POPULATION GENETIC STRUCTURE AND DIFFERENTIATION OF MONITOR LIZARD (*VARANUS BENGALENSIS)* FOR CONSERVATION AND ILLEGAL TRADE MONITORING

A Thesis

Submitted by

KUMUDANI BALA GAUTAM

for the award of the degree of

DOCTOR OF PHILOSOPHY

IN

WILDLIFE SCIENCE

Under the guidance of

Dr. ABHIJIT DAS

WILDLIFE INSTITUTE OF INDIA

Dehradun, Uttarakhand, India

Saurashtra University
Rajkot, Gujarat, India

August 2024

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Wildlife Institute of India

DECLARATION

I hereby declare that the work conducted under the thesis entitled "**Population Genetic Structure and Differentiation of Monitor lizard (*Varanus bengalensis)* for Conservation and Illegal Trade Monitoring**", is a record of original research work, done by me and subsequently submitted for the award of the degree of doctor of Philosophy in Wildlife Science to Saurashtra University, Rajkot. This research work has been carried out under the guidance and supervision of Dr. Abhijit Das, Scientist-E and co-supervision of Dr. Sandeep Kumar Gupta, Scientist-F Wildlife Institute of India, Dehardun. The work has not formed the basis for the award of any other degree, diploma or any other qualification. I also declare that the thesis embodies my own work, analysis, observation and understanding and the particulars given in it are true to the best of my knowledge.

(Ms. Kumudani Bala Gautam)
Place: Dehradun
Date: 29th August, 2024

02.09.24.

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CERTIFICATE

This is to certify that the thesis by **Ms. Kumudani Bala Gautam** entitled "**Population Genetic Structure and Differentiation of Monitor lizard (*Varanus bengalensis)* for Conservation and Illegal Trade Monitoring**" is an original and independent research work submitted to the **Saurashtra University, Rajkot (Gujarat)**, for the award of the degree of **Doctor of Philosophy in Wildlife Science**.

Ms. Kumudani Bala Gautam has put more than six semesters of research work embodied in this thesis under my guidance and supervision. The work presented in this thesis has not been submitted to any other University or Institute for the award of any degree, diploma or distinction.

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I certify that the research work was appreciated by all who were present, and the comments made by the faculty and researchers have been appropriately included in the thesis.

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Paper/Submission ID	2262964
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Total Pages, Total Words	93, 19627
Document type	Dissertation

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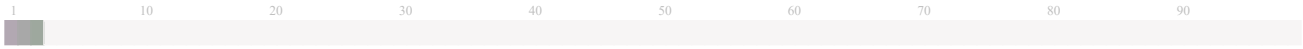

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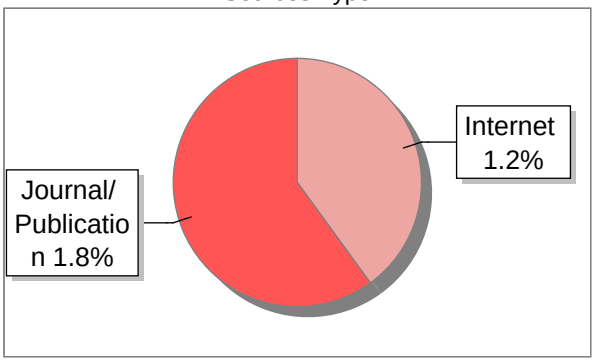

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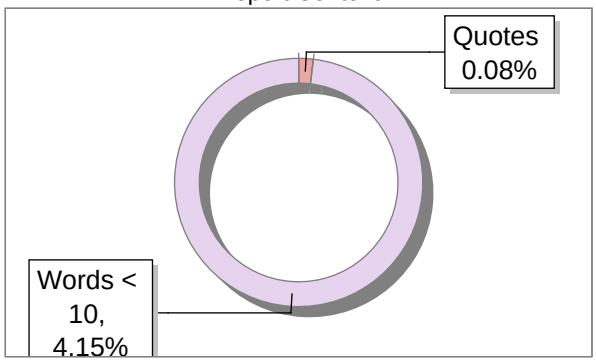

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ACKNOWLEDGEMENTS

This research marks the culmination of my academic journey, and I am deeply indebted to many individuals and institutions that have contributed to this achievement.

