Is the Javan tiger *Panthera tigris sondaica* extant? DNA analysis of a recent hair sample[†]

Wirdateti Wirdateti[*1] , Yulianto Yulianto[2]
Kalih Raksasewu[3] and Bambang Adriyanto[4]

Abstract The Javan tiger *Panthera tigris sondaica* and the Bali tiger *P. tigris balica* were categorized as Extinct on the IUCN Red List in both 2003 and 2008, leaving only the Sumatran subspecies *P. tigris sumatrae* extant in Indonesia. There have, however, been occasional, more recent reports of the Javan tiger but without conclusive evidence. Here, a potential observation in 2019 of a Javan tiger in a community plantation near the village of Cipendeuy in the forest of South Sukabumi, West Java, and a single hair found on a fence nearby, are assessed. The cytochrome b mitochondrial DNA (mtDNA) gene sequence of the putative Javan tiger hair were compared with that of a Javan tiger specimen in Museum Zoologicum Bogoriense, collected in 1930; hair samples of several tiger subspecies and the Javan leopard *Panthera pardus melas* were used as controls. The results showed that the genetic distances (d) of the putative Javan tiger hair with the Sumatran, Bengal *P. tigris tigris* and Amur *P. tigris altaica* tigers and the Javan leopard are $0.074 \pm SE\ 0.009$, $0.071 \pm SE\ 0.009$, $0.072 \pm SE\ 0.009$ and $0.088 \pm SE\ 0.010$, respectively, whereas the genetic distance of the putative Javan tiger hair with the Javan tiger museum specimen is $0.040 \pm SE\ 0.006$. In addition, phylogenetic trees showed that the putative Javan tiger hair sample belongs to the same group as the museum specimen of the Javan tiger, but is differentiated from other tiger subspecies and the Javan leopard. Whether the Javan tiger still occurs in the wild needs to be confirmed with further genetic and field studies.

Keywords Cytochrome b, DNA analysis, extinction, hair sample, Java, Javan tiger, *Panthera tigris sondaica*, phylogeny

There were formerly three subspecies of the tiger *Panthera tigris* in Indonesia: the Sumatran tiger *P. tigris sumatrae*, the Javan tiger *P. tigris sondaica* and the Bali tiger *P. tigris balica*. The Javan and Bali tigers were categorized as Extinct on the IUCN Red List in both 2003 and 2008, leaving only the Sumatran subspecies extant. The criterion for extinction used here is that a taxon has not been recorded in nature for 30 years (Tilson et al., 2004; Sanderson et al., 2010; Walston et al., 2010). The last positive confirmed sighting of the Javan tiger was in Meru Betiri National Park, East Java, in 1976 (Panko, 2017).

The Javan tiger was endemic to Java and was widespread in lowland forests, thickets and community gardens in the 18th and 19th centuries. However, it was hunted as a pest, and its habitat converted for agricultural use and infrastructure (Seidensticker, 1987). More recently, there have been occasional anecdotal reports of encounters with the Javan tiger in various locations, including Banjarnegara, Kuningan, Mount Prau, Meru Betiri, Baluran National Park and the Sukabumi Cikepuh Wildlife Reserve. These reports include alleged sightings, unidentified footprints larger than those of the leopard *Panthera pardus* and possible predation on livestock. The last survey for the subspecies was in 1999–2000 in Meru Betiri National Park, East Java, with 35 camera traps. No tigers were found, but some prey species and many poachers were recorded (Tilson, 1999; Breining, 2002).

On 18 August 2019, Ripi Yanur Fajar (a local resident and conservationist) reported seeing a Javan tiger in a community plantation near the village of Cipendeuy in the forest of South Sukabumi, West Java. This was reported to KR, who visited the site on 27 August 2019. He found a hair, potentially of a tiger, on a fence where an animal had apparently jumped between a village road and a plantation (Fig. 1). He and Bambang Adryanto (a local Forest Research and Development Department employee) then discovered footprints and claw marks that could be of a tiger, potentially corroborating the observation.

Based on our in-depth interview with Ripi Yanur Fajar, who saw the tiger, we believe the hair is from a Javan tiger. The interview was conducted during a survey on 15–19 June 2022 at the site where the hair was found. The hair sample was handed to geological staff conducting research in the area and forwarded to the West Java Nature Conservation Authority (BKSDA). On 4 March 2022, the BKSDA submitted the hair sample to the Biology Research Centre–National Research and Innovation (BRIN) for genetic analysis, along with several hair strands from Sumatran tigers from North Sumatra province for comparison.

