Session 4: Biodiversity and Environmental Rehabilitation

Haplotype Diversity of Wild Asian Elephants in Phu Khieo and Khao Ang Rue Nai Wildlife Sanctuaries, Thailand

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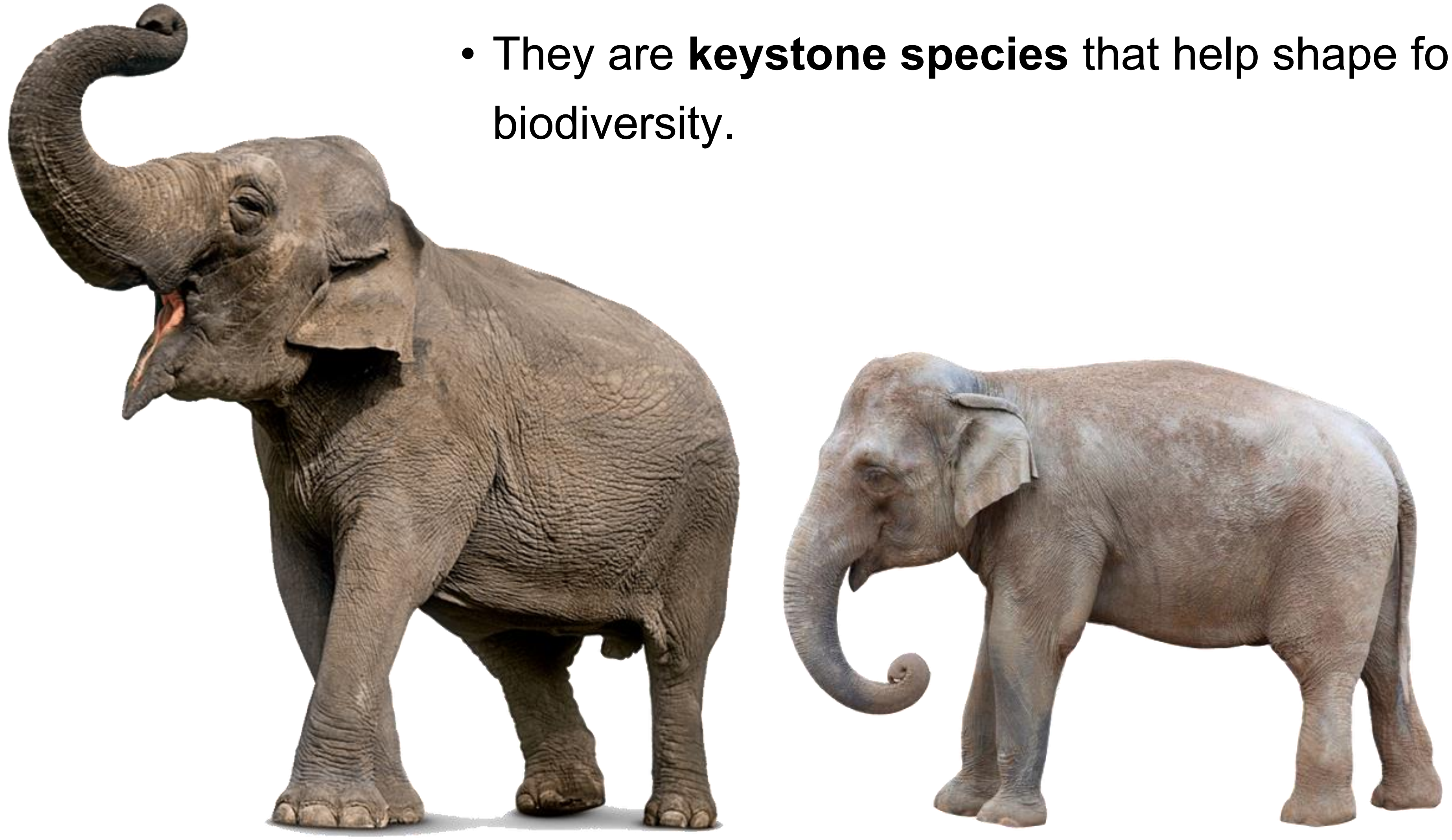
By Miss Supansa RERKDEE
Email: Supansa.re@ku.th
Department of Forest Biology,
Faculty of Forestry, Kasetsart University, Thailand

FACULTY OF FORESTRY
KASETSART UNIVERSITY

AGB Research Center

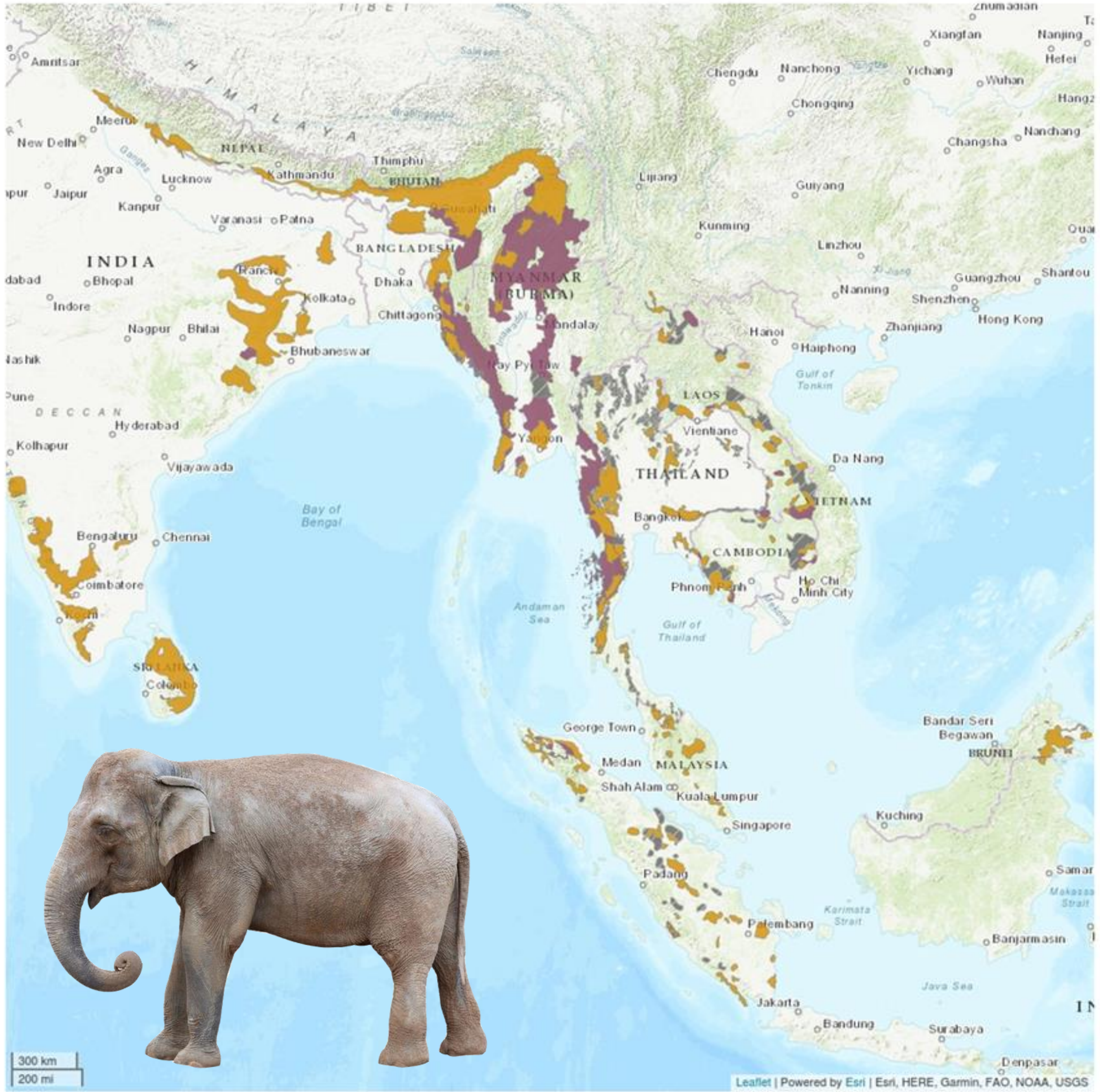
Introduction to Asian Elephants

- **Asian elephants** (*Elephas maximus*) have a deep historical and cultural connection with human societies, particularly in Southeast Asia.
  - Historically, elephants were used for transportation, agriculture, and religious ceremonies, making them deeply connected to human societies.
    - They are **keystone species** that help shape forests and maintain biodiversity.



Source: https://www.rzss.org.uk/asian-elephant
https://nationalzoo.si.edu/animals/asian-elephant

Conservation Status and Main Threat



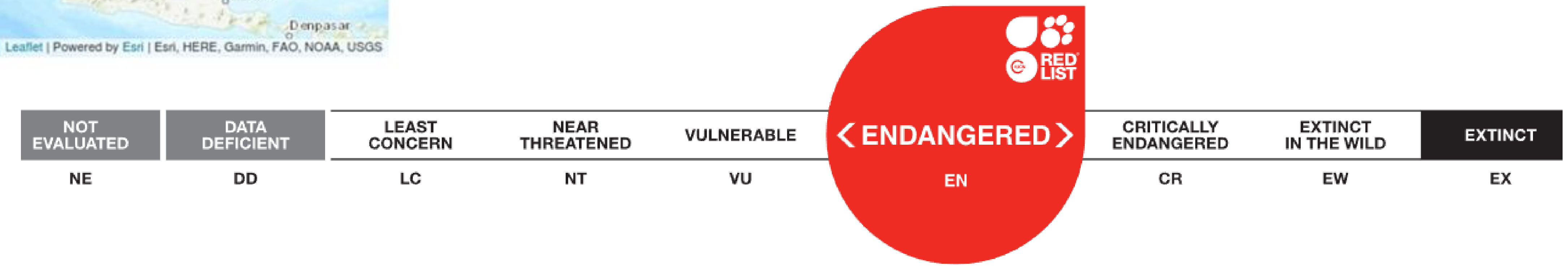
Legend

🟧 EXTANT (RESIDENT)

🟪 POSSIBLY EXTANT (RESIDENT)

⬛ POSSIBLY EXTANT & ORIGIN UNCERTAIN (RESIDENT)

- The IUCN Red List classifies them as **Endangered (EN)** due to habitat loss, poaching, and human-elephant conflicts.
- Asian elephants are widely distributed across South and Southeast Asia, but their populations are **highly fragmented**.



