

NONINVASIVE GENETIC MONITORING OF TIGER POPULATIONS IN THE PROTECTED AREAS OF INDIA

Case studies from Wildlife Genetics Laboratory, Aaranyak

Udayan Borthakur
Programme Head

Wildlife Genetics Programme

Aaranyak

A society for biodiversity conservation in Northeast India working since 1989

Mission

To foster conservation of biodiversity in Northeast India through research, environmental education, capacity building, legal advocacy and policy reform to usher in a new era of ecological security

Wildlife Genetics Programme (WGP) of Aaranyak

Aaranyak has established Wildlife Genetics Laboratory in the year 2008

- To build up a reference genetic database of the rich biodiversity of North East India.
- To standardize DNA based techniques for the identification of species, individuals and gender from various wildlife samples.
- To undertake genetic research in prioritized areas to provide answers to the questions of pressing wildlife management needs.

Facilities WGP have developed:

- Sample storage, DNA extraction and electrophoresis facility
- Physically separated faecal DNA handling facility
- PCR facility
- Genetic data analysis facility

Key areas of work

A. Tiger Genetics Projects

- To assist protected area managers in population monitoring, especially in low density Tiger Reserves

B. Asian Rhino Genetics Projects

- Population genetic monitoring of Greater One-horned Rhino in Assam and West Bengal
- Monitoring factors influencing dispersal in the Brahmaputra Valley, Assam
- Estimation of population size of critically endangered Javan rhinos in Indonesia

C. Avifauna projects

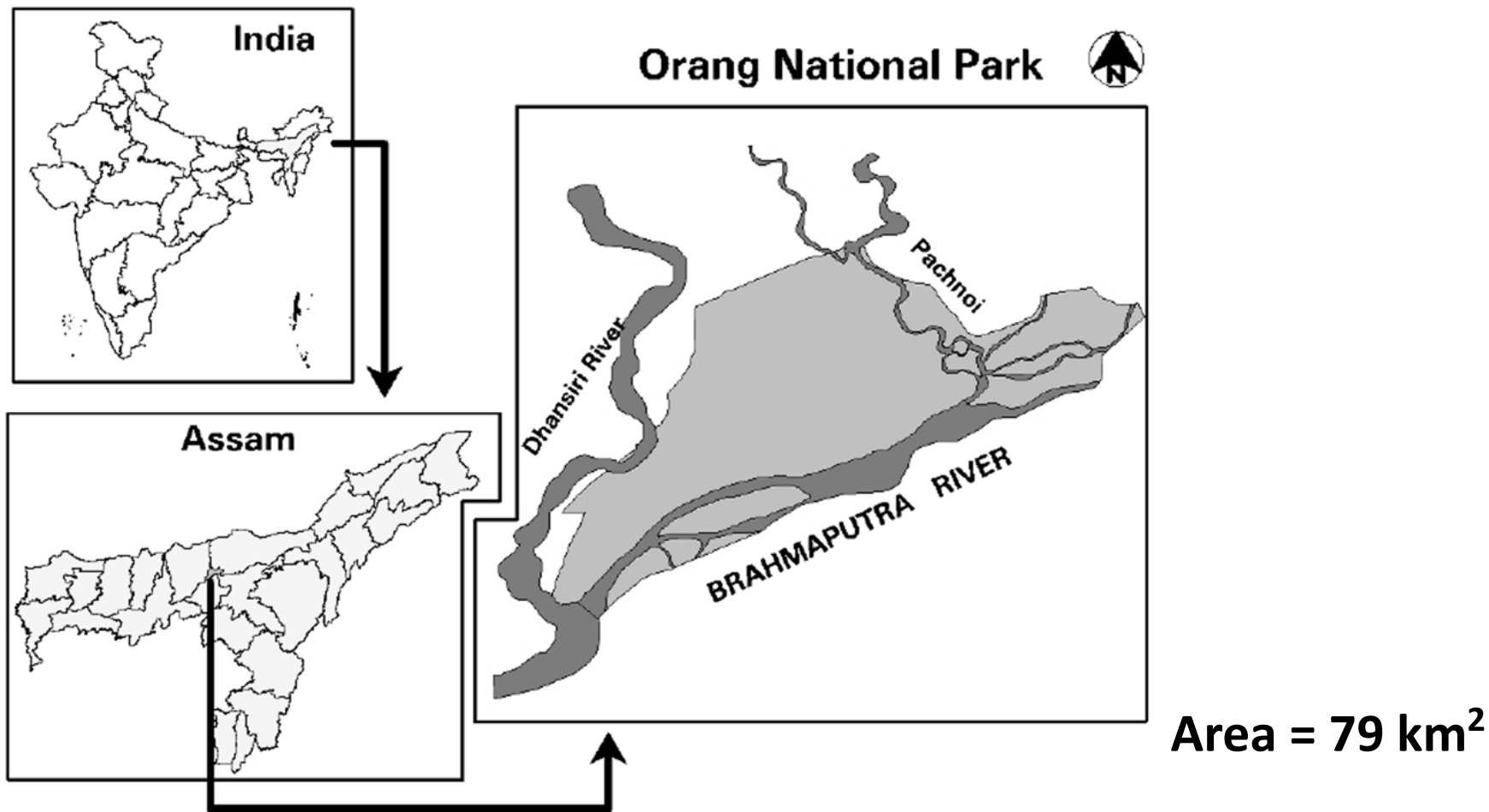
- Molecular phylogeny of Himalayan Avifauna (in collaboration with Univ. of Chicago)
- Survey of Owl species presence and distribution in north-east India



