (Cosson *et al.* 2007; Veron *et al.* 2020).

Many studies have suggested that mitochondrial markers are recommended for use as versatile molecular markers of wildlife classification (Arif and Khan 2009; Arif *et al.* 2011). *Cyt*B and CO1 have been widely explored as a barcode for many mammalian species (Luo *et al.* 2011; Kartavtsev 2011; Nicolas *et al.* 2012) because of their relatively constant mutation rate through evolution.

Therefore, in this study, we aim to uncover the phylogenetic relationships of the binturongs across the islands of Indonesia that have not yet been sampled by previous studies using cytochrome b (*Cyt*B) and cytochrome c oxidase subunit 1 (CO1). More specifically, we work with four binturong subspecies inhabiting different islands across Indonesia: *A. b. niassensis* (Sumatra), *A. b. kerkhoveni* (Bangka), *A. b. pageli* (Borneo), and *A. b. penicillatus* (Java). In addition, we aim to describe our qualitative assessment of the use of coat colour to group binturong individuals to supplement unresolved phylogeny. We also reported the type of tissue that yields the best DNA, which should be considered when sampling binturong for future genetic monitoring purposes.

2. Materials and Methods

2.1. Permit and Ethics

This study was performed under approval from The Ministry of Environment and Forestry Republic of Indonesia through document number SK.21/KSDAE/SET.3/KSA.2/2/2021. All procedures carried out in this study had been approved and under the supervision of the Ethical Committee Body of the Faculty of Veterinary Medicine Universitas Gadjah Mada through document number 00017/EC-FKH/Eks./2021.

2.2. Sampling Design

We initially collected 47 samples of binturong from Sumatra, Bangka, Java, and Borneo (Figure 1) but were only able to amplify mtDNA loci with our designed primer (see below) for only 28 individuals. After further scrutiny of the band thickness on the electrophoresis gel results and further verification of origin records, we sequenced 20 individuals for further analysis (Table 1). To place our results within the context of the two major clades of binturong previously discovered (Cosson *et al.* 2007; Veron *et al.* 2020), we included from GenBank NCBI eight additional sequences with known origins representing India, Myanmar, and Laos, and seven sequences of unknown origins to assess the diversity of binturong lineages already sequenced within the context of our new geographic coverage (Table 2).