First and foremost, I would like to express my deepest gratitude to Dr. Ajit Kumar, Dr. Bhim Singh, Subhashree Sahoo, Vishal Kumar Prasad and Diksha Kumari whose guidance, insight, love and unwavering support have been instrumental in the completion of this work. Your patience towards me and encouragement have been invaluable throughout this challenging process.

I am deeply grateful to my therapist Manasi Karanjkar who helped me to understand my sorrows, anxiety, confusion, sadness and many emotions so that I could steer my life ship without drowning in the ocean of this cruel society.

My deepest appreciation goes to my imperfect yet perfect family, particularly my parents (Mrs. Kiran Bala and Mr. Vishwanath), whose love, encouragement, and belief in me have been a constant source of motivation. Thanks to my stupid yet loveable siblings (Promodani and Hrydyungum), thank you for your patience (hahaha), understanding, and unconditional support during this long journey.

I am also grateful to Dr. Neeraj Mahar, Dr. Ninad Mungi, Dr. Ankita Bhattacharya, Dr. Sameeha Pathan, Amir Lone, Niazul Khan, Dr. Pratyush P. Mohapatra, Dr. V. P. Uniyal, Rajat Rastogi, Dr. Shivam Shrotriya, Dr. Anukul Nath and Dr. Christophe Dufresnes for their valuable feedback and constructive criticism, which have significantly enhanced the quality of my research.

A special thanks to Sapteswar Naik, Aradhana Rai, Ajay Maurya, Rachit Shukla, Dr. Mohd. Arman, Pratiksha Omary, Khalida Nasrin, Garima Singh, Pooja Chaudhary, Devesh Mishra, Deven Mehta, Manish Singanjude, Shagun Thakur, Amarjeet Kaur, Dr. Rahul Kumar, Jignasu Dolia, Himangshu Borah, Gaurav Sirola, Hariandra Baraiya, Ashish Jangid, Dhruv Jain, Naitik Patel, Bitupan Boruah, Shashank Nagarale, Mansi Kumar, Alex Jacob, Divya Parashar, Kuldeep, Anuja Mittal, Swati Nawani, Vivekanand, Sachin Suryavanshi and Amrit Balan whose

camaraderie and intellectual exchange made this journey more enjoyable and enriching. Your friendship and support have been a source of strength.

I am grateful to Deepika Mishra, Late Dilbahadur Thapa, Ms. Rajkumari Thapa, Deepak Bhaiya, Maneesha bhabhi, Narendra Bhaiya, Pooja bhabhi, Rahul Bhaiya, Nikhil Bhaiya, Kavita didi, Daleep jija ji, you all supported me in my difficult time as a family. All the cute munchkins Ayank, Ahana, Adhiraj, Mrunmay were stress busters during this journey.

I would like to express my special thanks to Late Dr. A. K. Kapoor (Former HOD, Dept. of Anthropology, University of Delhi) who always encouraged and appreciated me to become a vocal person. It is unfortunate for me to write my gratitude when he is not here with us. I will miss you Sir.

I would like to acknowledge my Supervisor Dr. Abhijit Das, Co-supervisor Dr. S. K. Gupta for providing me the platform for this research work. For providing me an opportunity to work in this prestigious Institute, I would like to thank current Director V. R. Tiwari and Dean Dr. Ruchi Badola as well as former Directors and Deans Dr. V. B. Mathur, Dr. G. S. Rawat and Dr. Y. V. Jhala. I am grateful to the Rufford Foundation for providing the resources and financial support necessary to carry out this research.

I sincerely credit Mr. A. Madhanraj for kind support during my sample preparation and laboratory work and technical assistance. The atmosphere of the Wildlife Forensic and Conservation Genetic Cell created by Dr. Prabaker Yadav, Preeti Singh, Mr. Ashok, Mr. Sanjay, Mr. Mahavir, Mr. Anil, Mr. Manoj was commendable for smooth working.

This work would not be feasible without the staff of state forest departments and NGOs, it is a long list and I am glad to know these many people who helped directly or indirectly.