Tiger Genetics Initiatives/ Projects at Aaranyak

1. Noninvasive genetic monitoring of tigers in Orang National Park, Assam
2. Estimation of tiger population size in Buxa Tiger Reserve, West Bengal
3. Estimation of tiger population size in Palamau Tiger Reserve, Jharkhand
4. Noninvasive genetic identity based monitoring of tigers in Manas Tiger Reserve, Assam
5. Confirmation of tiger presence in some of the Tiger Reserves in northeast India

Noninvasive genetic monitoring of tigers in Orang National Park, Assam



Noninvasive genetic monitoring of tigers in Orang National Park, Assam

Collection of scats from the study area and DNA extraction

Identification of genuine tiger scats using mitochondrial markers developed by Mukherjee *et al.* 2007

Selection of minimum number of polymorphic microsatellite markers for reliable individual identification

Generation of multilocus microsatellite genotypes of tiger scats and identification of individual tigers

Estimation of error in the process of microsatellite genotyping

Gender identification of tiger scats

Obtaining minimum number of individuals and sex ratio of tigers in an area

Selection of genetic markers

Individual identification


Three basic criteria:

1. Level of polymorphism (*marker selection statistics*)
2. Mean PCR success from scat DNA samples
3. Multiplexing compatibilities


Selection of genetic markers

Individual identification

38 microsatellite loci screened on 12 field collected scat samples and 5 reference tiger tissue samples



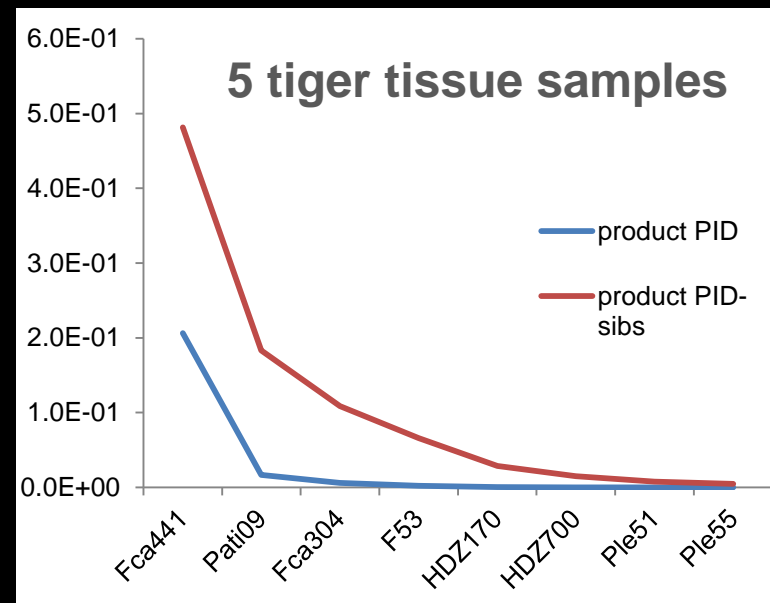
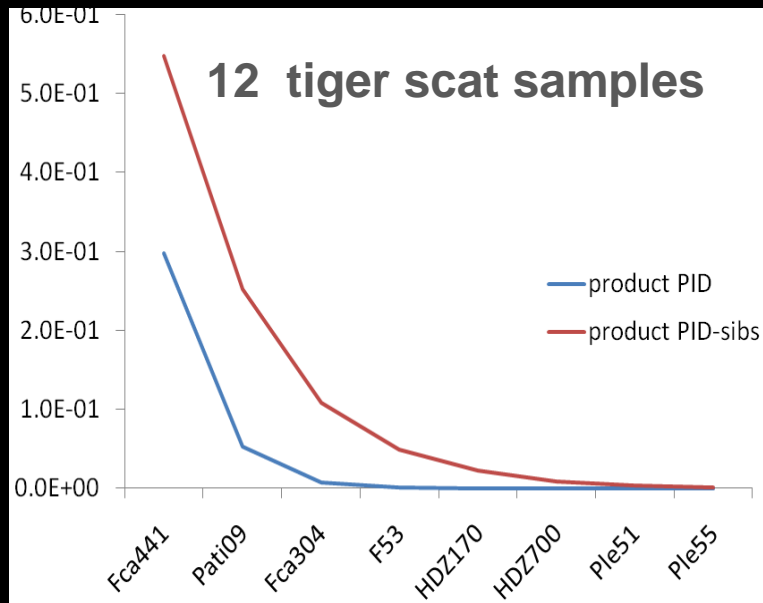
The level of polymorphism of all the microsatellites and rate of genotyping errors were quantified