Genetic analysis of tiger DNA is a powerful tool for addressing conservation questions and clarifying taxonomic uncertainties (Luo et al., 2004), reconstructing

*Corresponding author, teti_mzb@yahoo.com
[1]Research Center for Biosystematics and Evolution, National Research and Innovation Agency (BRIN), Cibinong, West Java, Indonesia
[2]Research Center for Applied Zoology, National Research and Innovation Agency (BRIN), Cibinong, West Java, Indonesia
[3]Yayasan Bentang Edukasi Lestari Bogor (BEL Foundation), Bogor, Indonesia
[4]Forest Ranger of Nature Conservation Agency (BKSDA), The Ministry of Environment and Forestry, Cikepuh Wildlife Reserve, West Java, Indonesia
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Fig. 1 The location where the putative Javan tiger *P. tigris sondaica* hair was found in the vicinity of Cipendeuy village, Sukabumi, West Java province, Indonesia.

phylogeography and demography (Mondol et al., 2009), and investigating the genetic ancestry of subspecies (Driscoll et al., 2009; Xue et al., 2015). Here we report the analysis of cytochrome b mitochondrial DNA (mtDNA) from the putative Javan tiger hair found near the village of Cipendeuy. We compared the sample with DNA from hairs of Sumatran tigers, the Javan leopard *Panthera pardus melas* and a museum specimen of a Javan tiger collected in 1930, all of known origin.

Total DNA extraction was performed using the Dneasy Blood & Tissue Kit according to the appropriate protocols (Qiagen, USA). The protocol was modified by adding proteinase K because of the high protein content in hair. PCR amplification of the entire cytochrome b mtDNA was performed with specific primers for the tiger. The primers were designed based on GenBank reference NC_010642.1 from the complete mitochondrial genome of *P. tigris*. The specific primary sequence was Forward: 5′-CTATAAGAACTTAATGACCAACATTCG-3′, Reverse: 5′-TTCATTTAAGGAGGC GGTTTT-3. The PCR kit Toyobo KOD FX (Toyobo, Japan) was used for PCR amplification. The PCR conditions were pre-denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 45 sec, annealing at 58 °C for 45 sec and extension at 72 °C for 60 sec, and a final extension at 72 °C for 10 min. The PCR products were sequenced with the same forward and reverse primer used for PCR amplification. Sequencing was performed at FirstBase, Singapore, using the Sanger method.

For verification, we used hair samples from 60–100 year old tiger specimens at the Museum Zoologicum Bogoriense (MZB) (catalogue numbers MZB 2428 for the Javan tiger, MZB 41742 and MZB 6832 for the Sumatran tiger), and from additional collections of Sumatran tigers from Simalungun Forest (North Sumatra, 2019) and Siak Forest

(Riau, 2018), and *P. pardus melas* from Mount Prau (Central Java, 2018) stored at the BRIN Genetic Laboratory in Bogor (Table 1). All samples were extracted and amplified using the same primer and method. Clarification was also performed using the cytochrome b sequence data of *P. tigris* subspecies from GenBank (Luo et al., 2004; Matrai & Csorba 2008; Xue et al., 2015).

All nucleotide sequence results were stored using *BioEdit 7.0.5* (Hall, 1999) and submitted to GenBank. The complement sequence between the forward and reverse primers was edited using *Chromas Pro 2.1.10.1* (Technelysium, Australia). All sequences were compared with the Genbank BLAST database. DNA alignment was performed using *Clustal X* (Thompson et al., 1997) and data were analysed using *MEGA 6.0* (Tamura et al., 2013). *MEGA* calculated the genetic distance and locational variation among the samples, and the phylogenetic trees were used to determine the position of the putative Javan tiger hair based on the Javan tiger museum specimen, Sumatran tigers, leopard, and several sequences from GenBank. The phylogenetic tree was constructed using the maximum likelihood method with a bootstrap of 10,000 replicates. *IQ-TREE 1.6.12* was used to construct the phylogenetic tree (Nguyen et al., 2015). The best fitting nucleotide substitution model for the gene was determined using *jModelTest 2.1.6* (Kalyaanamoorthy et al., 2017) based on the Bayesian Information Criterion. HKY+F+I was the model selection for the best fitting partitioned mitochondrial data, and was illustrated using *FigTree 1.4.4* (Rambaut, 2018). Bootstrap percentages were calculated with 10,000 replicates.