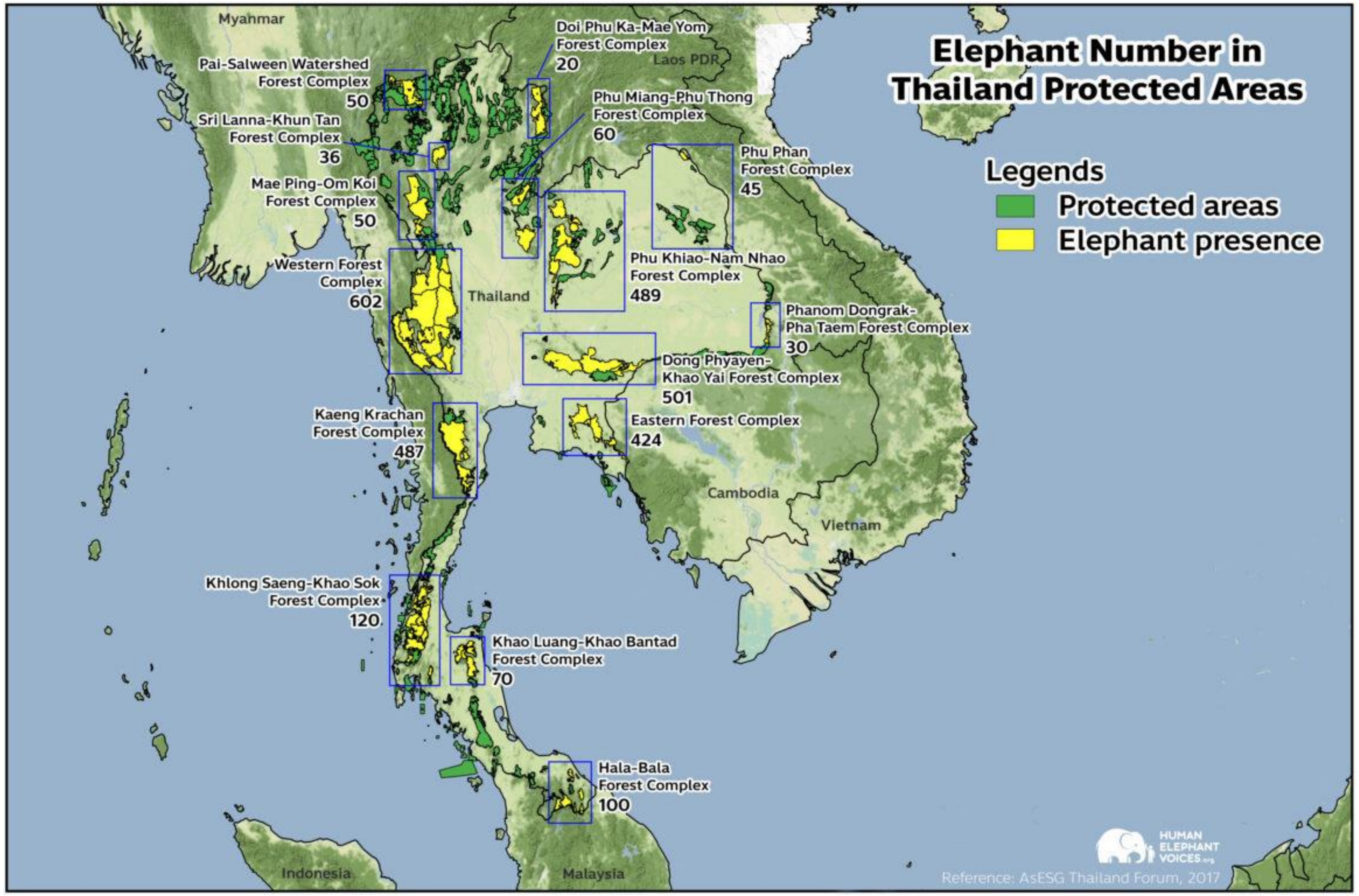
NOT EVALUATED	DATA DEFICIENT	LEAST CONCERN	NEAR THREATENED	VULNERABLE	ENDANGERED	CRITICALLY ENDANGERED	EXTINCT IN THE WILD	EXTINCT
NE	DD	LC	NT	VU	EN	CR	EW	EX

Challenges and Threats Facing Wild Elephants

- **Habitat destruction and fragmentation** are the most pressing threats to wild elephants, caused by deforestation, agriculture, and urban expansion.
- **Human-elephant conflicts**
- **Poaching and illegal wildlife trade**

www.vfaes.org/post/asian-elephant-habitat-threats
www.arabnews.com/node/1223986/world
www.thejakartapost.com/life/2021/06/27/march-of-the-elephants-chinas-rogue-herd-spotlights-habitat-loss

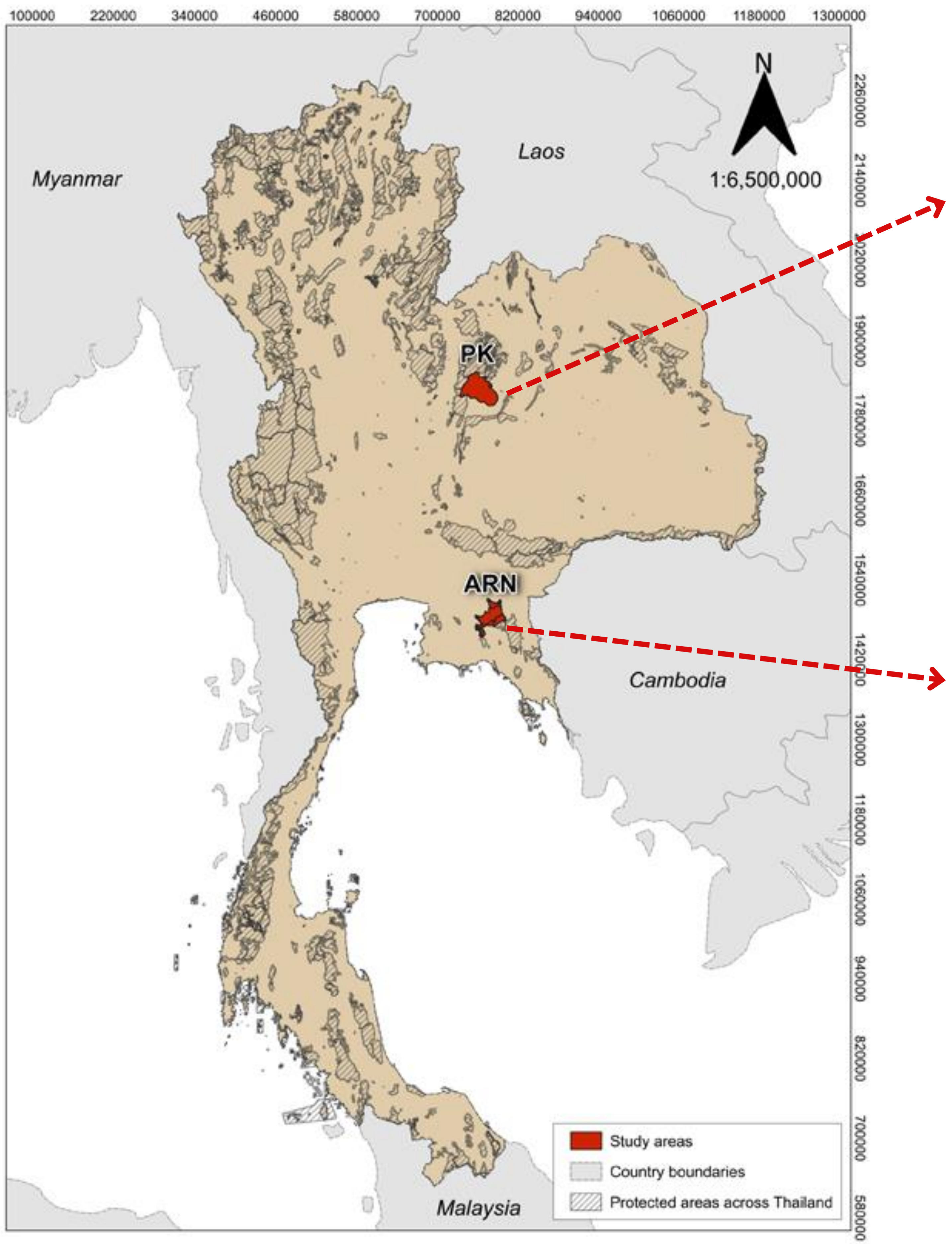
Distribution and Population in Thailand



Elephant Number in Thailand Protected Areas
Source: AsESG Thailand Forum (2017)

- Thailand’s wild elephant population is estimated to be between 4,013–4,422 individuals, distributed across 93 protected areas. (DNP, 2024)
- Despite being in protected areas, many populations are still at risk due to **habitat fragmentation** and **limited genetic exchange**.