8 polymorphic microsatellite loci were selected for reliable individual identification of tigers from scats

Selection of genetic markers

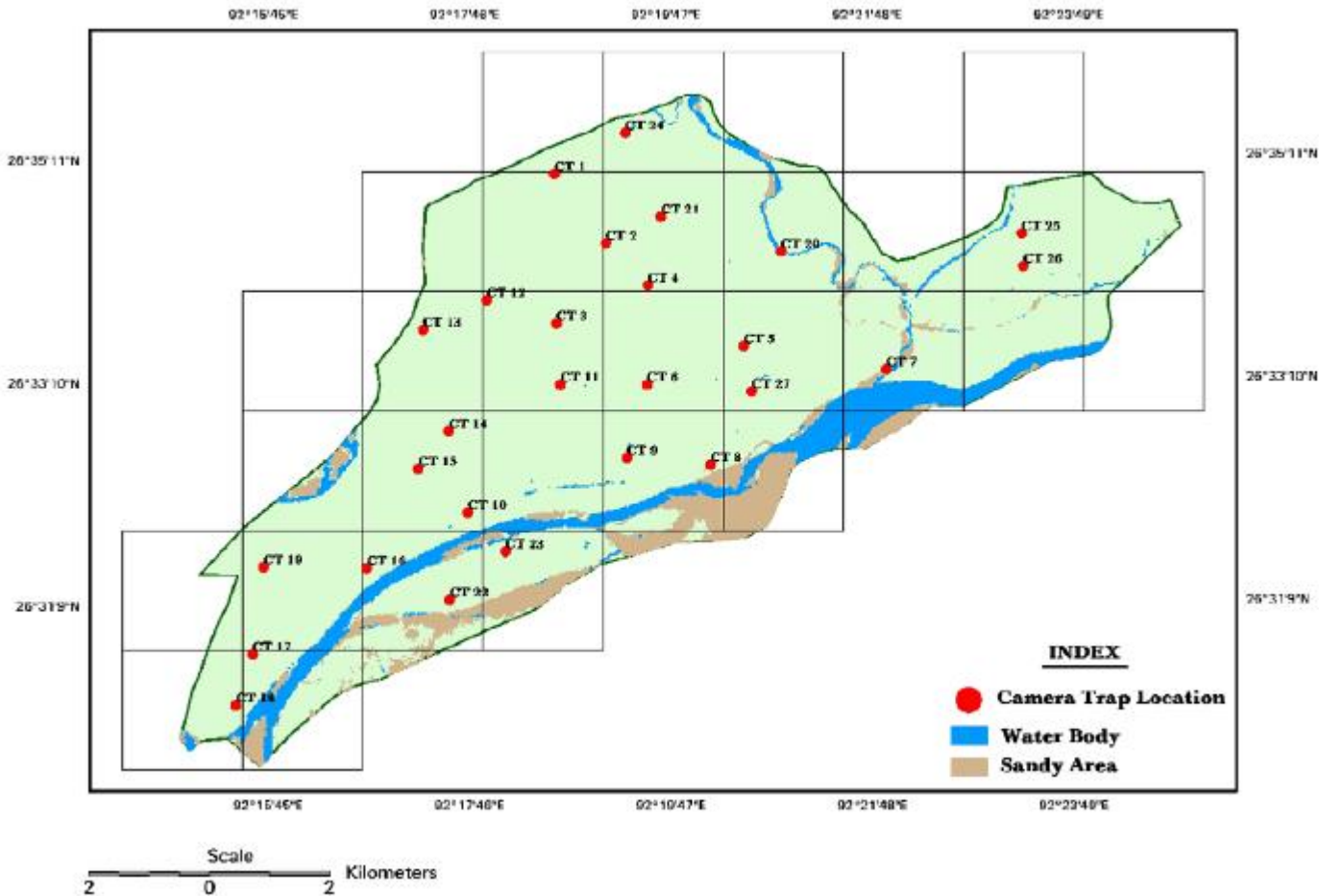
Cumulative **PID** of 6.29×10^{-6} and **PID-sibs** of 4.71×10^{-3} for the 8 selected markers



Comparison of Product PID and PID-sibs value of 8 selected polymorphic microsatellite loci