The resulting 1043 bp cytochrome b sequences of the putative Javan tiger hair and the Javan tiger museum specimen were compared with other tiger subspecies and the Javan leopard using the BLAST method. The putative tiger hair (Genbank accession no. OQ601561.1) had a sequence similarity of 97.06% with the Sumatran tiger (AF053054.1) and 96.87% with the Bengal tiger (AF053053.1), and the Javan tiger museum specimen (OQ601562.1) had a similarity of 98.23% with the Sumatran tiger (AF053054.1). The phylogenetic tree showed that the putative Javan tiger hair and the Javan tiger museum specimen belong to the same group. This group was separated from the Sumatran subspecies based on samples obtained from the MZB specimen (OQ629470.1, OQ629471.1), Medan (OQ629467.1), Riau (OQ629468.1, OQ629469.1) and GenBank JF357970.1 (Kitpipit et al., 2012), and from the Amur tiger *P. tigris altaica* (AF053031.1) and Bengal tiger *P. tigris tigris* (JF357968.1), with a bootstrap value of 100% (Fig. 2).

We also constructed a phylogenetic tree by analysing the 440 bp long cytochrome b sequences of *P. tigris* subspecies from Luo et al. (2004), of the Javan tiger from GenBank (Matrai & Csoba, 2008; Xue et al., 2015), and of the Javan leopard from Mount Prau and GenBank. These sequences

TABLE 1 The samples of subspecies of the tiger *Panthera tigris* and leopard *Panthera pardus* analysed in this study.

Accession no., by taxon[1]	Nucleotide bp length	Locality	Type	Note[2]
Leopard *P. pardus*				
JF720183.1	1043	GenBank (Ropiquet, 2011)		
MH588626.1	1043	GenBank (Paijmans et al., 2018)		
Javan leopard *P. pardus melas*				
MZBR.1414	1043	Mount Prau, Central Java, 2018	Faecal	Wild
Amur tiger *P. tigris altaica*				
AF053031.1	1043	GenBank (Cracraft et al., 1998)		
South China tiger *P. tigris amoyensis*				
AY736656.1	440	GenBank (Luo et al., 2004)		AMO3
AY736655.1	440	GenBank (Luo et al., 2004)		AMO2
Northern Indochinese tiger *P. tigris corbetti*				
AY736658.1	440	GenBank (Luo et al., 2004)		COR3
AY736635.1	440	GenBank (Luo et al., 2004)		COR7
Javan tiger *P. tigris sondaica*				
FJ403466.1	263	GenBank (Matrai & Csorba, 2008)		
FJ403467.2	263	GenBank (Matrai & Csorba, 2008)		
KJ686504.1	267	GenBank (Xue et al., 2015)		
KJ686505.1	267	GenBank (Xue et al., 2015)		
OQ601561.1	1043	Cipendeuy village, West Java, 2019; GenBank (Wirdateti et al., 2023)	1 hair	Putative
OQ601562.1	1043	West Java, 1930 (Museum Zoologicum Bogoriense); GenBank (Wirdateti et al., 2023)		MZB 2428
Sumatran tiger *P. tigris sumatrae*				
AY736648.1	440	GenBank (Luo et al., 2004)		SUM8
AY736642.1	440	GenBank (Luo et al., 2004)		SUM3
AY736644.1	440	GenBank (Luo et al., 2004)		SUM4
JF357970.1	1043	GenBank (Kitpipit et al., 2012)		
OQ629467.1	1043	GenBank (Wirdateti et al., 2023)	Hairs	Medan/ wild
OQ629468.1	1043	GenBank (Wirdateti et al., 2023)	Bone	Riau1, 2020/ wild
OQ629469.1	1043	GenBank (Wirdateti et al., 2023)	Bone	Riau2, 2020/ wild
OQ629470.1	1043	Bukit Tinggi, West Sumatra, 1956 (Museum Zoologicum Bogoriense); GenBank (Wirdateti et al., 2023)	Dry skin	MZB 41742
OQ629471.1	1043	Pagar Alam, South Sumatra, 1941 (Museum Zoologicum Bogoriense); GenBank (Wirdateti et al., 2023)	Dry skin	MZB 6832
Bengal tiger *P. tigris tigris*				
AY736651.1	440	GenBank (Luo et al., 2004)		TIG2
AY736653.1	440	GenBank (Luo et al., 2004)		TIG4
JF357968.1	1043	GenBank (Kitpipit et al., 2012)		

[1]GenBank accession numbers, except for MZBR 1414, which is an accession number in BRIN Zoology Laboratory.
[2]AMO3, AMO2, COR3, COR7, SUM8, SUM3, SUM4, TIG2, TIG3: GenBank sequence names; MZB, specimen in Museum Zoologicum Bogoriense.

show clear genetic differentiation of the putative Javan tiger hair and the Javan tiger reference samples from Museum Zoologicum Bogoriense (Fig. 3).