Study Sites



Phu Khieo Wildlife Sanctuary (PK)

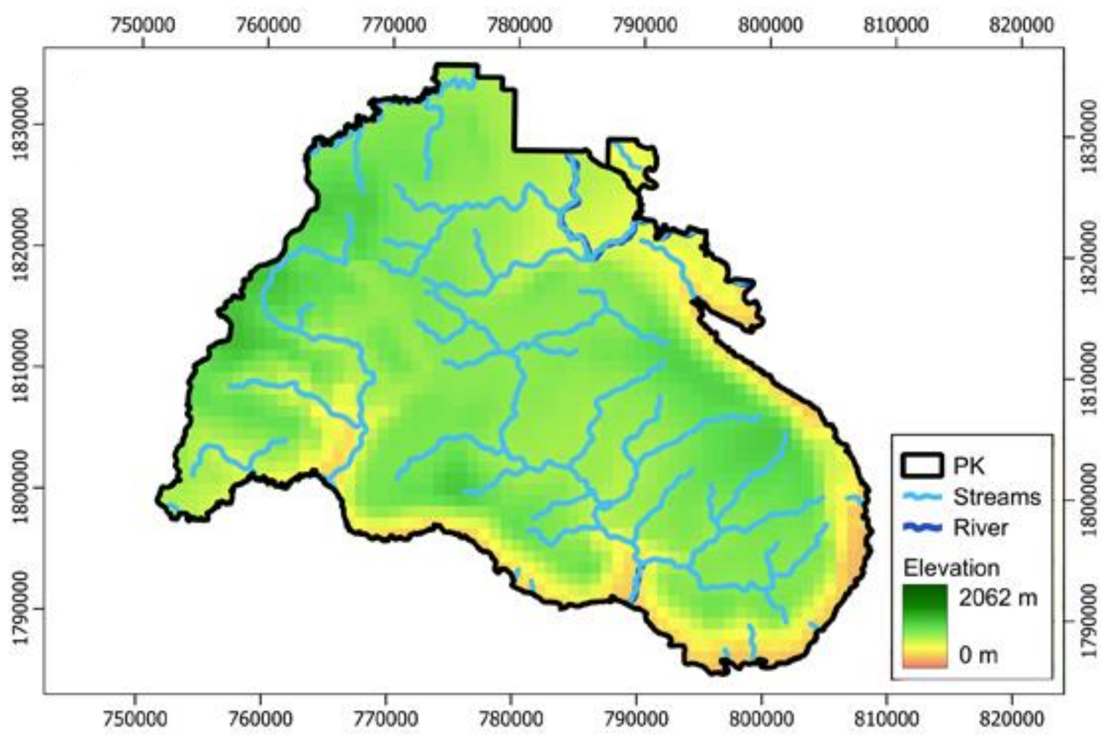
- Located in Northeast Thailand.
- Part of a large, well-connected forest complex.
- Generally experiences low anthropogenic resistance (less human development).

Khao Ang Rue Nai Wildlife Sanctuary (ARN)

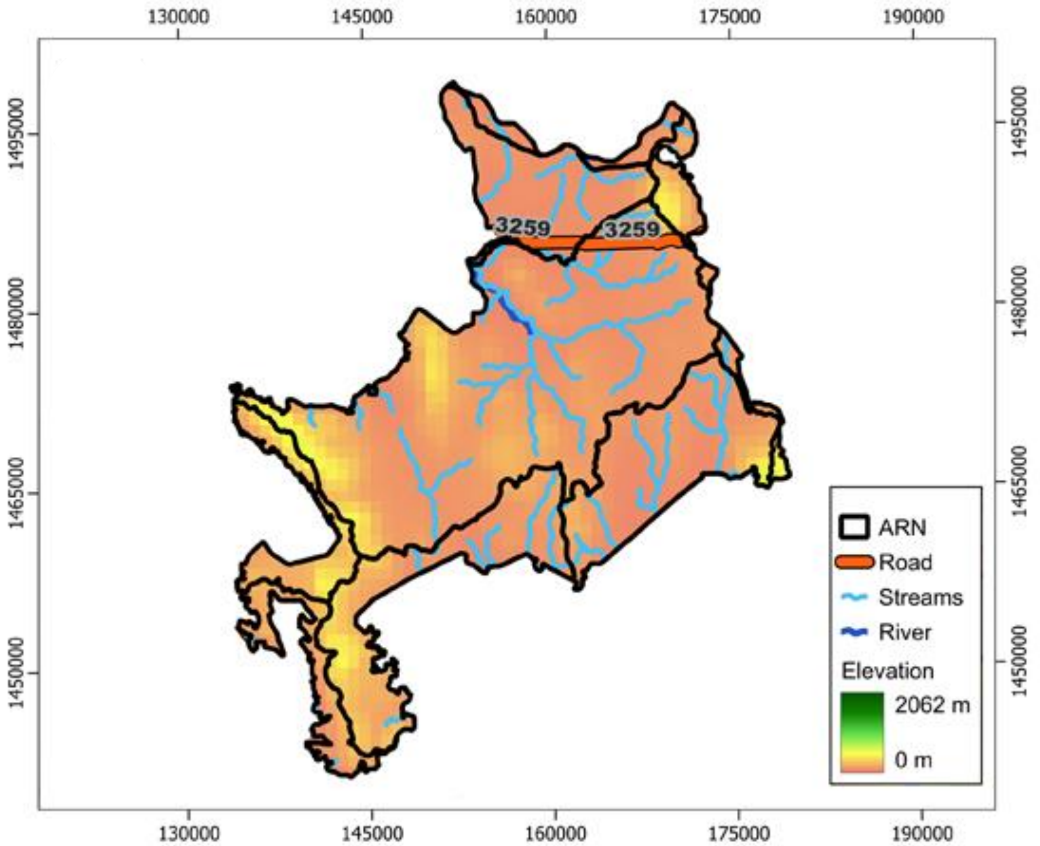
- Located in Eastern Thailand.
- Surrounded by extensive human land use (agriculture, settlements).
- Site of increased Human-Elephant Conflict (HEC).

Study Sites

PK



ARN



https://thailandtourismdirectory.go.th/attraction/3420
https://mgronline.com/business/detail/9620000080933

https://www.tnews.co.th/social/social-news/619572
https://www.thaipbs.or.th/news/content/262013

Study Sites

- **Phu Khieo (PK) and Khao Ang Rue Nai (ARN) Wildlife Sanctuaries** are a habitat for wild elephants, **with an increasing population trend**.
- Although elephant numbers are growing, **habitat fragmentation limits movement**, raising concerns about genetic diversity and long-term viability (Htet *et al*., 2021).
- **Evaluating genetic diversity** in these sanctuaries can help ensure that the population remains viable in the long term.

https://www.nationtv.tv/news/region/378887914
https://www.banmuang.co.th/news/region/386938

Study gap

Previous research **lacks genetic baseline data** needed to guide the rehabilitation of elephant population connectivity in Thailand's main protected areas.

Objectives

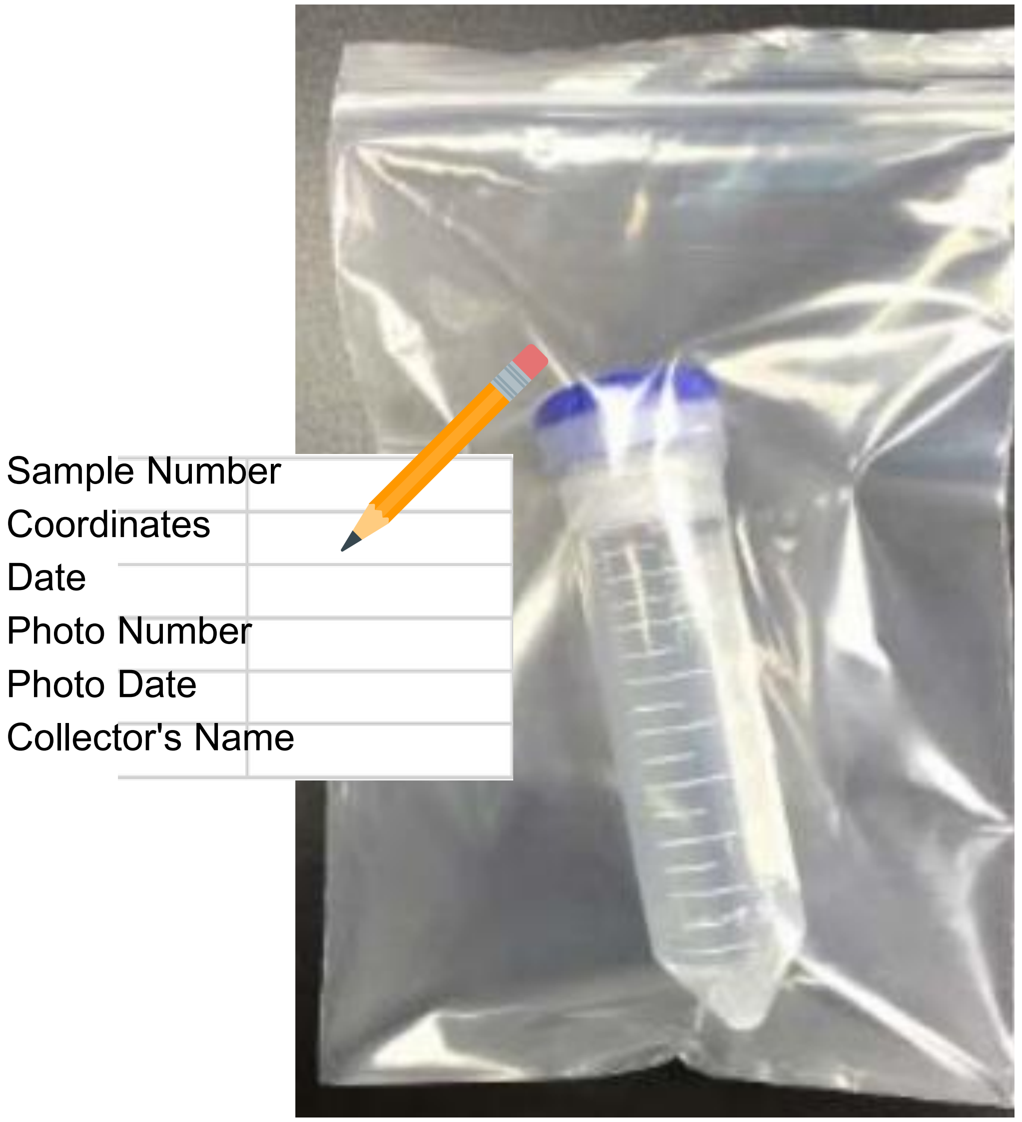
1. To determine the **mitochondrial DNA (mtDNA) D-loop haplotype diversity** of wild Asian elephants in Phu Khieo and Khao Ang Rue Nai Wildlife Sanctuaries.