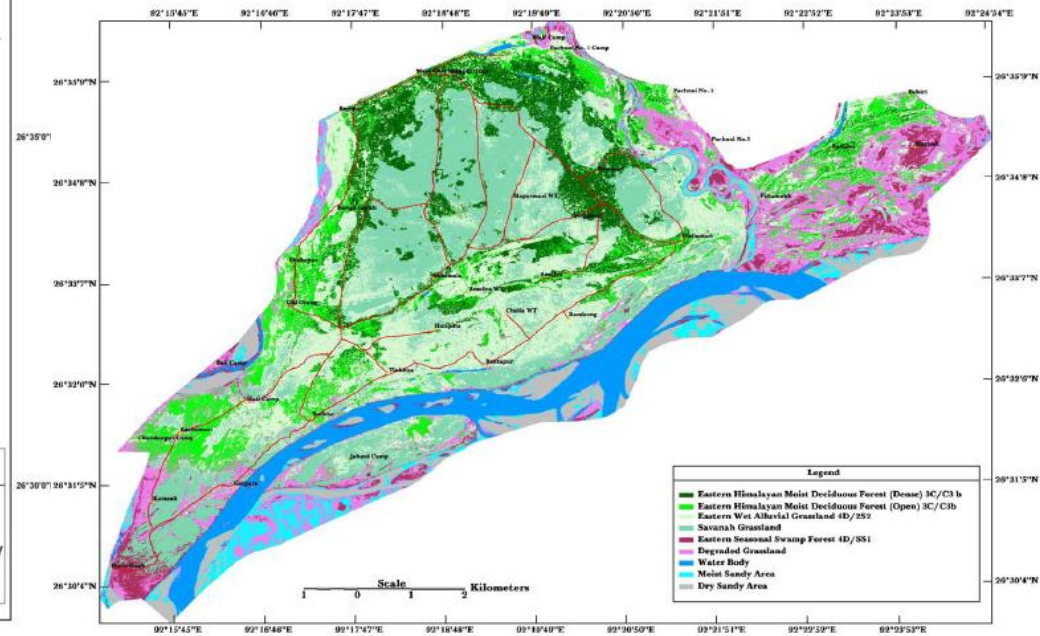
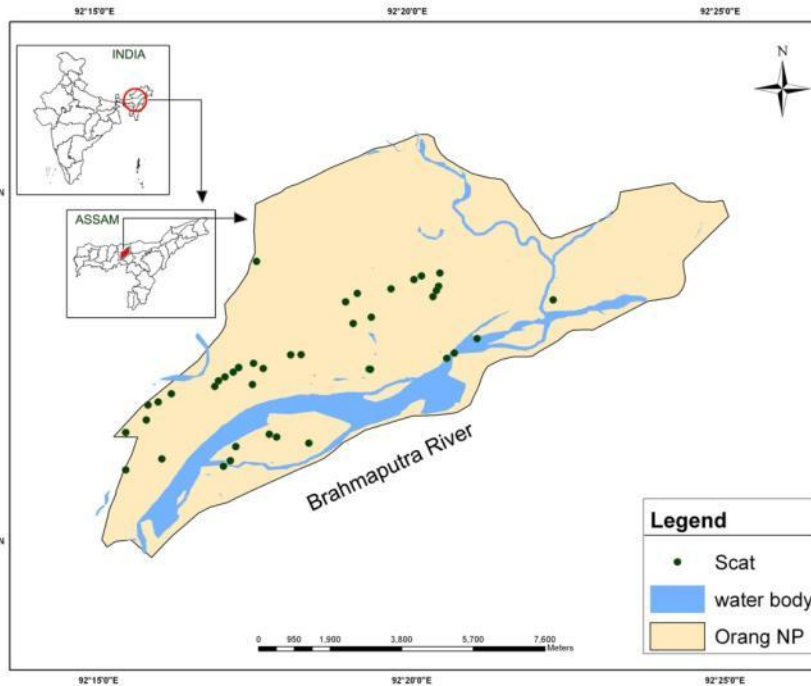
Results from Orang National Park