The *MEGA* analysis shows that the genetic distance (d) between the putative Javan tiger hair and the other tiger subspecies (i.e. Bengal, Amur and Sumatran tigers) and the Javan leopard is higher than between the Bengal, Amur and Sumatran tiger subspecies (Table 2.). The average difference is 78,883 nucleotides (7.4%); with leopards it is 86,500 nucleotides (8.2%), with the Bengal tiger 71,000 nucleotides (7.1%) and with the Amur tiger 72,000 nucleotides (6.9%). The nucleotide difference between the putative Javan tiger hair and the Javan tiger museum specimen was 41,000 nucleotides (3.9%).

To verify the status of the putative Javan tiger hair, a comparative analysis was conducted based on Luo et al. (2004). The genetic distances between the presumed Javan tiger hair and other tiger subspecies are 0.037–0.041 (3.7–4.1%), with the leopard 0.042 (4.2%) and with the museum specimen of the Javan tiger 0.003 (0.3%). This differentiation was more pronounced in the longer cytochrome b sequence (1043 bp). In addition, our results suggest that the genetic distance between the putative Javan tiger hair and the other tiger subspecies from Sumatra and continental

(a)   (b)
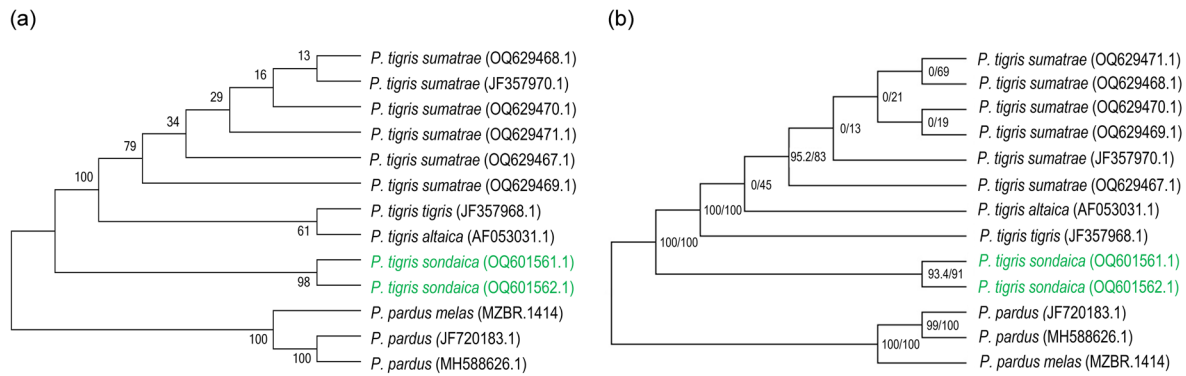

FIG. 2 Phylogenetic trees, constructed using (a) the maximum likelihood method with the Kimura 2-parameter model in *MEGA*, and (b) the maximum likelihood phylogram in *IQ Tree*, for the putative Javan tiger hair and specimens of the Sumatran tiger *P. tigris sumatrae*, Bengal tiger *P. tigris tigris*, Amur tiger *P. tigris altaica* and leopard *Panthera pardus* based on the mtDNA cytochrome b gene (1043 bp nucleotide length). The two analyses have a consistent profile, with the putative Javan tiger hair (OQ601561) in the same clade as the Javan tiger museum specimen (OQ601562). The number on each branch represents the bootstrap value. See Table 1 for details of the samples.
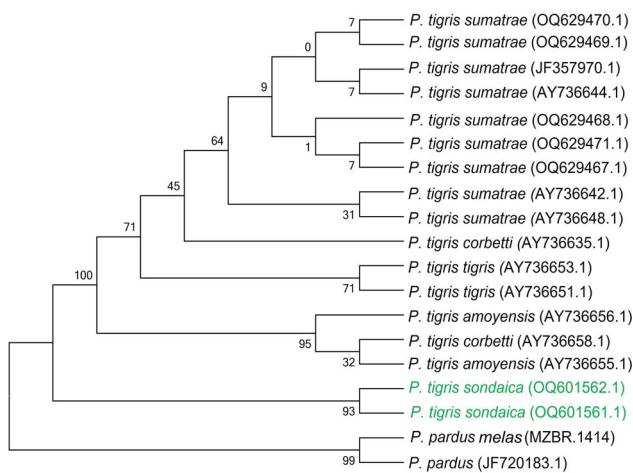