2. To assess the level of **genetic differentiation** between the PK and ARN populations.

Methodology

Sample collection

- **Collect fresh feces samples within 24 hours** to ensure DNA quality
- Wear gloves to prevent contamination
- Label samples with date, time, and GPS coordinates for tracking
- Store samples in a cool, dry environment or freeze to maintain DNA integrity



Sample Number
Coordinates
Date
Photo Number
Photo Date
Collector's Name

Source: Airubon | Dreamstime.com

Methodology

The study collected 133 samples from Phu Khieo and 192 samples from Khao Ang Rue Nai.

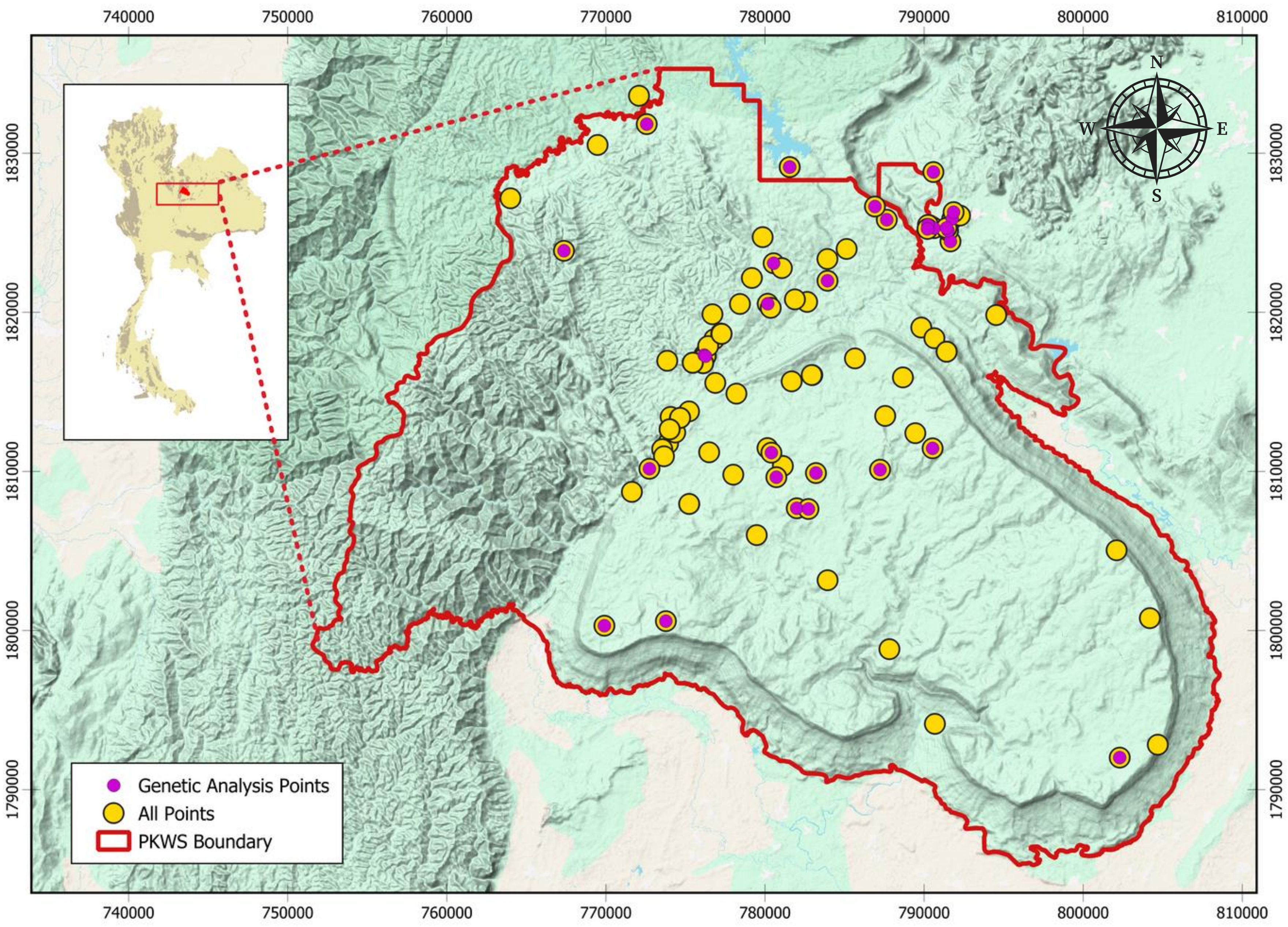


Figure Map of elephant sample sites in Phu Khieo Wildlife Sanctuary

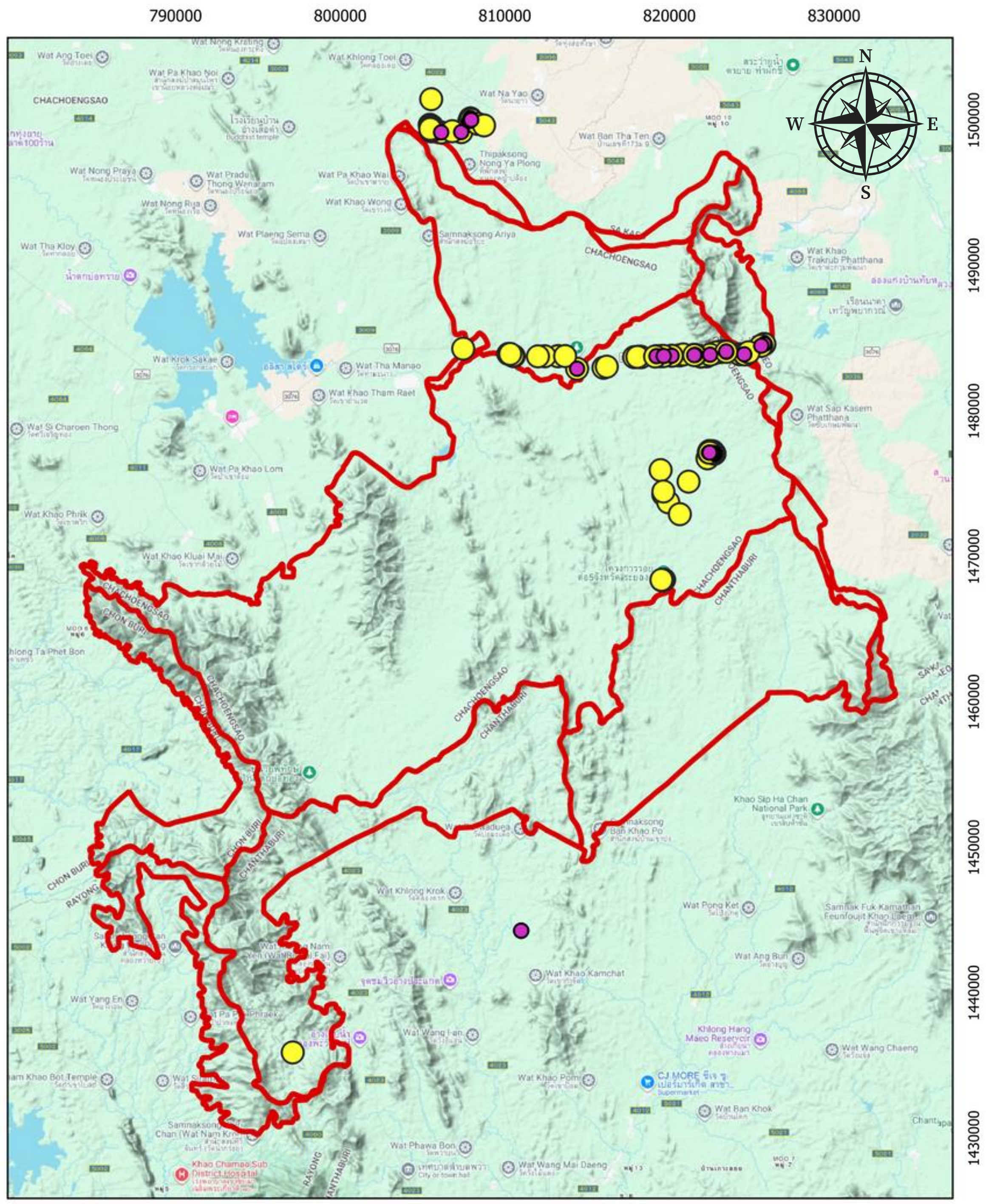
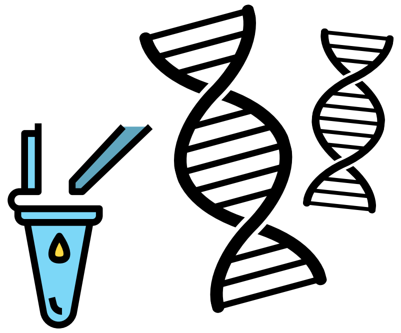


Figure Map of elephant sample sites in Khao Ang Rue Nai Wildlife Sanctuary

Methodology

Genetic analysis of elephant diversity

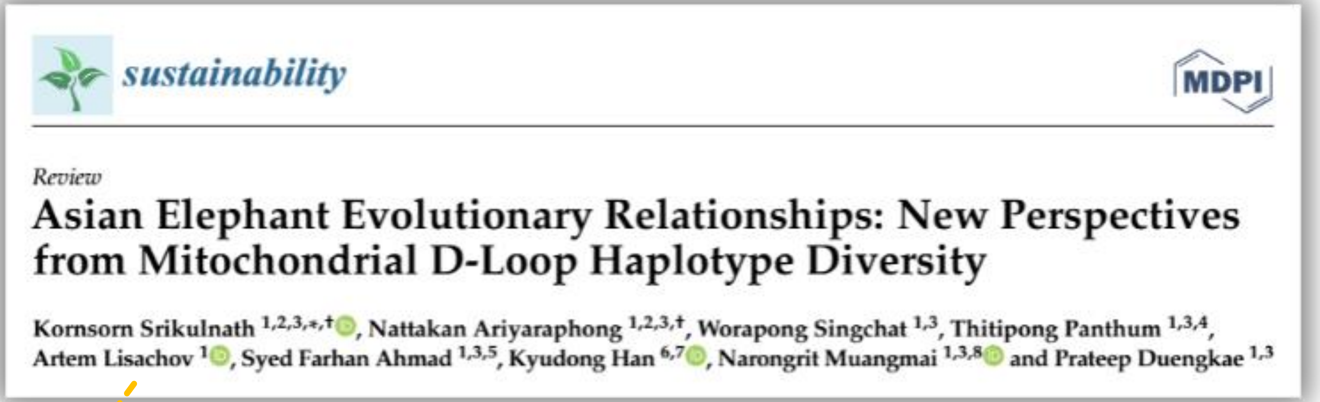
Sample collection



DNA extraction

PCR amplification

(mitochondrial D-loop region)