- 57 scats collected by TRCI team during early 2009



Results from Orang National Park

- 48 scats were genetically identified as of tiger

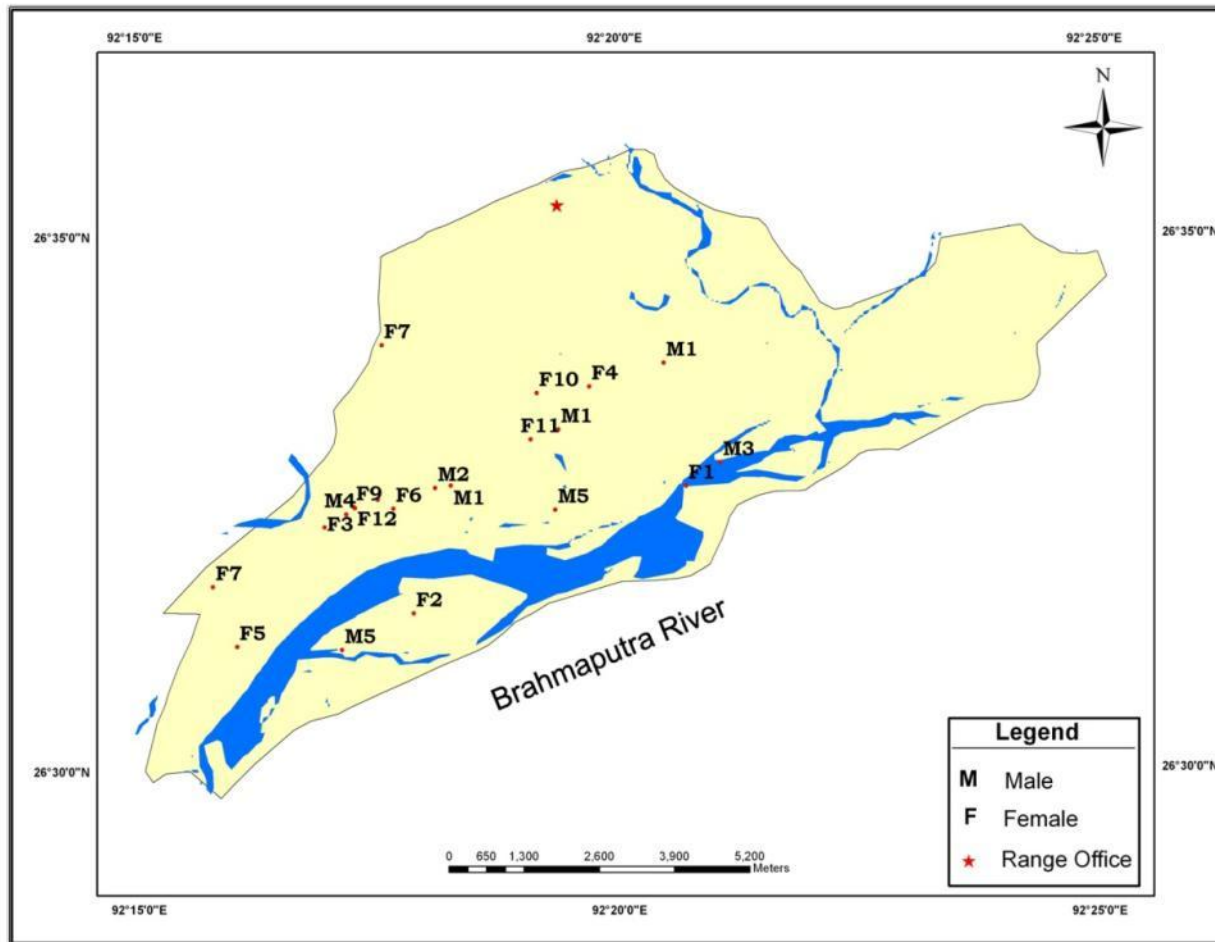


Results from Orang National Park

- Microsatellite genotypes could be obtained for 34 tiger scats
- Applying stringent quality criteria (Miquel *et al.* 2006) allowed accepting data for 24 scats

Results from Orang National Park

- 17 individual tigers in Orang National Park during 2008
- 12 female and 5 male



Results from Orang National Park

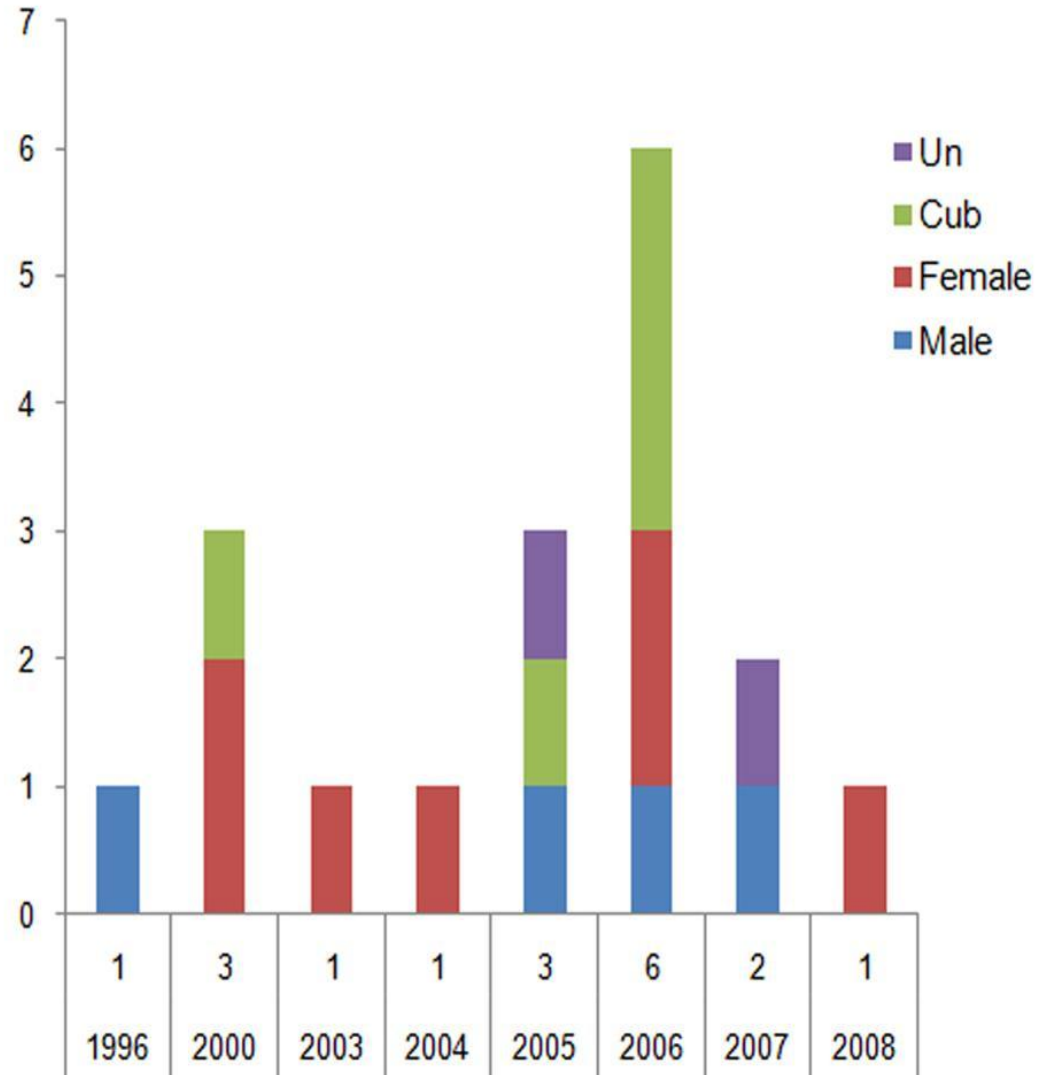
- No additional individual tiger in scats that did not qualify in our data quality criteria
- The rate of genotyping error per sample was 3.2%