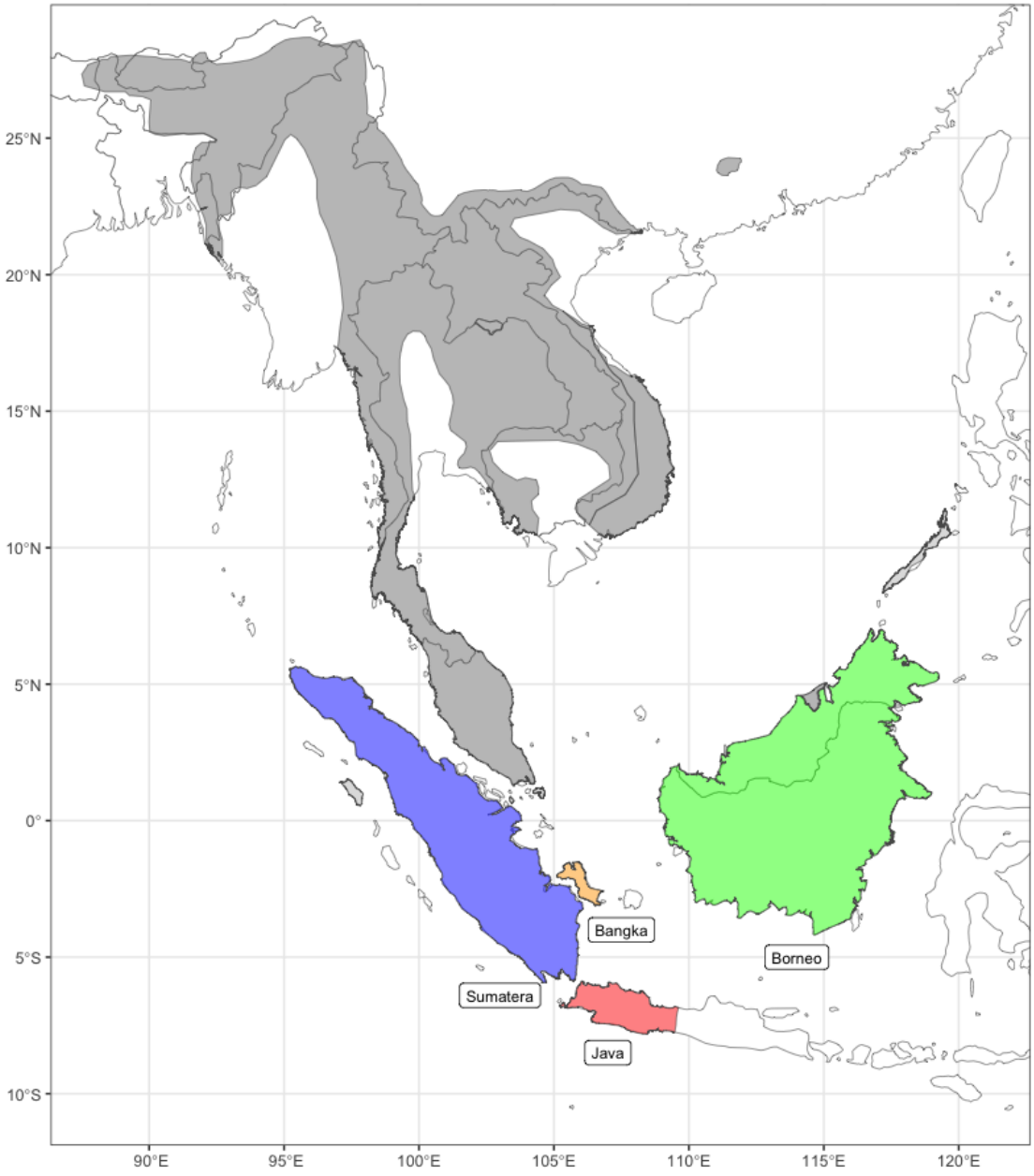

Figure 1. Map of binturong showing distribution according to IUCN Red List (Wilcox *et al.* 2016) with the distribution borders marked by a solid black line and areas of distribution coloured grey except for the area which we have newly sequenced samples, i.e. Sumatra (blue), Bangka (orange), Borneo (green), and Java (red)

Table 1. Information of binturong individuals involved in this study

Individual name	Origin*	Sampling site
JAVA1	Java Island	Wildlife Rescue Centre (WRC) Jogja, Yogyakarta, Java
JAVA2	Java Island	Pusat Penyelamatan Satwa (PPS) Tegal Alur, Banten, Java
JAVA3	Java Island	Pusat Penyelamatan Satwa (PPS) Tegal Alur, Banten, Java
JAVA4	Java Island	Wildlife Conservation Centre (WCC) Cikananga, West Java
JAVA5	Java Island	Wildlife Conservation Centre (WCC) Cikananga, West Java
JAVA6	Java Island	Bali Wildlife Rescue Centre (BWRC), Bali
SUMATRA1	Sumatra Island	Wildlife Rescue Centre (WRC) Jogja, Yogyakarta, Java
SUMATRA2	Sumatra Island	Pusat Penyelamatan Satwa (PPS) Tegal Alur, Banten, Java
SUMATRA3	Sumatra Island	Private ownership, Semarang, Central Java
SUMATRA4	Sumatra Island	Private ownership, Semarang, Central Java
SUMATRA5	Sumatra Island	Private ownership, Semarang, Central Java
SUMATRA6	Jambi, Sumatra Island	Pusat Penyelamatan Satwa (PPS) Alobi, Bangka Belitung Islands
SUMATRA7	Jambi, Sumatra Island	Pusat Penyelamatan Satwa (PPS) Alobi, Bangka Belitung Islands
BANGKA1	Bangka Induk Island	Pusat Penyelamatan Satwa (PPS) Alobi, Bangka Belitung Islands
BANGKA2	West Bangka Island	Pusat Penyelamatan Satwa (PPS) Alobi, Bangka Belitung Islands
BANGKA3	West Bangka Island	Pusat Penyelamatan Satwa (PPS) Alobi, Bangka Belitung Islands
BORNEO1	Borneo Island	Sinka Zoo, Singkawang, West Kalimantan
BORNEO2	Borneo Island	Sinka Zoo, Singkawang, West Kalimantan
BORNEO4	Borneo Island	Sinka Zoo, Singkawang, West Kalimantan
BORNEO3	Borneo Island	Sinka Zoo, Singkawang, West Kalimantan

*Based on procurement records and physical appearances

Table 2. Details of the origin of additional *Cyt*B and CO1 sequences from NCBI GenBank used in this study

Origin	Accession number	Locus	Source of tissue	Source
India	NC_043895.1	Complete mitochondrion genome	Sepahijala Zoo, Tripura	Mitra *et al.* (2019)
India	KX449332.1	Complete mitochondrion genome	Sepahijala Zoo, Tripura	Mitra *et al.* (2019)
Laos	MK681030.1	CYTB	Laos Wildlife Rescue Center/Lao Zoo	Veron *et al.* (2020)
Laos	MK681031.1	CYTB	Laos Wildlife Rescue Center/Lao Zoo	Veron *et al.* (2020)
Laos	MK681032.1	CYTB	Laos Wildlife Rescue Center/Lao Zoo	Veron *et al.* (2020)
Laos	MK681033.1	CYTB	Lao Zoo	Veron *et al.* (2020)
Myanmar	MK680982.1	CYTB	Yangoo Zoo	Veron *et al.* (2020)
Myanmar	MK680983.1	CYTB	Yangoo Zoo	Veron *et al.* (2020)
Unknown origin	MK680985.1	CYTB	Taipei Zoo, Taiwan	Veron *et al.* (2020)
Unknown origin	MK681004.1	CYTB	Singapore Zoo	Veron *et al.* (2020)
Unknown origin	MK681005.1	CYTB	Singapore Zoo	Veron *et al.* (2020)
Unknown origin	MK681006.1	CYTB	Halle Zoo, then Dortmund Zoo, Germany	Veron *et al.* (2020)
Unknown origin	KJ852005	CYTB	Singapore Zoological Garden	Veron *et al.* (2015)
Unknown origin	NC_053969.1	Complete mitochondrion genome	NMS RL78/97, Southport Zoo UK	Hassanin *et al.* (2021)
Unknown origin	MW257218.1	Complete mitochondrion genome	NMS RL78/97, Southport Zoo UK	Hassanin *et al.* (2021)