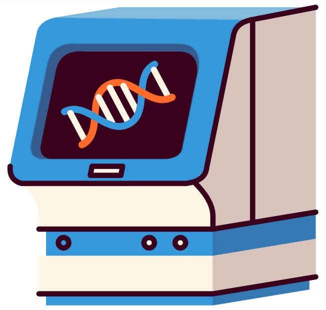
sustainability MDPI

Review

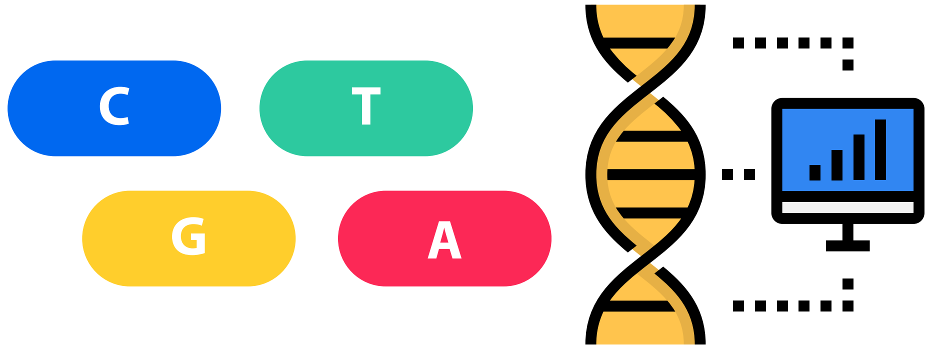
Asian Elephant Evolutionary Relationships: New Perspectives from Mitochondrial D-Loop Haplotype Diversity

Kornsorn Srikulnath [1,2,3,*,†], Nattakan Ariyaraphong [1,2,3,†], Worapong Singchat [1,3], Thitipong Panthum [1,3,4], Artem Lisachov [1], Syed Farhan Ahmad [1,3,5], Kyudong Han [6,7], Narongrit Muangmai [1,3,8] and Prateep Duengkae [1,3]

PCR product purification



Sequencing



Sequence editing and alignment

Data analysis

MEGA Molecular Evolutionary Genetics Analysis

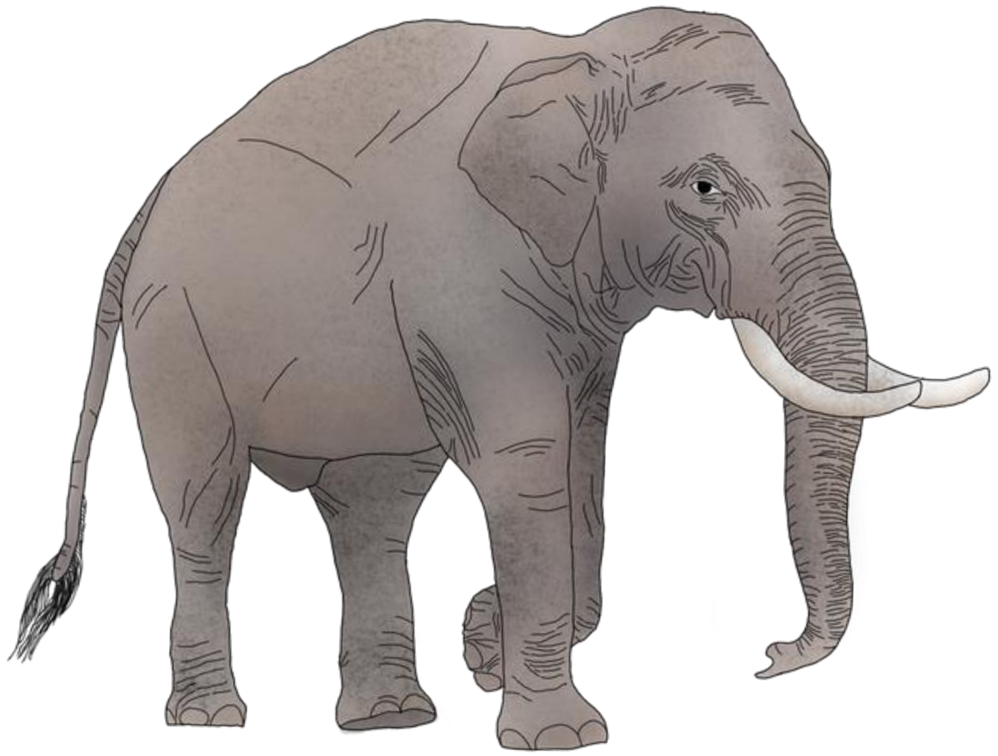
DnaSP

Genetic diversity analysis

- Nucleotide diversity (π)
- Haplotype diversity (Hd)
- Haplotype network

genetic differentiation analysis

- AMOVA



Methodology

MaxEnt modeling for habitat suitability

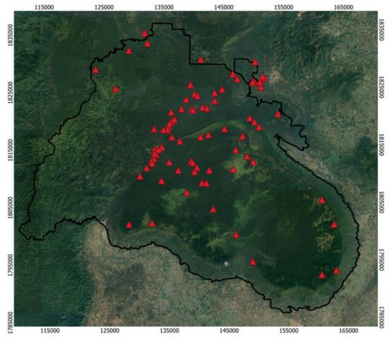
Species Distribution Model (MaxEnt)

Occurence dataset

GPS locations from field surveys

QGIS

Arrangement of sample points



Environmental variables

WorldClim

anthropogenic factor

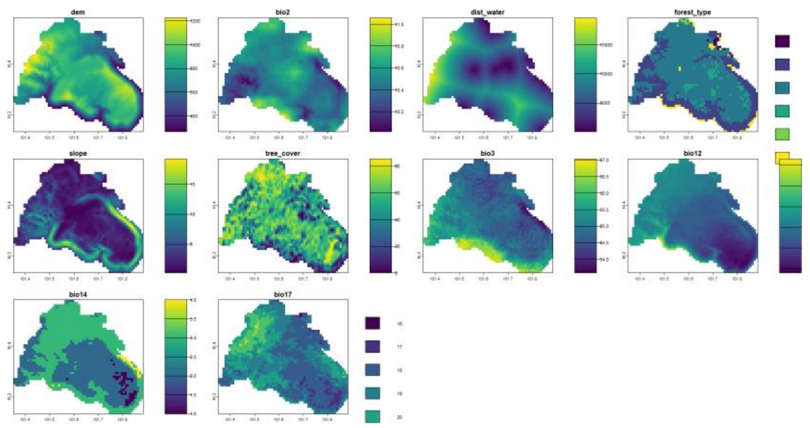
Topographical

Vegetation

Hydrological

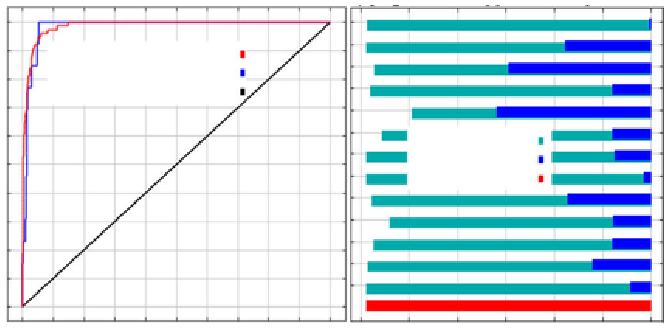
Resampling & Variance inflation factor (VIF) calculations

RStudio



Model delvelopment using **dismo** package in R

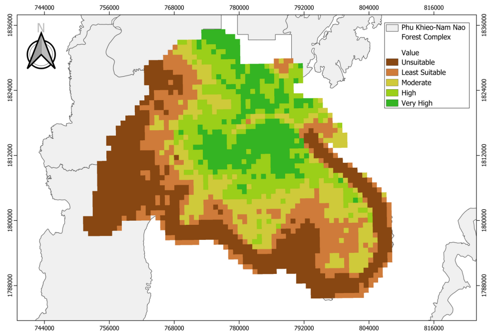
(Hijmans *et al.*, 2024)



Model outputs

1. AUC & ROC Value
2. JackKnife test

1. Classification of predicted habitat
2. Evaluating variable importance



Results and discussion

Genetic analysis of elephant diversity

- A total of 14 distinct mtDNA haplotypes were identified across all 66 individuals.
- The Haplotype Network clearly shows distinct clusters, with **TH2 being the only bridge**.
- This limited sharing strongly indicates genetic isolation between PK and ARN.