Haryana forest department: DFO Shiv Rawat (Kalesar), Insp. Rajesh Kumar Chahal (Kalesar), Paramjeet (Sirsa), Parvez Ahmed (Kalesar), Sukhwinder Forester (Sirsa).

Uttar Pradesh forest department: A.K Dwivedi (DFO Kanpur Dehat), A. K. Khan (Mailani Buffer Zone), A.K. Kashyap (DFO Amebedkar nagar), A.K. Siddiqui (RO Abdullah Ganj, Bahraich), Akashdeep Padhawan (DFO Katarniyaghat), Anantram Forester (Rupaidiha,

Bahraich), Anil Kumar Patel (DFO, North Kheri), Arvind Yadav (DFO Kanpur), Arwind (ACF Kanpur Zoo), Ashish (Forester Pratapgarh), Ashok kumar Yadav (RO Pratapgarh), Atul Kumar Gupta (Forester Barabanki), Atul Kant Shukla (DFO, Etawah), B.N Singh (Biologist Lion safari Etawah), D.P Singh (RFO Ayodhya), Varun (DFO Pratapgarh), Divya (DFO Faizabad), D.K. Singh (SDO Bahraich), Dr. Daya Shankar (Vet Dudhwa TR), Dr. Mohd. Nasir (Vet Kanpur Zoo), Jai Ram (FG, Gola Range South Kheri), MohanRam Chaulta (Forester Dudhwa TR), Indrabhaan Sonkar (Tanda range, Ambedkar Nagar), K. P. Singh (RO Mailani), Manish Singh (DFO Bahraich), Manoj Sonkar (DFO Dudhwa TR), Mithilesh (FG, Katherniaghat), Nagendra Pandey (Forester South Kheri), Panna Lal (RFO Ayodhya), R.K. Singh (CCF Lucknow), R.S. Negi (Staff in PCCF office), Rajiv Kumar Sharma (Mailani Range), Ram Kumar (RO, Katerniaghat), Dr. Ravi (DFO Awadh), Rishabh Khanna (Kanpur Zoo), Ashok Kumar (RO, Ayodhya), Rustam PArvej (DFO Barabanki), Sameer Kumar (DFO South Kheri), Sanjay Pathak (Dir. Dudhwa TR), Satayjit Singh (Forester, Bahraich), S.P. Tripathi (Dy. Ranger Mailani), Taufiq S(DO Dudhwa TR), Vinay Rana (Forester Rupaidiha Bahraich), Vinod (Forester Pratapgarh).

Uttarakhand forest department: Bijulal (DFO nainital), Sudarsan Pawar (QRT), Shankar Sati (FG, Terai West), Lalit (staff to PCCF).

Bihar: Amita (ACF, VTR), Neeraj Narayan (DFO VTR), Prabhat Gupta (PCCF), Saurabh Verma (Biologist VTR).

NGOs: Anil Gandass, Kushi Mohd. Qureshi (Team Snakeman Sirsa), Suraj (Suraksha trust, Sirsa), Rajeev Chauhan (SCoN), Sanjeev Chauhan (SCoN), Aditya Tiwari (Paryavaran).

MOEF&CC: Aditya Bisht, Erashree Gautam, Shashi Kumar

All the support above people has provided in the field is priceless.

Finally, I would like to dedicate this dissertation to all the **females** around the globe, whose stories and strength have been an inspiration and a driving force behind my pursuit of knowledge.

Thank you all for being part of this journey.