FIG. 3 Phylogenetic tree of the putative Javan tiger hair (OQ601561), Javan tiger museum specimen (OQ601562), other tiger subspecies (Luo et al., 2004) and leopard based on the mtDNA cytochrome b gene (440 bp nucleotide length) using the maximum likelihood method with the Kimura 2-parameter model. The number on each branch represents the bootstrap value. See Table 1 for details of the samples.

Asia was greater than with the Javan tiger museum specimen.

Using cytochrome b sequences of Javan tigers from GenBank, which are 263 bp long (Matrai & Csoba, 2008) and 267 bp long (Xue et al., 2015), the putative Javan tiger hair and Javan tiger museum specimens are in the same group. However, the Javan tiger sample from GenBank was assigned to the same clade as the Sumatran tigers (Fig. 4). The genetic distance (data not shown) between the putative Javan tiger hair and the GenBank sequences from Xue et al. (2015; 0.038) and Matrai & Csoba (2008; 0.052 and 0.049) were comparable. The genetic distances between the Sumatran and Javan tiger sequences from GenBank are 0.000–0.003 (0.00–0.03%), indicating a misclassification of the latter.

The successful amplification of the 1043 bp long mitochondrial cytochrome b gene from just one hair indicates that the DNA material is fresh rather than preserved. In contrast, we were unable to isolate the 1043 bp mtDNA fragment from hair of historical museum samples in Museum Zoologicum Bogoriense that had been preserved with

TABLE 2 Genetic distance (d) between the putative Javan tiger hair and samples from the Bengal, Amur, Sumatran and Javan tigers and leopards, based on the mtDNA cytochrome b gene (1043 bp) (see Table 1 for details of the samples).

Taxon (reference no.)	1	2	3	4	5	6	7	8	9	10	11	12	13
P. tigris tigris (JF357968.1)		0.002	0.002	0.002	0.002	0.002	0.003	0.002	0.010	0.011	0.011	0.009	0.010
P. tigris altaica (AF053031.1)	0.003		0.002	0.002	0.002	0.002	0.003	0.002	0.011	0.011	0.011	0.009	0.010
P. tigris sumatrae (OQ629471.1)	0.004	0.005		0.001	0.001	0.000	0.002	0.000	0.010	0.010	0.011	0.009	0.010
P. tigris sumatrae (OQ629470.1)	0.005	0.006	0.001		0.001	0.001	0.003	0.001	0.011	0.011	0.011	0.009	0.010
P. tigris sumatrae (OQ629467.1)	0.005	0.006	0.001	0.002		0.001	0.003	0.001	0.010	0.010	0.011	0.009	0.010
P. tigris sumatrae (OQ629468.1)	0.004	0.005	0.000	0.001	0.001		0.002	0.000	0.010	0.010	0.011	0.009	0.010
P. tigris sumatrae (OQ629469.1)	0.010	0.011	0.006	0.007	0.007	0.006		0.002	0.010	0.010	0.011	0.009	0.010
P. tigris sumatrae (JF357970.1)	0.004	0.005	0.000	0.001	0.001	0.000	0.006		0.010	0.010	0.011	0.009	0.010
P. pardus (JF720183.1)	0.112	0.115	0.111	0.113	0.113	0.111	0.115	0.111		0.002	0.007	0.010	0.011
P. pardus (MH588626.1)	0.108	0.112	0.108	0.109	0.109	0.108	0.111	0.108	0.003		0.006	0.010	0.011
P. pardus melas (MZBR.1414)	0.109	0.113	0.111	0.113	0.113	0.111	0.115	0.111	0.044	0.041		0.010	0.011
P. tigris sondaica (OQ601561.1)	0.071	0.072	0.073	0.074	0.073	0.073	0.076	0.073	0.089	0.086	0.089		0.006
P. tigris sondaica (OQ601562.1)	0.090	0.091	0.092	0.093	0.092	0.092	0.095	0.092	0.107	0.103	0.102	0.040	