2.3. Tissue Collection Protocol

All specimen collection protocols were performed under anaesthesia using a combination of ketamine (19 mg/kg) and xylazine (1.3 mg/kg) preceded with atropine sulphate (0.04 mg/kg) for premedication if necessary. The body weight was estimated by observation only and by the latest medical examination data collection. Either the darting method or restraining-cage-assisted direct jabbing was performed in the drug. In case of delayed anaesthetic effect, an additional quarter to half dosage of anaesthetic drug was administrated in order to prolong the anaesthetic period if needed during physical examination. The anaesthetic plane was determined by loss of reflex and control in the extremities with observed deep regular respiration rate.

About 1 ml of blood was taken from the cephalic vein, saphenous vein, and/or coccygeal vein, considering the thickness of the subcutaneous fat layer underneath. The cephalic vein was often twisted medially to the inner aspect of the forelimb. Adult individuals with moderate subcutaneous fat layer will need 23-21 gauge needles, whereas the smaller individuals need smaller gauges to access the veins. Hair follicle specimens were collected by plucking the hairs around the shoulder and observing the presence of hair bulbs. All specimens were placed in a 70% ethanol-contained 1.5 ml tube for prolonged preservation. The blood specimen would easily coagulate soon after in contact with the preservative.

2.4. DNA Extraction

Our method used 70% commercial ethanol for preservation, even in very remote areas, and it worked well under room temperature to guarantee the best yield of subsequent DNA extraction. Simpler blood collection to collect a small amount of dried blood, such as filter paper, might be useful for certain situations in remote areas. We should note that DNA was not successfully extracted where only hair follicles or arsenic-preserved museum specimens are used as sources of tissue. DNA extraction from blood yielded the best outcome compared to hair follicle DNA extraction and, hence, was more accurate and useful for subspecies identification by origin for genetic rescue and/or relocation purposes.

A small portion of the preserved blood specimen was taken from the main container and replaced with a new 1.5-microcentrifuge tube. The tube was then remained lid-open at room temperature for about 72 hours to evaporate the preservative. After the blood was completely dried, the specimen underwent the DNA extraction protocol following the manufactured protocol. We used Geneaid™ Blood DNA Extraction Kit for DNA extraction with modification of incubation time in a water bath for the tissue lysis stage, which took time overnight. For DNA extraction from hair follicles, three to five hair bulbs were placed in a microcentrifuge tube and genome extractions were done using Geneaid™ Tissue DNA Extraction Kit following the manufacture protocol.

2.5. Amplification and Sequencing

We designed our primers for Cytochrome B (*Cyt*B) and Cytochrome C Oxidase Subunit 1 (CO1) encoding gene amplification based on available sequences in GenBank NCBI as follows: CbF (5'-CACATGGAATCTAACCATGACCAA-3') and CbR (5'-TTCAGCTTTGGGTGCTGATG-3') for *Cyt*B; CO1ABINF (5'- ACA GTC TAA TGC TTT ACT CAG C -3') and CO1ABINR (5'- GCT GGT TCT TCA AAT GTA TGG T -3') for CO1. The thermal cycler was run under the following conditions: initial phase of pre denaturation 94°C for 5 minutes, 35 cycles of denaturation (94°C for 30 seconds), the annealing temperature of 49°C for *Cyt*B primer and 48°C for CO1 (30 seconds), elongation (72°C for 90 seconds), and ended with post elongation 72°C for 5 minutes. The amplicons were then run in an electrophoresis apparatus and proceeded for sequencing using the Sanger sequencing method with Applied Biosystems™ 3730xl DNA Analyzer.

2.6. Sequence Analysis

All sequences were compiled and aligned in MEGA 10.1.8 using the ClustalW alignment method. Private single nucleotide polymorphisms (SNPs) were identified for each presumptive island group by eye. The substitution model for nucleotide substitution type was determined using jModelTest and MEGA 10.1.8 with the lowest Bayesian information criterion (BIC), resulting in the Hasegawa-Kishino-Yano (HKY) model. Phylogeny reconstructions were performed using the bootstrap method in the same substitution model with 1,000 bootstrap replications and maximum likelihood for the statistical method. The phylograms were converted in Newick format and improved in FigTree v1.4.3 for setting the polar

tree layout as well as grouping the clades and taxa by colour. Haplotype analysis was performed in DnaSP v6.12.03 and NETWORK 10.2.0.0 to construct a median-joining haplotype network. Genetic distances among binturongs were estimated using the p-distance model using MEGA 10.1.8. All nucleotide sequences were translated to be protein sequences based on vertebrate mitochondrial genetic code (Elzanowski and Ostell 2023).