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Abbreviation

ASVR	Assam *Varanus* Reference
BI	Bayesian Inference
BRVR	Bihar *Varanus* Reference
CI	Confidence Interval
CITES	Convention on International Trade in Endangered Species
COI	Cytochrome c oxidase I
CYT *b*	Cytochrome *b*
DAPC	Discriminant Analysis of Principal Components
DNA	Deoxyribonucleic acid
ESU	Evolutionary Significant Unit
FINS	Forensically Informative Nucleotide Sequencing
GJVR	Gujarat *Varanus* Reference
HRVR	Haryana *Varanus* Reference
IUCN	International Union for Conservation of Nature
MAVR	Maharashtra *Varanus* Reference
MOEF&CC	Ministry of Environment, Forest and Climate Change
MPVR	Madhya Pradesh *Varanus* Reference
MYA	Million Years Ago
NCBI	National Centre for Biotechnology Information
ND1	NADH dehydrogenase subunit 1
ND2	NADH dehydrogenase subunit 2
ND4	NADH dehydrogenase subunit 4
ND5	NADH dehydrogenase subunit 5
NP	National Park
PCG	Protein Coding gene
PCR	Polymerase chain reaction
RNA	Ribonucleic acid
ROM	Remainder of Mainland
SNP	Single Nucleotide Polymorphism
STR	Short Tandem Repeats
TAL	Terai Arc Landscape
UKVR	Uttarakhand *Varanus* Reference
UPVR	Uttar Pradesh *Varanus* Reference
VF	*Varanus flavescens*
VG	*Varanus griseus*
VH	*Varanus* Hemipenis
WCCB	Wildlife Crime Control Bureau
WFCGC	Wildlife Forensics and Conservation Genetic Cell
WII	Wildlife Institute of India
WLS	Wildlife Sanctuary
WOE	Whole Organism Equivalents
WPA	WildLife (Protection) Amendment Act, 2022

Chapter-1

INTRODUCTION

Background

The Monitor lizards, belongs to family Varanidae and categorised under the genus *Varanus* are widely distributed throughout the Old-World tropics and subtropics (Auliya and Koch, 2020). The family Varanidae, represented by a single genus *Varanus* Merrem, comprises 88 extant species (Uetz et al., 2024). The genus *Varanus* is further recognized by 11 subgenera namely, *Empagusia*, *Euprepiosaurus*, *Hapturosaurus*, *Odatria*, *Papusaurus*, *Philippinosaurus*, *Polydaedalus*, *Psammosaurus*, *Solomonsaurus*, *Soterosaurus* and *Varanus* (Ziegler and Böhme, 1997). Although the subgeneric classification by Mertens (1942, 1963) was merely phenetic, the proposed subgeneric classification by Ziegler and Böhme (1997) remains largely reflective of modern views on varanid phylogeny, though some subgeneric classifications are still debated. With the advancement of morphological and molecular taxonomic studies, 25 species in the genus are described in the twenty-first century (Gaulke and Curio, 2001; Böhme and Jacobs, 2001; Böhme et al., 2002; Jacobs, 2003; Bohme and Ziegler, 2005; Eidenmüller and Wicker, 2005; Aplin et al., 2006; Ziegler et al., 2007; Koch et al., 2009; Welton et al., 2010a; Weijola and Sweet, 2010; Koch et al., 2010; Welton et al., 2014; Maryan et al., 2014; Doughty et al., 2014; Böhme et al., 2015; Weijola et al., 2016; Böhme et al., 2019; Weijola et al., 2020; Pavón-Vázquez et al., 2022; Weijola and Kraus, 2023). Varanids have adapted to an array of habitats from mangrove swamps to dense forests to savannas to arid deserts, and are being semi-aquatic to terrestrial to semi or true arboreal in their habitat preferences (Pianka and King, 2004). India has four extant species of Varanids, such as *Varanus bengalensis* (Daudin, 1802) and *V. flavescens* (Hardwicke and Gray, 1828) under the subgenus *Empagusia*, *V. koniecznyi* (Böhme et al., 2023) under *Psammosaurus* and *V. salvator* (Laurenti, 1768) under the subgenus *Soterosaurus* (Koch et al., 2013). The Bengal monitor lizard (*V. bengalensis*) is a widespread

species distributed throughout the Indian plains and is much more adaptable in its habitat use, including human habitations (Cota et al., 2021). The Yellow monitor lizard (*V. flavescens*) is largely known from the Terai landscape and the Gangetic floodplains on the eastern and western India (Das et al., 2021). Among the Indian varanids, the Desert monitor lizard (*V. griseus*) represented by the subspecies *V. g. koniecznyi* Mertens is the smallest and is marginally distributed in Indian deserts in the states of Gujarat and Rajasthan of India, which is now elevated to species status as *V. koniecznyi* (Soorae et al., 2021; Böhme et al., 2023). The Water monitor lizard (*V. salvator*), which is represented by two subspecies: *V. salvator macromaculatus* Deraniyagala and *V. salvator andamensis* Deraniyagala, is the largest among the Asian varanids; the former subspecies is distributed in the Eastern and Northeast India whereas the later in the Andaman & Nicobar Islands (Quah et al., 2021).