(a)   (b)
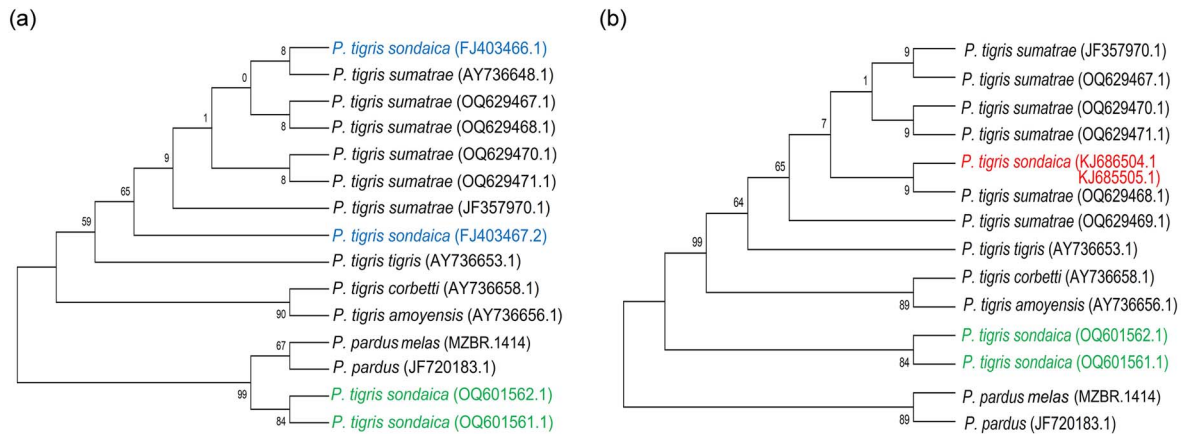

FIG. 4 Phylogenetic tree of the putative Javan tiger hair (OQ601561.1), Javan tiger museum specimen (OQ601562.1) and sequences of *P. tigris* subspecies, and *P. pardus* from GenBank using the maximum likelihood method with the Kimura 2-parameter model: (a) using two *P. tigris sondaica* sequences of 263 bp (Matrai & Csoba, 2008), and (b) using two *P. tigris sondaica* sequences of 267 bp (Xue et al., 2015). Both trees show that the *P. tigris sondaica* sequences from GenBank ((a): FJ403466.1, FJ403467.2; (b): KJ686504.1, KJ686505.1) are in the same group as *P. tigris sumatrae*, whereas the putative Javan tiger hair is in the same group as the Javan tiger museum specimen. The number on each branch represents the bootstrap value. See Table 1 for details of the samples.

arsenic. From this comprehensive mtDNA analysis we conclude that the hair sample from South Sukabumi belongs to the Javan tiger, and that it falls in the same group as the Javan tiger museum specimen collected in 1930. Whether the Javan tiger actually still occurs in the wild needs to be confirmed with further genetic and field studies.

References

CRACRAFT, J., FEINSTEIN, J., VAUGHN, J. & HELM-BYCHOWSKI, K. (1998) Sorting out tigers (*Panthera tigris*): mitochondrial sequences, nuclear inserts, systematics, and conservation genetics. *Animal Conservation*, 1, 63–74.

DRISCOLL, C.A., YAMAGUCHI, N., BAR-GAL, G.K., ROCA, A.L., LUO, S. et al. (2009) Mitochondrial phylogeography illuminates the origin of the extinct Caspian tiger and its relationship to the Amur tiger. *PLOS One*, 4, e4125.

HALL, T.A. (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series*, 41, 95–98

KALYAANAMOORTHY, S., MINH, B.Q., WONG, T.K.F., ARNDT, V.H. & LARS, S.J. (2017) ModelFinder: fast model selection for accurate phylogenetic estimates. *Nature Methods*, 14, 587–589.

KITPIPIT, T., TOBE, S.S. & LINACRE, A. (2012) The complete mitochondrial genome analysis of the tiger (*Panthera tigris*). *Molecular Biology Report*, 39, 5745–5754.

LUO, S.J., KIM, J.H., JOHNSON, W.E., VAN DER WELT, J., MARTENSON, J. et al. (2004) Phylogeography and genetic ancestry of tigers (*Panthera tigris*). *PLOS Biology*, 2, 2275–2293.