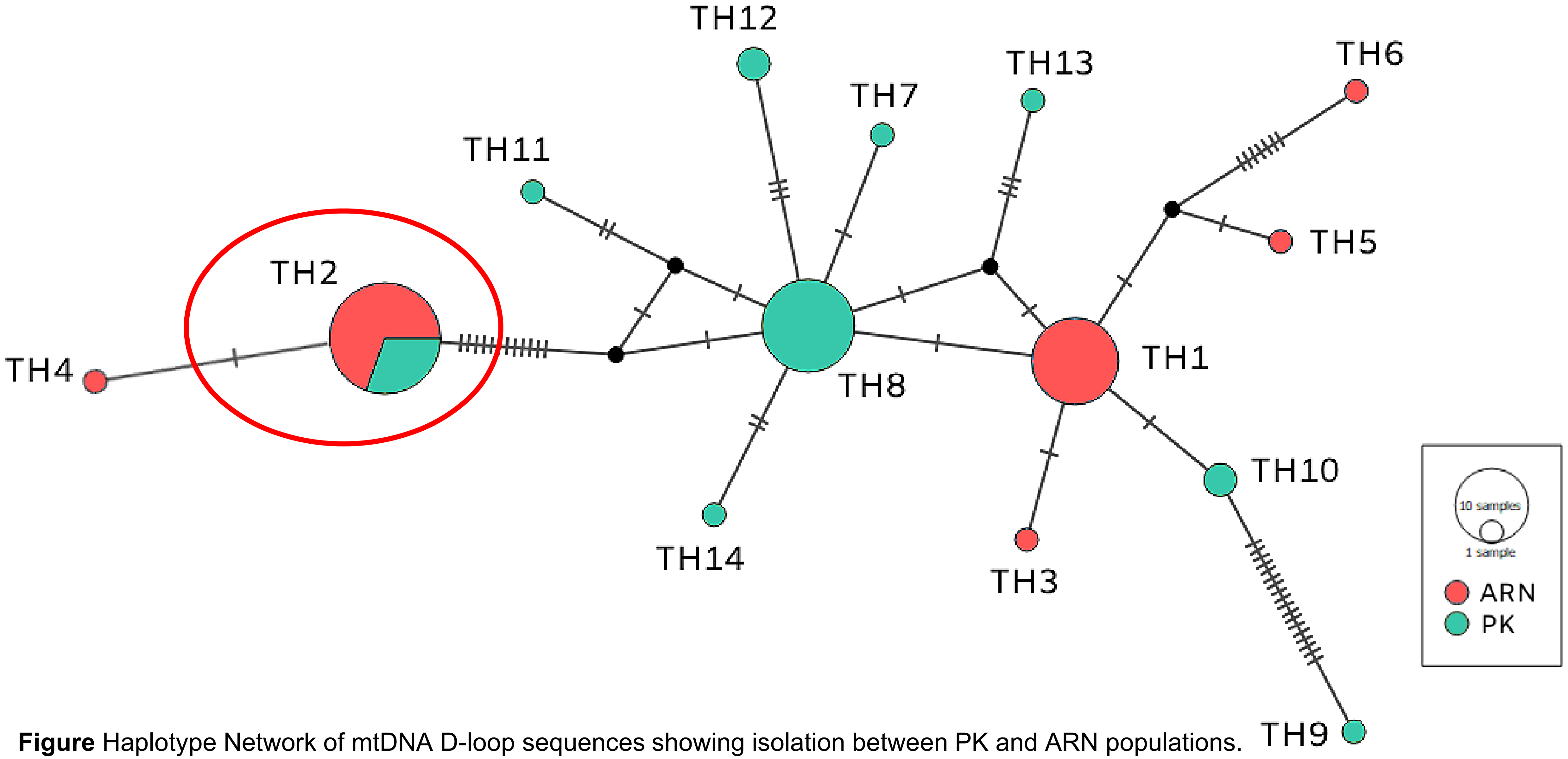


Figure Haplotype Network of mtDNA D-loop sequences showing isolation between PK and ARN populations.

Results and discussion

Thai Elephant Genetic Lineages

- The analysis reveals three distinct genetic groups in Thailand: Alpha, Beta-1, and Beta-3.
- The two populations we studied primarily belong to the Alpha and Beta-1 groups.
- This confirms that elephant populations in Thailand are highly structured, with deep, separate evolutionary histories.

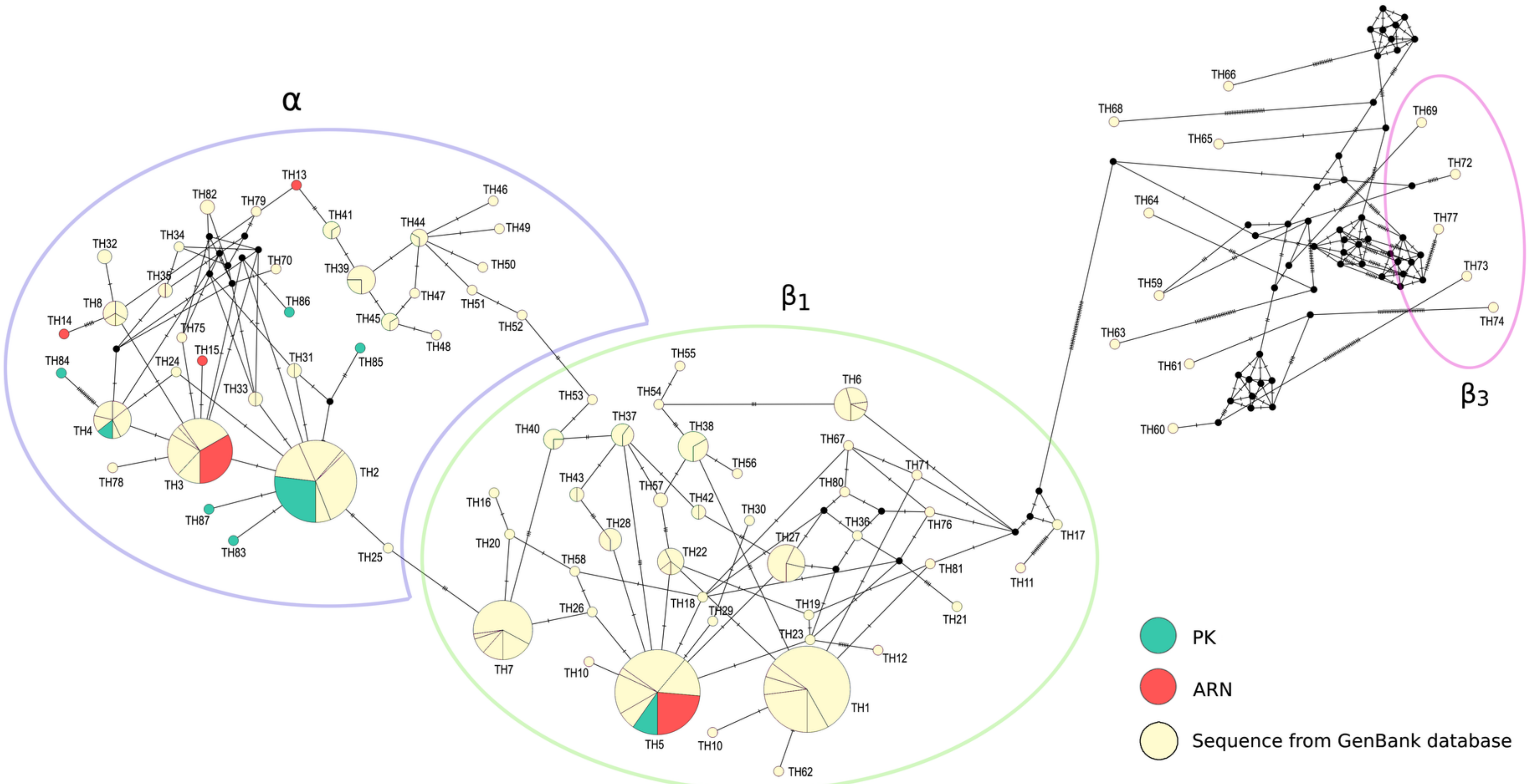


Figure Haplotype Network showing the three major genetic groups of Thai elephants

Results and discussion

Genetic Diversity Health Check

- PK shows high genetic variety (Hd=0.712), reflecting stability and good historical movement.
- ARN shows lower variety (Hd=0.624) and slightly higher Nucleotide Diversity (π=0.022).
- This lower variety in ARN suggests that this population is more vulnerable to genetic loss (genetic drift) in the face of fragmentation.

Table Comparison of genetic diversity between PK and ARN elephant populations

Population	Sample Size	Number of Haplotype (*H*)	Haplotype Diversity (Hd)	Nucleotide Diversity (*π*)	Average Nucleotide Differences (*k*)
PK[1]	32	9	0.712	0.020	7.012
ARN[2]	34	6	0.624	0.022	7.914
Total	**66**	**14**	**0.783**	**0.022**	**7.983**

[1] Phu Khieo Wildlife Sanctuary

[2] Khao Ang Rue Nai Wildlife Sanctuary

Results and discussion

Genetic Differentiation: The Quantified Barrier

- AMOVA showed that **11.65%** of the total genetic variation occurred **between** the PK and ARN elephant populations.
- This moderate partitioning (>5%) that the two elephant groups are functioning as two separate evolutionary units.

Table AMOVA results for mitochondrial DNA D-loop sequences

Source of variation	df	Sum of squares	Variance components	Percentage of variation	*p*-value
Among populations	1	20.890	0.51516	11.65%	0.007
Within populations	64	249.936	3.90525	88.35%	
Total	65	270.826	4.42041	100.00%	

Results and discussion

Spatial Factors Driving Elephant Presence

MaxEnt

- MaxEnt species distribution model shows which factors influence elephant presence in each sanctuary.
- In PK, factors are mainly related to terrain: Distance to Major Roads, Slope, and Terrain Ruggedness (TRI).
- In ARN, the key factors are human-related: Distance to Major Roads and Nighttime Lights (Human Activity).