The monitor lizards, across their distribution range, are facing varied potential threats such as habitat destruction, human consumption, traditional medicine, and collection for the global leather industry and pet trade (Schlaepfer et al., 2005; Bennet, 1995). TRAFFIC report (1990-2014) stated that nearly 55 million specimens of *Varanus* species were reportedly traded internationally over the last 25 years. As per the report, approximately 20 million specimens were exported and approximately 30 million specimens were re-exported over this period. Surprisingly, 54 species of monitor lizards were reported in trade, of which *V. salvator*, *V. niloticus*, *V. exanthematicus* are the most traded species comprising around 99% of the total volume of trade, over a period of 25 years. Tikader (1983) reported about 2,00,00,000 skins of *V. salvator* were shipped from Calcutta (now Kolkata) to Europe between 1930 to 1982, due to its high demand in the leather industry. Heavy exploitation for skin has been documented of *V. salvator* due to its patterned skin texture followed by skins from *V. bengalensis* which

diminishes its population at a greater extent and faster rate (Inskipp, 1981). In 1985, around 6.7 million pounds worth of *V. bengalensis* skin were exported to Europe, this export quantity gradually increased from 6.7 million pounds to 9.1 million pounds in between 1985 to 1990. Of late, a new threat is becoming prevalent for the monitor lizards in India, the trade of male intromittent organ (hemipenis) in the trade name "Hatha Jodi" and has been sold in the name of a plant material (Sharma et. al., 2019). The online price of "Hatha Jodi" may reach up to 90 US$ per set. However, trade on "Hatha Jodi" and other products of Monitor lizards are considered to be for domestic consumption, as there is no report of international trade. All four species of monitor lizards in India are protected by the Indian Wild Life (Protection) Amendment Act, 2022 and are listed under Schedule I of the Act. Similarly, all the Indian varanids are also listed in the Appendices of the Convention on International Trade in Endangered Species (CITES), prohibiting the international trade in the species. As per the Red List Assessment by the International Union for Conservation of Nature (IUCN), out of the four Indian species, one species is in the Endangered category, one is Near Threatened and the rest two are of Least Concern category. The conservation status of the Indian species of monitors is listed in Table 1.

Table 1: Protection status and geographical range of Indian Varanids

Common Name	Scientific Name	Range	IUCN	CITES	WPA
Bengal monitor lizard	*V. bengalensis*	Bangladesh, Bhutan, India, Iran, Myanmar, Nepal, Pakistan, Sri Lanka	Near Threatened	Appendix I	Schedule-I
Yellow monitor lizard	*V. flavescens*	Bangladesh, India, Nepal, Pakistan	Endangered	Appendix I	Schedule-I
Water monitor lizard	*V. salvator*	Bangladesh, Cambodia, China, India, Indonesia, Laos, Malaysia, Myanmar, Singapore, Sri Lanka, Thailand, Vietnam	Least Concern	Appendix II	Schedule-I
Desert monitor lizard	*V. koniecznyi*	India and Pakistan	Least Concern	Appendix I	Schedule-I

Bengal Monitor Lizard

Varanus bengalensis (Daudin, 1802)

Synonyms and Chresonyms

Tupinambis bengalensis — Daudin 1802
Tupinambis cepedianus — Daudin 1802 (fide Mertens 1963)
Lacerta argus— Daudin 1802 (in part)
Varanus punctatus— Merrem 1820
Varanus taraguira — Merrem 1820 (in part)
Monitor gemmatus— Guérin-Méneville 1829
Monitor heraldicus — Gray in Griffith 1831: 27 (fide Mertens 1963)
Varanus bengalensis — Duméril & Bibron 1836
Monitor inornatus — Schlegel 1839
Monitor cepedianus — Schlegel 1839
Varanus bibronii — Blyth 1842
Uaranus lunatus — Gray 1845
Varanus dracaena — Theobald 1868
Varanus lunatus — Anderson 1871
Varanus dracaena — Anderson 1871
Varanus (*Varanus*) *bengalensis* — Mertens 1942