MATRAI, E. & CSORBA, G. (2008) *DNA technology in the examination of mammal skins kept in museums. The first genetic data of the Javan tiger (*Panthera tigris sondaica*).* GenBank, National Institutes of Health, Bethesda, USA. ncbi.nlm.nih.gov/nuccore/FJ403466.1 [accessed September 2023].

MONDOL, S., KARANTH, K.U. & RAMAKRISHNAN, U. (2009) Why the Indian subcontinent holds the key to global tiger recovery. *PLOS Genetics*, 5, e1000585.

NGUYEN, L.T., SCHMIDT, H.A., VON HAESELER, A. & MINH, B.Q. (2015) IQ-TREE: a fast and effective stochastic algorithm for estimating maximum likelihood phylogenies. *Molecular Biology and Evolution*, 32, 268–274.

PANKO, B. (2017) *Long thought extinct, Javan tiger may have been spotted in Indonesia.* Smithsonian Magazine, 18 September 2017. smithsonianmag.com/smart-news/island-tiger-thought-extinct-may-have-been-spotted-180964920 [accessed September 2023].

PAIJMANS, J.L.A., BARLOW, A., FOERSTER, D.W., HENNEBERGER, K., MEYER, M., NICKEL, B. et al. (2018) *Historical biogeography of the leopard (*Panthera pardus*) and its extinct Eurasian populations.* GenBank, National Institutes of Health, Bethesda, USA. ncbi.nlm.nih.gov/nuccore/MH588626.1 [accessed September 2023].

RAMBAUT, A. (2018) *FigTree 1.4.4.* tree.bio.ed.ac.uk/software/figtree [accessed 5 August 2022].

ROPIQUET, A. (2011) *Tolerance versus translocation for ensuring the genetic diversity of leopard?* GenBank, National Institutes of Health,

Bethesda, USA. ncbi.nlm.nih.gov/nuccore/JF720182.1 [accessed September 2023].

SANDERSON, E.W., FORREST, J., LOUCKS, C., GINSBERG, J., DINERSTEIN, E. et al. (2010) Setting priorities for tiger conservation 2005–2015. In *Tigers of the World*, 2nd edition (eds R. Tilson & P.J. Nyhus), pp. 143–161. William Andrew Publishing, Norwich, USA.

SEIDENSTICKER, J. (1987) Bearing witness: observations on the extinction of *Panthera tigris balica* and *Panthera tigris sondaica*. In *Tigers of the World: The Biology, Biopolitics, Management, and Conservation of an Endangered Species* (eds R.L. Tilson & U.S. Seal), pp. 1–8. Noyes Publications, New Jersey, USA.

TAMURA, K., STECHER, G., PETERSON, D., FILIPSKI, A. & KUMAR S. (2013) MEGA 6: molecular evolutionary genetics analysis version 6.0. *Molecular Biology Evolution*, 30, 2725–2729.

THOMPSON, J.D., GIBSON, T.J., PLEWNIAK, F., JEANMOUGIN, F. & HIGGINS, D.G. (1997) The Clustal_X windows interface: flexible strategies for mutiple sequence alignment aided by quality analysis. *Nucleid Acids Research*, 25, 4876–4882.

TILSON, R., HU, D., MUNTIFERING, J. & NYHUS, P.J. (2004) Dramatic decline of wild South China tigers *Panthera tigris amoyensis*: field survey of priority tiger reserves. *Oryx*, 38, 40–47.

WALSTON, J., ROBINSON, J.G., BENNETT, E.L., BREITENMOSER, U., DA FONSECA, G.A.B. et al. (2010) Bringing the tiger back from the brink: the six percent solution. *PLOS Biology*, 8, e1000485.

WIRDATETI, W., YULIANTO, Y., RAKSASEWU, K. & ADRIYANTO, B. (2023) *Preliminary study of suspected javan tiger at south Sukabumi Forest, West Java, based on molecular genetics*. GenBank, National Institutes of Health, Bethesda, USA. ncbi.nlm.nih.gov/nuccore/OQ601561 [accessed September 2023].

XUE, H.R., YAMAGUCHI, N., DRISCOLL, C.A., HAN, Y., BAR-GAL, G.K. et al. (2015) Genetic ancestry of the extinct Javan and Bali Tigers. *Journal of Heredity*, 106, 247–257.