Why is monitoring tigers in Orang so interesting?



Why is monitoring tigers in Orang so interesting?

- 19 tigers in 2000 Forest Department census
- 19 reported tiger deaths during 2000-2009
- 14+3.6 tigers in 2009 Aaranyak camera trapping estimate
- 17 tigers in DNA analysis of 2009 scats



Recorded mortality of tigers in Orang by the Forest Department

Why is monitoring tigers in Orang so interesting?

Orang is maintaining a steady number of tigers for past one decade. Is it because:

- (i) Orang has a healthy breeding population?
- (ii) A source-sink dynamics, i.e., immigration from nearby tiger populations such as Kaziranga?
- (iii) Or both?

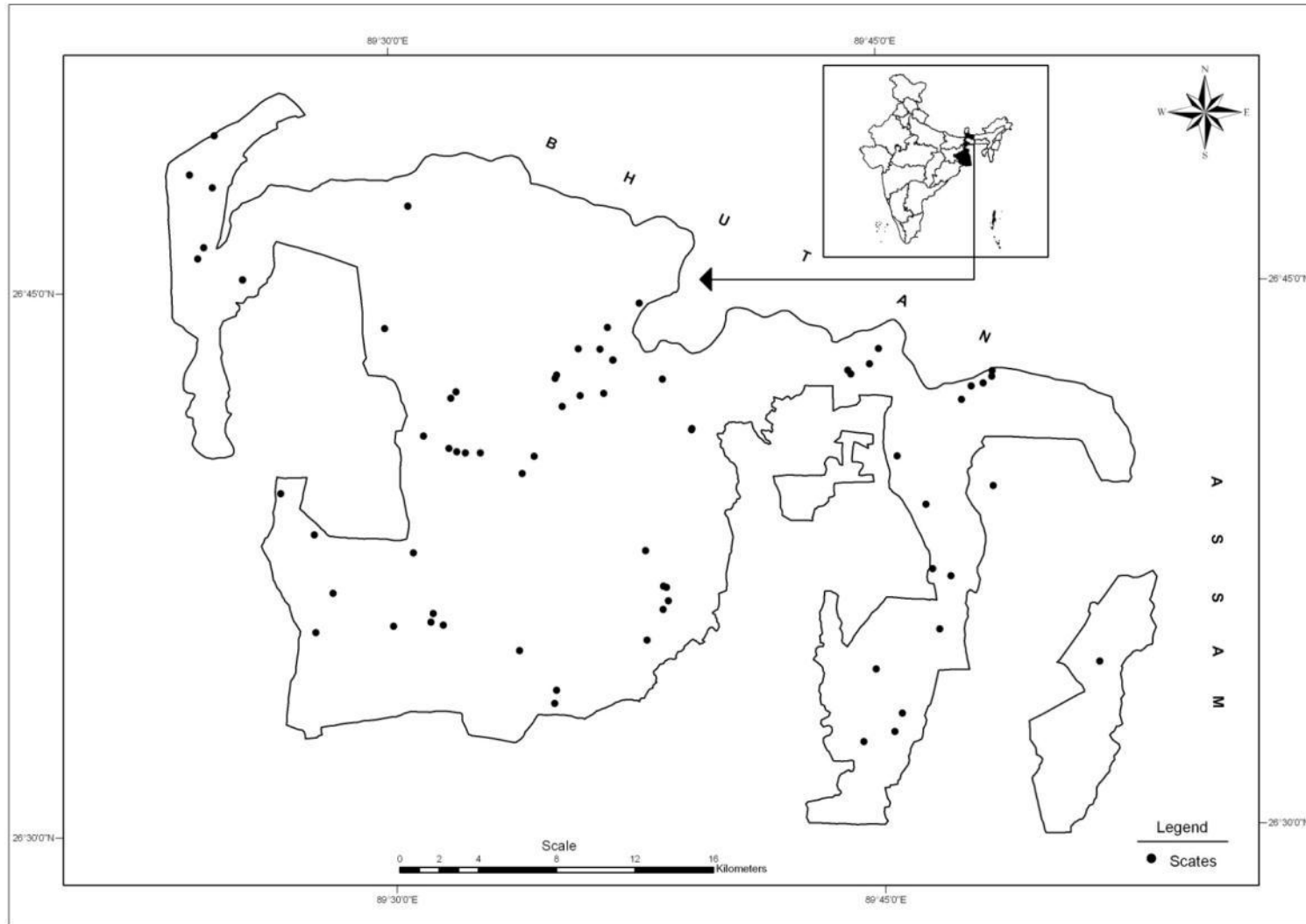
Noninvasive genetic identification of individual tigers in Buxa Tiger Reserve, West Bengal (2010)