3. Results

In all of the 20 binturong samples in our study, DNA extraction from hair follicles resulted in a lower yield than blood DNA extraction. Amplification using our primers yielded excellent bold and thick amplicon bands around 1,000 and 1,250 bp ladder bands of both encoding genes (Figure 2). We successfully recovered 1140 base pairs (bp) of complete *Cyt*B and 1457 of 1545 base pairs of CO1 encoding gene sequences from all 20 specimens collected. Sequence haplotype analysis found that we have, in total, 18 and 10 haplotypes from *Cyt*B and CO1 sequences, respectively. All Indonesian binturong sequences encoded 380 and 485 amino acid residuals of *Cyt*B and CO1 encoding genes, respectively, with no residual difference in CO1 and two differences in *Cyt*B. This difference comes from Bornean binturongs with private isoleucine residual at site 212 and methionine at site 360 of *Cyt*B amino acid sequence private to Bornean and Bangka binturongs.

Both *Cyt*B and CO1 nucleotide sequences revealed that Bangka binturong is the most easily discriminated against with private SNPs at both genes, with several potential SNPs for other islands. From *Cyt*B sequence alignment, five SNPs are unambiguously private, mostly for Bangka and Borneo (Table 3), corroborating the amino acid residual results. The first two are private to Bangka binturongs, which comprise a thymine (T) residual at site 105 for all Bangka binturongs (Table 3) and a T residual at site 919 for West Bangka (see Table 1 and 3). The third SNP is a T residual at site 636 for the Bornean binturongs (Table 3). The fourth and fifth are SNPs at sites 285 (T) and 1106 (C, cytosine), which are private to a subgroup of Sumatran binturong (Table 3). Alignment of the partial CO1 encoding gene presented more potential discriminative SNPs for Javan binturongs, i.e. two SNPs that are A residual at site 429 and T at site 1242 (Table 4). There is one potential discriminative SNP for Bornean binturong, which is a C residual at site 142 of the CO1 gene (Table 4). Bangka binturong again obtained a private A SNP at site 813 for this gene (Table 4).

Both phylogram topology and haplotype networks support the idea that Bornean and Bangka binturongs apparently diverged from Sumatran binturongs. Phylograms of both *Cyt*B and partial CO1 sequences further corroborated the distinctness of Borneo and Bangka clusters (Figure 3). Binturong from Sumatra is apparently polyphyletic, as shown by its widespread occurrence in both Borneo and Bangka

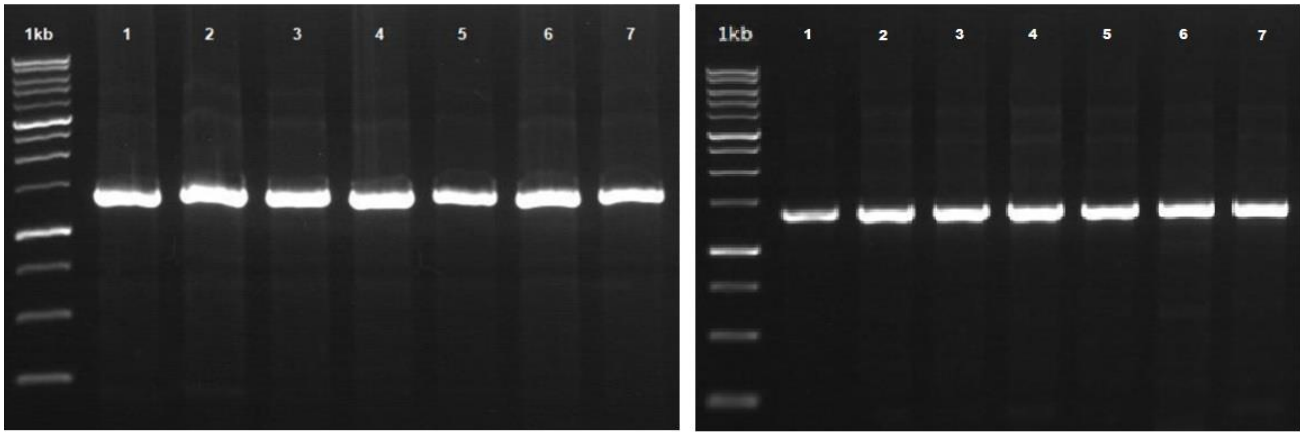

Figure 2. A sample of electrophoresis gel results of *Cyt*B (left, lane 1-7: Sumatra, Java, Java, Sumatra, Sumatra, Sumatra, Sumatra) and CO1 (right, lane 1-7: Sumatra, Bangka, Bangka, Sumatra, Bangka, Borneo, Borneo)