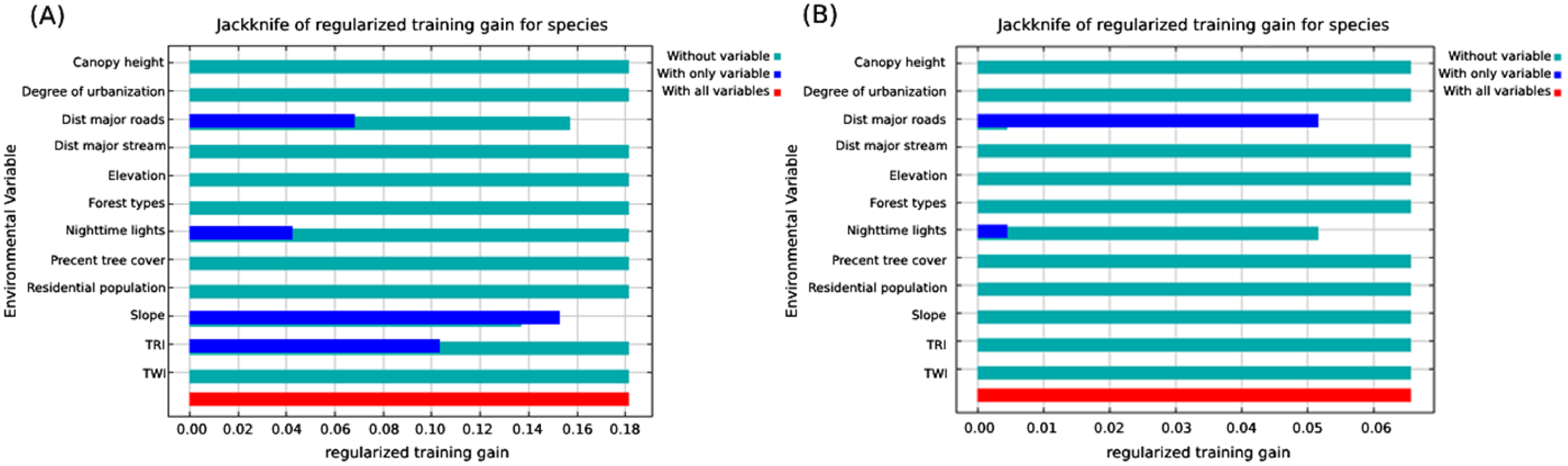
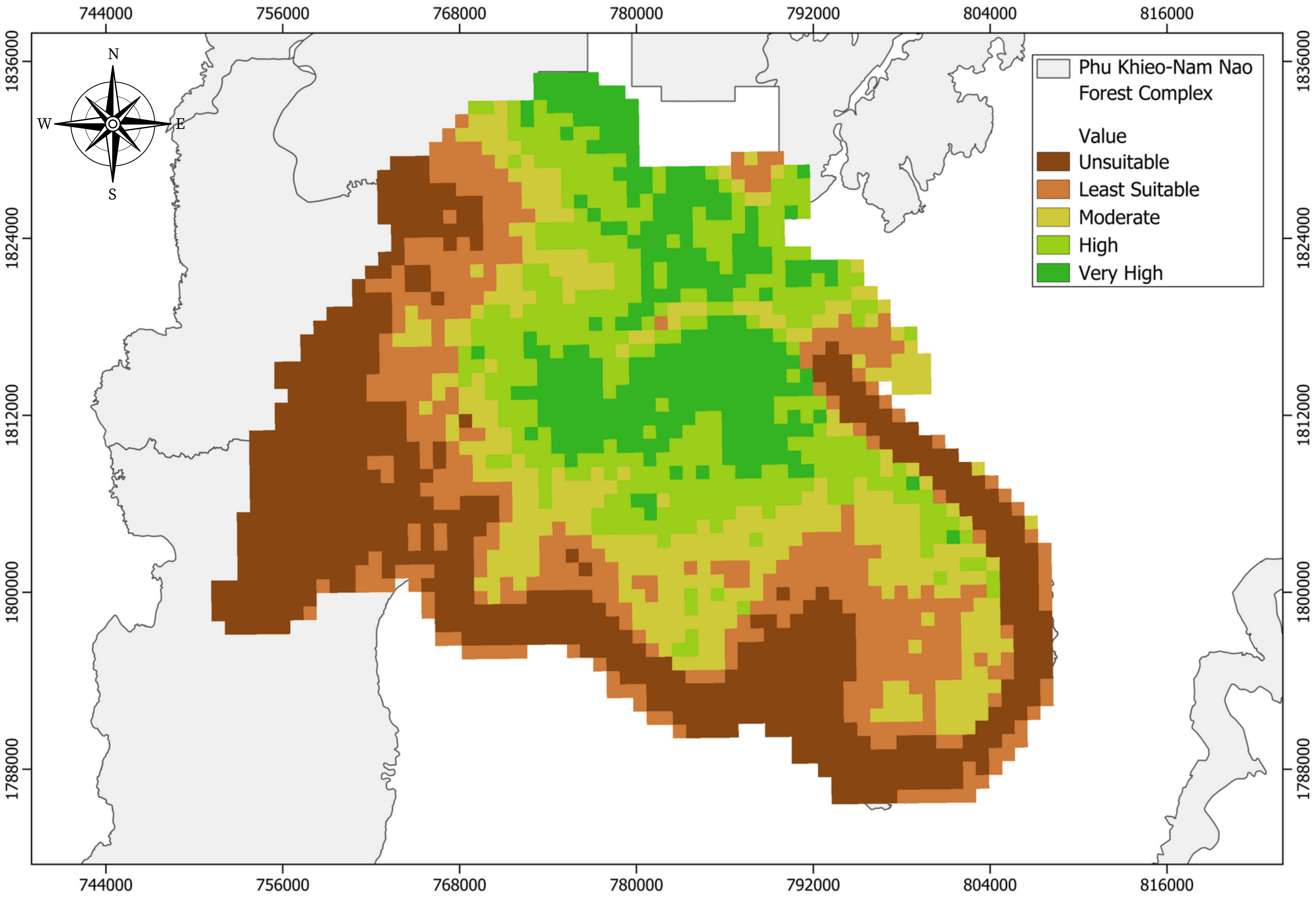


Figure Jackknife analysis showing the relative importance of environmental variables on elephant presence in Phu Khieo (A) and Khao Ang Rue Nai (B).

Results and discussion

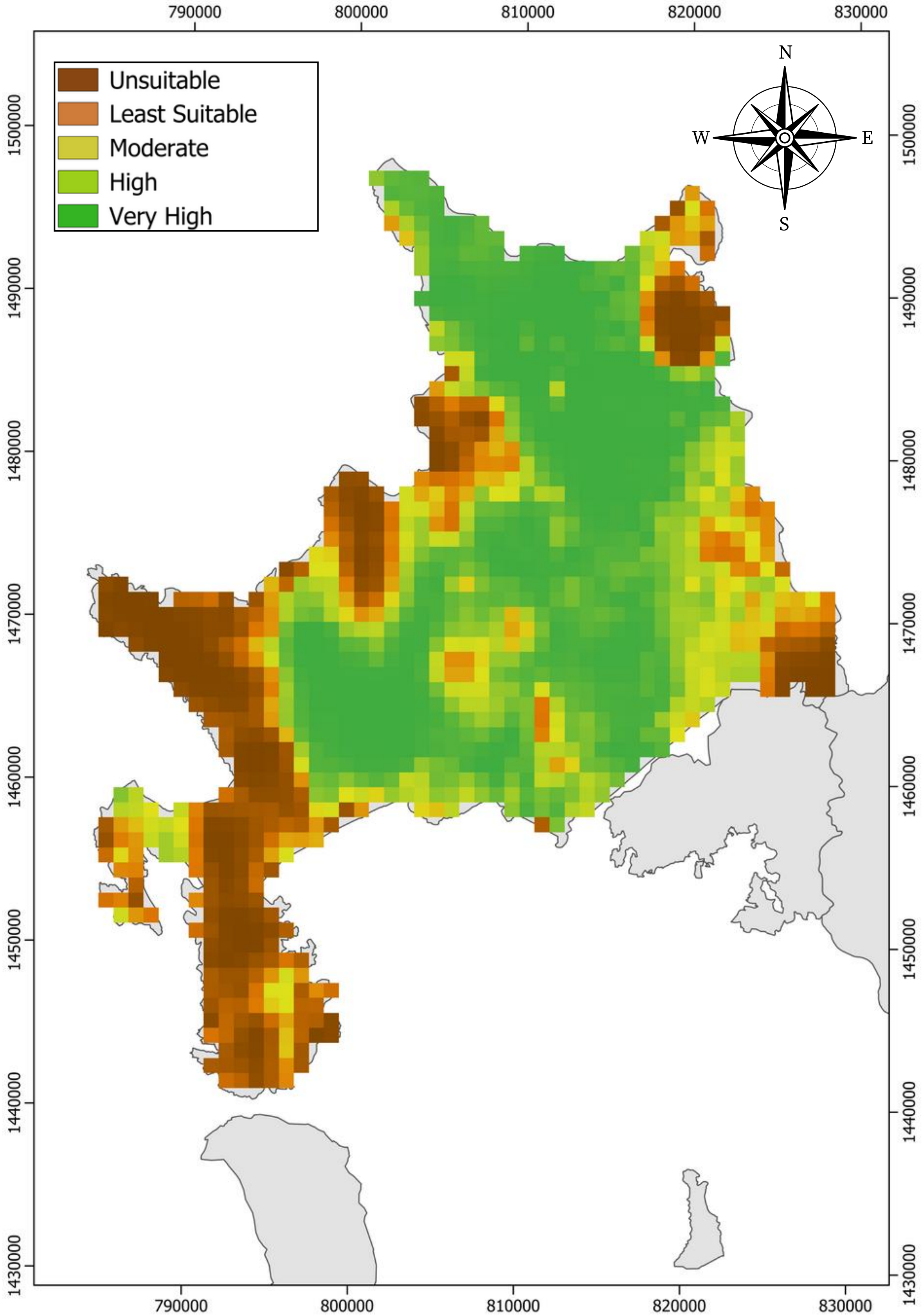
MaxEnt model for species distribution to predict habitat suitability of an Asian elephant



Study area 1772.81 km²

- unsuitable 566.41 km² (31.96% of total area)
- least suitability 349.09 (19.70% of total area)
- moderate suitability 325.13 (18.34% of total area)
- high suitability 349.94 (19.75% of total area)
- **very high suitability 182.24** (10.27% of total area)

Results and discussion



MaxEnt model for species distribution to predict habitat suitability of an Asian elephant

Study area 1232.11 km²

- unsuitable 256.64 km² (20.83% of total area)
- least suitability 176.25 (14.03% of total area)
- moderate suitability 170.10 (13.80% of total area)
- high suitability 262.27 (21.28% of total area)
- **very high suitability 366.85** (29.77% of total area)