- Work undertaken upon request and financial support from West Bengal Forest Department
- 38 genuine tigers scats were obtained from a total of 72 scats handed over by the BTR authority
- 7 microsatellite loci were used to genotype all the 38 tiger scats
- After Assigning quality criteria (Miquel *et al.* 2006), data for 27 scats selected for final analysis

Results from Buxa 2010 analysis

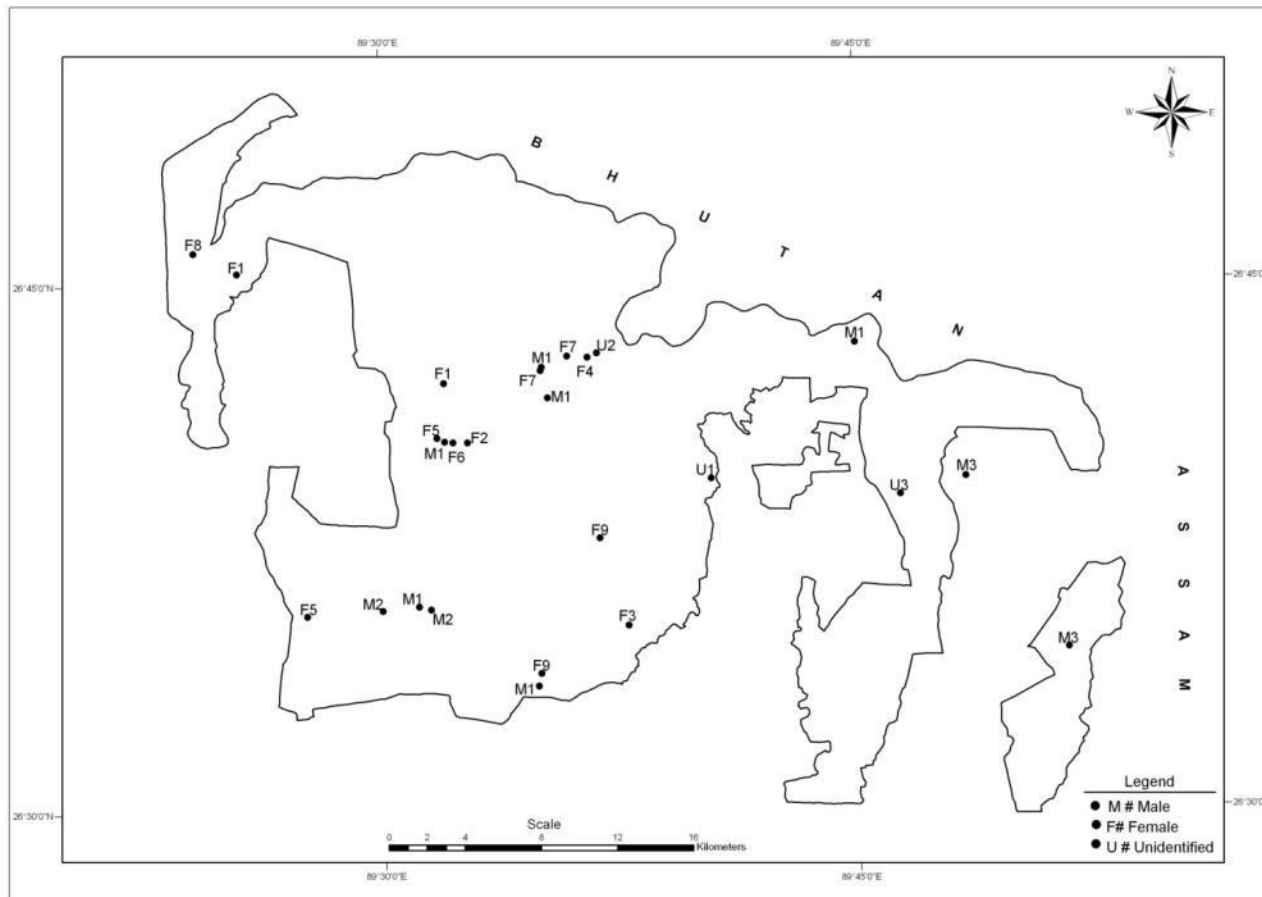
N = 72

Area = 761 km²



Results from Buxa 2010 analysis

- 15 individual tigers were identified from 27 scats
- Product P_{ID} of 8.21×10^{-6} and P_{ID} -sibs of 8.32×10^{-3}
- 9 female, 3 male and 3 unidentified gender