Table 3. Putative SNPs in *Cyt*B encoding gene sequences of Indonesian binturongs. Numbers on the heading (up to down) indicate the site number of the sequence

	42	105	216	285	636	660	693	715	724	744	816	919	1078	1106
JAVA1	T	C	T	C	A	T	T	T	T	T	G	C	C	T
JAVA4	.	.	.	.	.	.	.	.	.	.	.	.	.	.
JAVA5	.	.	.	.	.	.	.	.	.	.	.	.	.	.
JAVA6	.	.	.	.	.	.	.	.	.	.	.	.	.	.
BORNEO4	.	.	.	.	.	.	.	.	.	.	.	.	.	.
JAVA2	C	.	.	.	.	.	.	.	.	.	.	.	.	.
JAVA3	C	.	.	.	.	.	.	.	.	.	.	.	.	.
SUMATRA6	C	.	.	.	.	.	.	.	.	.	.	.	.	.
SUMATRA4	C	.	.	.	.	.	.	.	.	.	.	.	.	.
SUMATRA1	C	.	.	.	.	.	.	.	.	.	.	.	.	.
SUMATRA2	C	.	.	.	.	.	.	.	.	.	.	.	.	.
SUMATRA3	C	.	C	T	.	C	C	.	C	C	A	.	.	C
SUMATRA5	C	.	C	T	.	C	C	.	C	C	A	.	.	C
BANGKA1	C	T	C	.	.	C	C	.	C	C	A	.	A	.
BANGKA2	C	T	C	.	.	C	C	.	C	C	A	T	A	.
BANGKA3	C	T	C	.	.	C	C	.	C	C	A	T	A	.
BORNEO1	.	.	C	.	T	C	C	.	C	C	A	.	A	.
BORNEO2	.	.	C	.	T	C	C	C	C	C	A	.	A	.
BORNEO3	.	.	C	.	T	C	C	C	C	C	A	.	A	.

Table 4. Putative SNPs in CO1 encoding gene sequences of Indonesian binturongs. Numbers on the heading (up to down) indicate the site number of the sequence

	142	378	429	693	813	921	1044	1056	1107	1242	1248
JAVA1	T	G	A	C	G	A	T	C	C	T	C
JAVA4	.	.	.	.	.	.	.	.	.	.	.
JAVA5	.	.	.	.	.	.	.	.	.	.	.
JAVA6	.	.	.	.	.	.	.	.	.	.	.
BORNEO4	.	.	.	.	.	.	.	.	.	.	.
JAVA2	.	.	G	.	.	G	.	.	.	C	.
JAVA3	.	.	G	.	.	G	.	.	.	C	.
SUMATRA2	.	.	G	.	.	G	.	.	.	C	.
SUMATRA6	.	.	G	.	.	G	.	.	.	C	.
SUMATRA3	.	A	G	T	.	G	C	.	T	C	T
SUMATRA7	.	A	G	T	.	G	C	T	T	C	T
BANGKA1	.	A	G	T	A	G	C	T	T	C	T
BANGKA2	.	A	G	T	A	G	C	T	T	C	T
BANGKA3	.	A	G	T	A	G	C	T	T	C	T
BORNEO1	C	A	G	T	.	.	C	T	T	C	T
BORNEO2	C	A	G	T	.	.	C	T	T	C	T
BORNEO3	C	A	G	T	.	.	C	T	T	C	T

clade. Its apparent higher relatedness to the more basal Java lineages (Figure 3). The four individuals from Java and one individual from Borneo that consistently form a clade in phylogram from both loci (Figure 3) interestingly shared similarly bright hair colouration that is typical to Javan binturong (Figure 4). Median-joining networks support the established grouping of Javan, Bornean, and Bangka group as one monophyletic clade distinct from the Indochinese clade as also shown in the phylogram (Figure 3). Haplotype networks from both *Cyt*B and CO1 also support the distinctness of the Javan clade, Borneo clade, and Bangka clade, with *Cyt*B grouping Bangka and Borneo more closely than CO1. Binturong of unknown origin apparently spread across the clusters.