Results and discussion

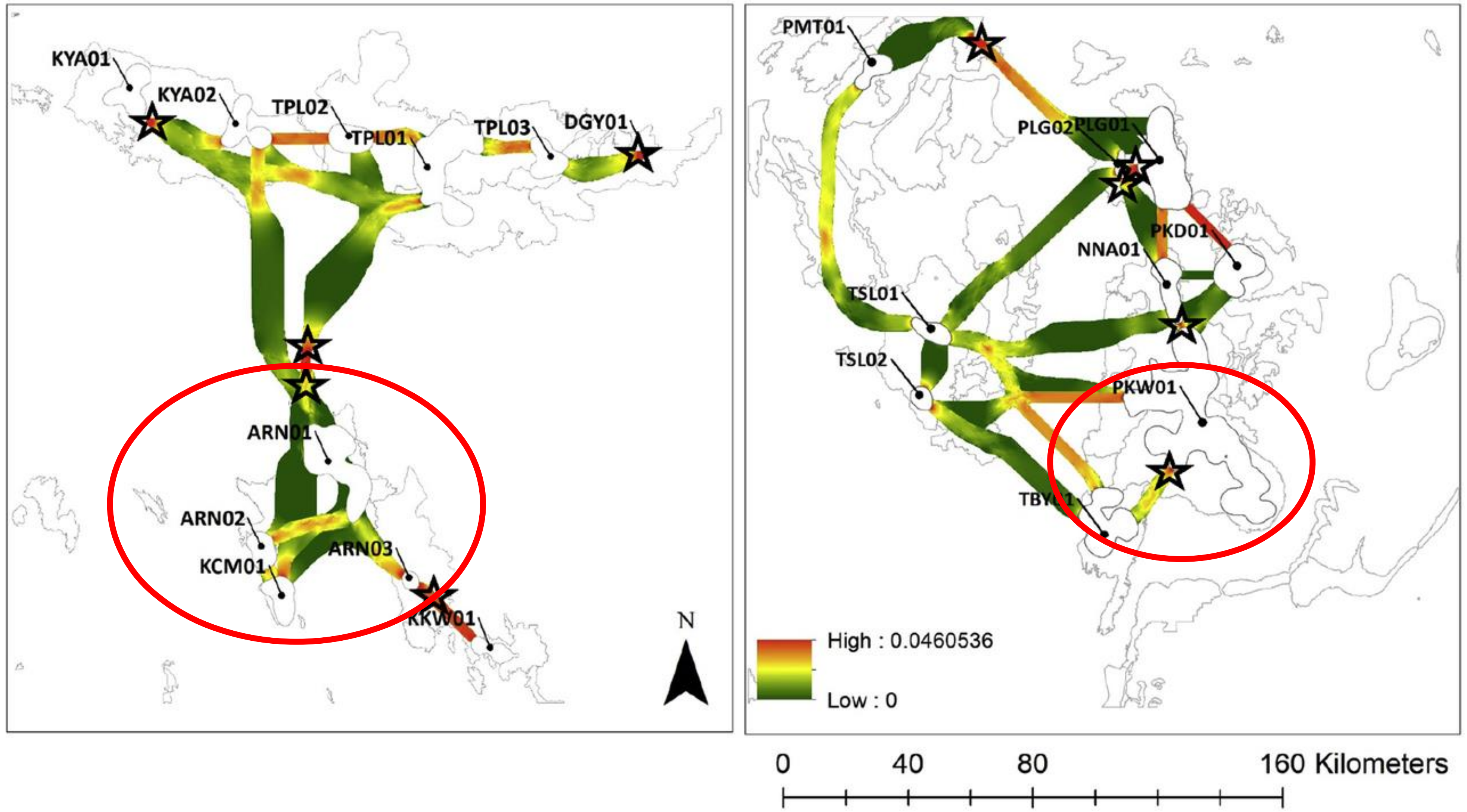


Figure Accumulative Current Flow Map Highlighting Critical Bottlenecks (Red) and Elephant Corridors (Green) between Core Habitats.

Suksavate et al. (2019)

- To address the fragmentation shown in ARN, our results, aligning with Suksavate et al. (2019), clearly indicate the **critical paths for elephant movement.**

- This visualization confirms the **importance of creating corridors between habitats** to support genetic exchange, which is vital for improving **haplotype and nucleotide diversity** within these isolated populations.

Main conclusion

- The contrast between the **wide, continuous forest of PK** and the r**oad-segmented landscape of ARN** (cut by nearly 100 km of major road) confirms fragmentation as the leading cause of population decline and genetic isolation.

- The physical barrier posed by the road in ARN has resulted in the separation of elephant family lines. This directly threatens **haplotype and nucleotide diversity**, which is a critical factor for the long-term survival of the population.

- The strong presence of **cultivated farmland and agricultural water sources** at the forest periphery acts as a pull factor, causing some elephants to **permanently refuse re-entry** to the main forest, intensifying human-elephant conflict (HEC).

Conservation Implications

- Prevent the irreversible loss of genetic diversity by safeguarding existing intact core habitats.

- Restore gene flow across fragmented landscapes to mitigate the risks of inbreeding and low genetic diversity.

- Stabilize elephant populations within protected areas to ensure that natural movement patterns for foraging and breeding are safe and unhindered.

- Ensure all conservation investments are strategically targeted for maximum genetic benefit.

Acknowledgments

Thank you for your attention

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เขตรักษาพันธุ์สัตว์ป่าภูเขียว
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เขตรักษาพันธุ์สัตว์ป่าเขาอ่างฤาไน
KHAO ANG RUENAI WILDLIFE SANCTUARY

Assoc.Prof.Dr. Prateep Duengkae
Department of Forest Biology,
Faculty of Forestry, Kasetsart University

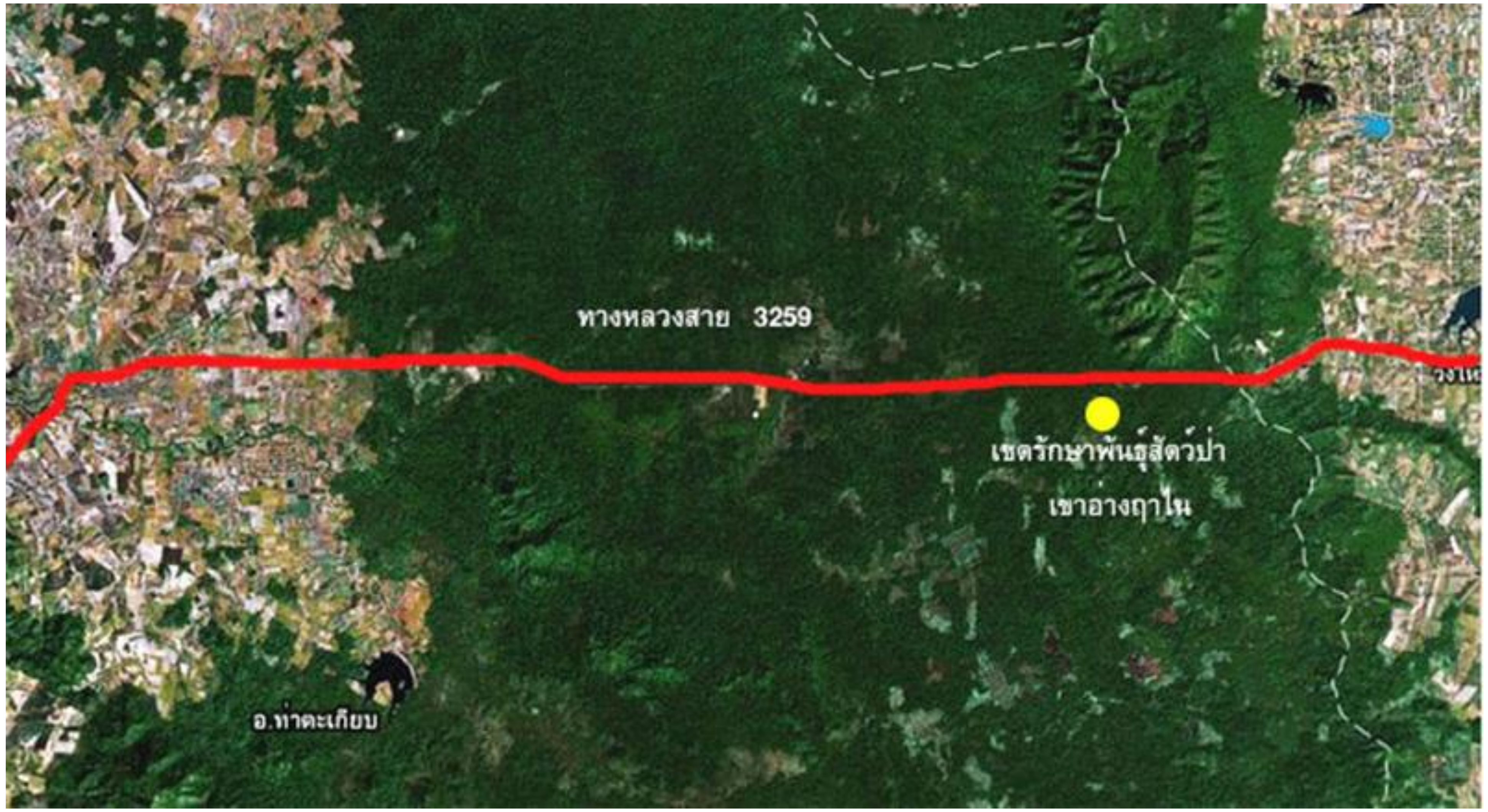
Assoc.Prof.Dr. Kornsorn Srikulnath
Department of Genetics,
Faculty of Science, Kasetsart University

Dr. Worapong Singchat
Department of Genetics,
Faculty of Science, Kasetsart University

Asst.Prof.Dr. Warong Suksavate
Department of Forest Biology,
Faculty of Forestry, Kasetsart University

Dr. Thitipong Panthum
Department of Genetics,
Faculty of Science, Kasetsart University

https://www.oneearth.org/species-of-the-week-asian-elephant/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