Noninvasive genetic identification of individual tigers in Palamau Tiger Reserve, Jharkhand (2011)

- Work undertaken upon request and financial support from Palamau Tiger Reserve Field Directorate
- Scats collected by the Field Directorate during April to July 2011
- 38 out of the 45 collected scats were used for analysis

Results from Palamau 2011 analysis

- Genetic analysis confirmed 8 scats as tiger, 13 as non-tiger and rest (45%) failed to produce any results
- Genotyping results using 7 polymorphic microsatellite loci shows the presence of 6 individual tigers

Confirmation of tiger presence some of the Tiger Reserves in northeast India

Confirmation of tiger presence through genetic species identification

1. Dampa Tiger Reserve, Mizoram in 2010
2. Namdapha Tiger Reserve, Arunachal Pradesh in 2011

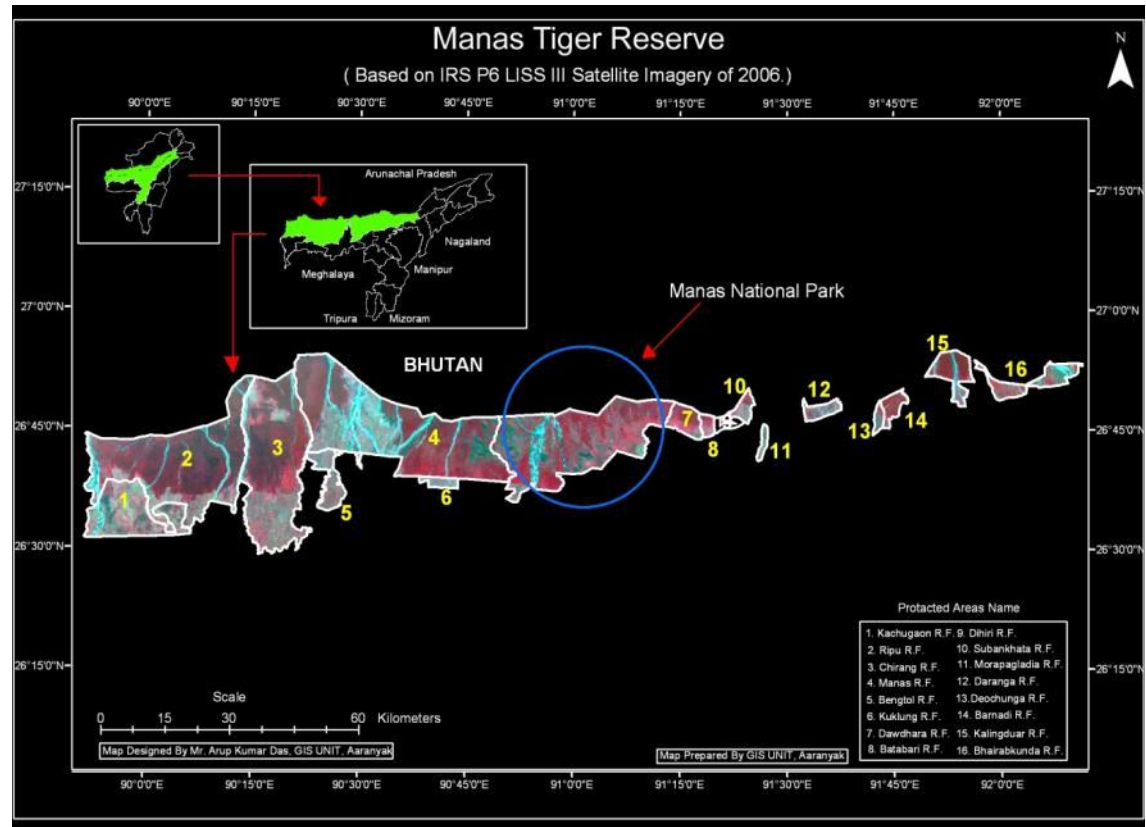
Noninvasive genetic identity based monitoring of tigers in Manas Tiger Reserve, Assam

Investigators: Udayan Borthakur

M Firoz Ahmed

Funding Agency: USFWS

Area = 2837 km²



Noninvasive Genetic monitoring of Tigers in Tiger Reserves of Arunachal Pradesh

Investigators: Udayan Borthakur
Bibhab Kumar Talukdar
Pranjit Kumar Sarma

Funding Agency: National Tiger Conservation Authority, Gol

1. Training frontline Forest staff for genetic sampling
2. Confirmation of tiger presence
3. Identification of individual tigers and estimation of population size

Acknowledgements

- *Ministry of Environment and Forests, Government of India*
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- *IRF*
- *IFS*
- *Rufford Small Grants*
- *MBZ-SCF*
- *ARP, Australia*



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