Genetic distance analysis supported the notion that Borneo binturong was more closely related to

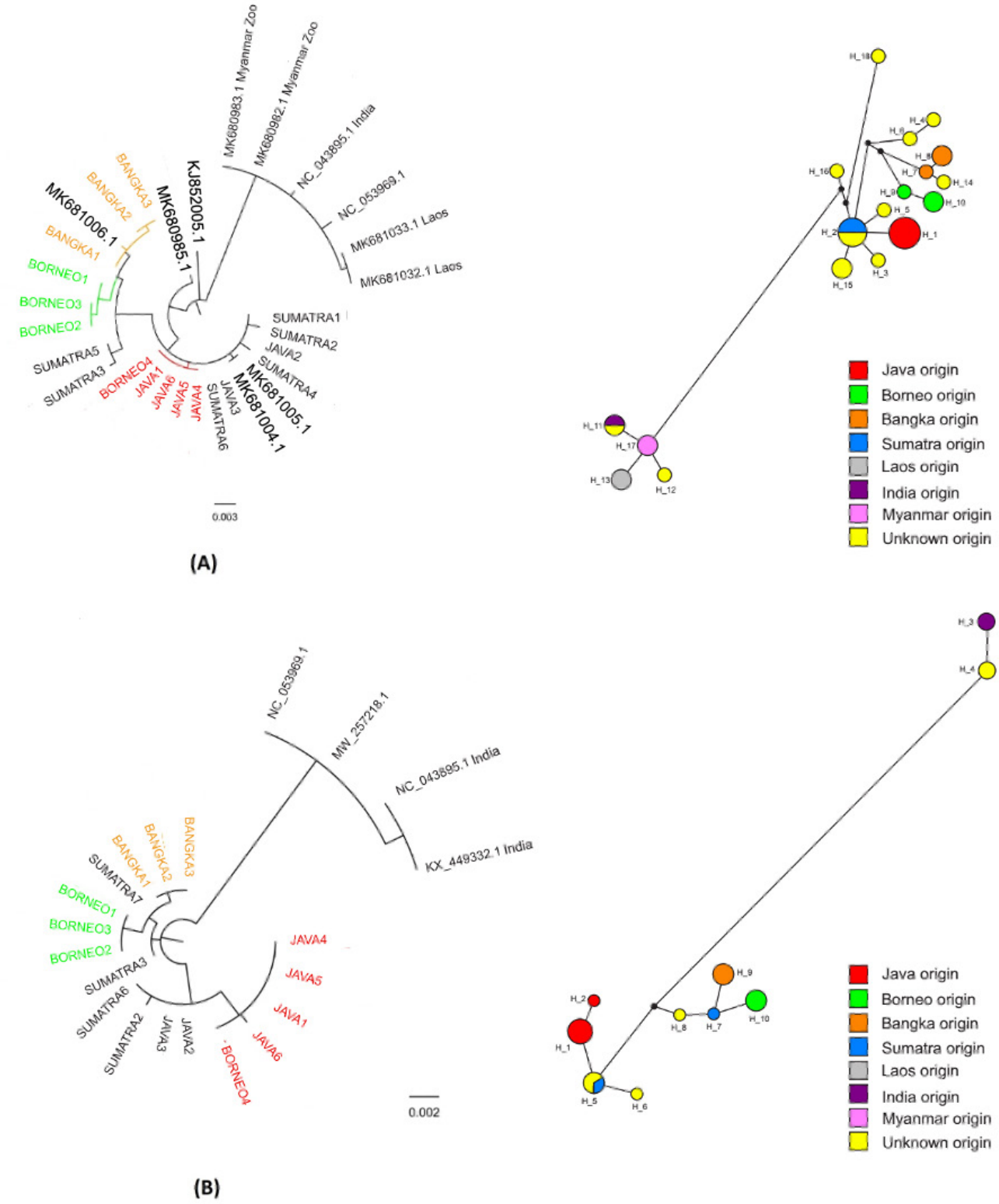

Figure 3. The phylogram (left) and median-joining haplotype network (right) of *Cyt*B (A) and partial CO1 (B) encoding gene sequences of Indonesia binturong with maximum likelihood reconstruction supporting the clustering of Java group (red), Borneo group (green) and Bangka group (orange) as distinct

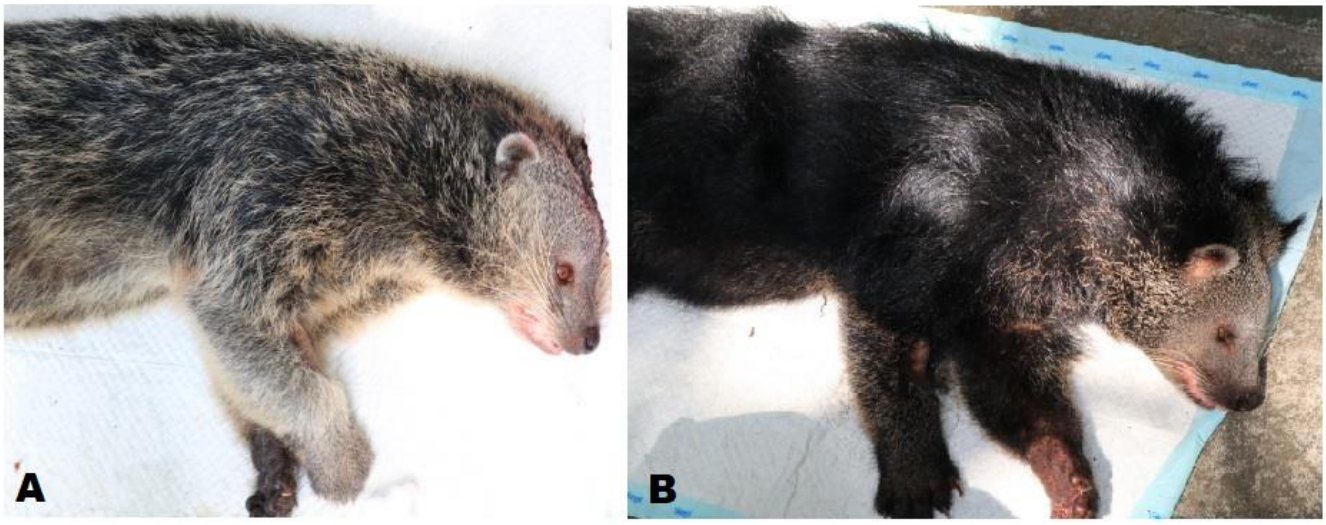

Figure 4. The general physical appearance of the binturong pelage of an individual from Java (A) and Sumatra (B) shows that Javan binturong possess more diffuse greyish to brownish colouration while Sumatran binturong has darker colouration (personal documentation)

Bangka Island binturong, although Bangka Island is closer to Sumatra compared to Borneo geographically (see Figure 1). Bangka binturong genetic distance to Borneo for CO1 is 0.002 and for *Cyt*B is 0.004, relatively smaller than when compared to Javan binturong (*Cyt*B = 0.008, CO1 = 0.007) or Sumatran binturong (CO1 = 0.003, *Cyt*B = 0.005). Javan binturong distinctness is corroborated by Bornean binturong's closeness to Sumatran binturong (CO1 = 0.004, *Cyt*B = 0.005), is relatively higher than its closeness to Javan binturong (CO1 = 0.006, *Cyt*B = 0.008). Considering the polyphyletic nature of Sumatran binturong, we did not treat it as a separate clade and did not calculate its genetic distance relative to other clades further.

4. Discussion

This study corroborates the distinctness of the Sundaic clade previously reported (Cosson *et al.* 2007; Veron *et al.* 2020). This indicates a long-standing separation from Indochina binturong populations, supported by mainland Asia binturongs, which are evolutionarily distant from Indonesian binturongs. A further look at the phylogenetic relationship within Indonesian binturongs further confirmed the distinctness of island lineages from Indonesia. The maximum likelihood (ML) phylogenetic reconstruction of *Cyt*B and CO1 consistently shows the separation between the Bornean-Bangka group and the Java-Sumatran group. Moreover, the genetic distance analysis revealed that binturongs from Bangka were relatively closer to those from Borneo rather than to Sumatra, which is geographically closer to Bangka.

Our study further shows the distinctness of three clades of binturong: Bangka, Borneo, and Java. The separation of Bangka from either Sumatra or Borneo and its consistent monophyletic grouping across the phylogenetic and haplotype network analysis may support the distinctness of binturong lineages from this island. Genetic distance of as much as 0.2% at least (from Borneo) is within the same magnitude as what is found in Palawan (0.38%) and all other Indonesian binturong (0.3%) previously studied in Veron *et al.* (2020). Similar conclusions can also be made for Javan and Bornean binturong, although not as strong as Bangka, considering that individuals recorded as having originated from Java and Borneo are sometimes still grouped with another geographic origin, such as Sumatra or Java and Borneo. Whether the three distinct island lineages warrant further distinct classification into subspecies would require further investigation involving more relevant characters, such as cranial morphology, ecological niche, and more genetic markers (e.g. Wilting *et al.* 2015).

Considering the lack of taxonomic work on binturong, the potential of fur colour as a proxy and other phenotypic characteristics that are readily observed is yet to be fully understood. An intriguing case where the Borneo individual that is consistently

grouped with Java in both phylogram and private SNPs has a similar phenotypic appearance with Javan individuals might represent a case of falsely recorded Javan binturong. This is not implausible considering the rate of interisland wildlife trade (authors' anecdotal experiences). Interisland trading might also be behind the lack of island clustering for individuals recorded from Java and Sumatra, in addition to the possibility of mixed genetic features caused by haphazard breeding. Fur colouration has less variation to be used as a distinguishing character as we also observed that the remaining Bornean binturongs that formed a monophyletic clade possess coat colour that resembled presumptive Sumatran binturongs. The inclusion of other characters with more variations, such as morphometric geometrics or a larger sample size, might be able to resolve this (Veron *et al.* 2020).

The distinctness of island lineages of Bangka, Borneo, and Java, with an emphasis on the closeness of Bangka to Borneo, can be explained by the possible history of migration of binturong across Southeast Asia. The connection of the Indonesia archipelago with the Malayan Peninsular through land bridges as part of the Sundaland subcontinent during the Pleistocene (Bird *et al.* 2005) allowed the migration of animals from mainland Asia to colonise islands across the Indonesian archipelago consecutively. We deduce that Sumatra and Java are among the islands initially settled in by binturongs based on their geographic proximity and conserved genetic properties. This dispersal pattern is also reported to resemble Southeast Asian pig-tailed macaques (*Macaca nemestrina*) based on mitochondrial DNA haplotype analysis (Rosenblum *et al.* 1997; Abegg and Thierry 2002). Significant partition of gene flow among Indochina and Sundaland populations was also reported in other species, such as the Asian feline family (Luo *et al.* 2004). Our results, however, supported the idea that migration to Bangka was coming from Borneo instead of Sumatra, which was geographically closer. This event might be identical to what is already proposed by Veron *et al.* (2020) in Palawan binturongs.

Therefore, the different island lineages each represent a unique evolutionary history and warrant attention as a conservation entity. Conservation intervention, however, more often than not, is left with a limited budget, and lineage identification should be made as cost-effective as possible. In addition to the private SNPs recovered for each island group, haplotype-based clustering might be a more definitive method, considering the cluster consistency we found. Haplotype-based marker from *Cyt*B has also been reported to be successfully separating western and eastern Testudo graeca populations in Morocco (Alvarez *et al.* 2000) and three clades of *Procapra picticaudata* from Tibet (Zhang and Jiang 2006). Haplotype analysis of mitochondrial control region (CR) also reportedly does similar things to several species, such as separation of two geographically separated black muntjac (*Muntiacus crinifrons*) (Wu *et al.* 2006) and three local clades of brush-tailed rock-wallaby (*Petrogale penicillata*) in Southeastern Australia (Eldridge *et al.* 2001). Eldridge *et al.* (2001) also tracked the origin of New Zealand's brush-tailed rock-wallaby population, which came from central New South Wales.

The evidence of the distinct genetic makeup of the island lineages should push towards a careful disposition of confiscated binturong by the authority and founder selection for breeding efforts. Reintroduction and other conservation translocations without genetic testing must be reconsidered to prevent mixed breeding and loss of genetic purity among binturongs with geographically distinct genetic features. At the very least, individual recording is of utmost importance to ensure lineage conservation. Genetic analysis must be included as a compulsory procedure prior to binturong reintroduction and release. Though the procedure will be time-consuming and laborious, failure to identify the species' origin will result in potential mixed breeding in the wild. Such unintentional indiscriminative breeding could lead to the loss of specific lineages (Berthouly-Salazar *et al.* 2012), occurrence of genetic-associated disease (Adavoudi and Pilot 2022), and loss of fitness due to potential inbreeding (Keller and Waller 2002). Decreasing body size among inbreeds in multiple generations has been observed in many animal breeding programs, including mice, dogs, and some birds, which affects their fittest in the wild (Keller and Waller 2002; Casellas 2011; Yordy *et al.* 2020).

Preserving the genetic diversity of binturongs might help raise concerns about conservation priorities to protect their home range as well as those who live within it. The evidence that they coexist with several flagship species, such

as Sumatran tigers and orangutans, becomes an undeniable reason to uphold habitat sustainability for supporting ecosystem capacity as well as directing national conservation priority (Smith and Sutton 2008; Jepson and Barua 2015; Krause and Robinson 2017). Conserving habitats will reduce the contact between humans and wild animals in the case of zoonosis transmission from wildlife. It was reported that most infectious zoonotic diseases came from wild animals, and those were due to land use alteration that converted natural landmarks to commercial, industrial sites (Taylor *et al.* 2001; Bengis *et al.* 2004; Chomel *et al.* 2007; Hayman 2011; Gebreyes *et al.* 2014).

Understanding the genetic richness of these wild carnivores and conserving them in their natural habitat will require great effort in coordinating comprehensive action to protect the land and manage its use due to overlapping discretions among institutions. Our improved understanding of the phylogenetic richness of the binturongs will ultimately increase concern towards binturong conservation as well as improve scientific-based *ex-situ* conservation efforts such as genetically informed breeding or rehabilitation for release. The confirmed binturong origin will assist the authority to direct the conservation program intensively to a specific area or population in order to preserve local genetic flow in the wild. Development of a shorter procedure to detect the binturong origin through multiple-loci amplification with amplicon band pattern analysis through multiple primers or specific endonuclease restriction enzymes might improve the detection panel to make a shortcut to the identification process (Bravi *et al.* 2004; Guan *et al.* 2018).

In conclusion, our study provides grounds to further the systematic study of the binturong, especially in much of the unsampled region in Indonesia and the importance of collecting genetic information in ex-situ management such as breeding, rehabilitation, as well as the conservation reintroduction and conservation translocations. Bornean, Bangka, and Javan binturongs are genetically distinct, and a genetic test needs to be performed to identify binturong origins for conservation purposes accurately. Proper sample size and well-maintained recording will increase accuracy and support a wider scope of binturong genetics in Indonesia. Evidence-based conservation needs to be taken to ensure the sound wildlife conservation strategy and support scientific policy. We strongly recommend that all institutions and foundations involved in binturong conservation in Indonesia put more concern on the importance of the genetic test of each binturong group based on geographic origin.

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