Varanus (*Indovaranus*) *bengalensis* — Mertens 1963
Varanus (*Empagusia*) *bengalensis* — Koch et al. 2013

Description

The Bengal monitor lizard (*V. bengalensis)* was first described by Daudin in 1802 and was named after its occurrence in Bengal, India (Figure 1, Figure 2 and Figure 3). According to the Brygoo (1987), Syntype: MHNP 2179 (1504) was sent by naturalist Massé from Bengal to the Natural History Museum in Paris and also noted by Guibé (1954) who incorrectly designates lectotype as a holotype. Previously Bengal monitor has been divided into two subspecies according to the distribution range and morphological features namely *V. bengalensis bengalensis* and *V. bengalensis nebulosus*. Gray (1831) recognizes its subspecies from Java as *V. bengalensis nebulosus*. Mertens (1937) resurrected *V. nebulosus* to specific level due to position of supraocular scales. The species is known by several vernacular names in different parts of India such as Goh, Goh-saap, Gohira, Gohita, Bis-Kopada, Gopar, Udumbu etc. and interestingly this species is also known by different names in its life stages. Although morphologically males and females are of same colouration, the adult males grow larger than the females. The male Bengal monitor attains a total length of ~175 cm and weighs up to 2.7 kg whereas the females grow up to ~120 cm and weighs around ~1.5 kg (Auffenberg, 1994). The adults have a strong tail which is laterally compressed with keel and goes 1.5 times more than Snout-Vent length. The nostrils are slit shaped in adults while oval shaped in juveniles and are located exactly at the middle of the eye and tip of the snout. The ear opening is an elongated oval with the longest axis in the horizontal plane. The recognizable feature of *V. bengalensis* is presence of small undifferentiated scales above the eye i.e., on the supraocular region. The colour of *V. bengalensis* hatchlings is most intense as compared with adults and the pattern is also evident. Young ones have a series of narrow and dark crossbars on neck, throat and back and the tail is light and dark

banded. Additionally, between these crossbars there is a transverse series of lighter spots known as ocelli. Due to a large distribution range with a wide variety of habitat and climatic conditions the colour pattern of adults shows differences from very dark to light shade. The dorsal body of an adult is brown to black usually patterned with more or less many small light or dark scales/spots while the ventral side of the body is light and dark speckled. There is considerable geographical variation in colour and pattern of juveniles and adults of *V. bengalensis*. It is observed that sexual maturity is reached at the age of 2½-3 years in the natural environment (Auffenberg, 1994). Mating occurs during the monsoon i.e. June-July and eggs are laid in August after the careful selection of oviposition sites. The eggs are laid inside earthen embarkments, rotten tree trunks and in termite mounds. The clutch size is associated with the age of the reproductive life. In their first year, females lay about 1 to 8 eggs, while those in their second reproductive year produce 3 to 11 eggs. Older females, beyond these stages, can lay between 5 to 42 eggs. As a result, the overall average clutch size is approximately 20 (Auffenberg, 1994). The incubation period (~168-254 days) of eggs is winters and they hatch in early March. Juveniles stay close to their hatching site for using it as safe retreats for several days before dispersing. The survivorship of neonates is low; around half of the clutch perishes by the end of their second year (Bennett, 1995). *V. bengalensis* rarely breeds in captivity and delayed reproduction has been documented (Bennett, 1995). The Bengal monitor is diurnal, mainly ground-dwelling and strictly carnivorous. All monitors have similar body plan and several morphological synapomorphies, still achieved diversity within while overall morphology makes it an ideal radiation for investigating and comparing the evolution of *Varanus*.

Study of *Varanus* evolution is in vulnerable state due to its non-monophyletic subgeneric taxonomy and lack of well-sampled, well–corroborated phylogenetic hypothesis. Phylogenetic

relationships on subgeneric level have been attempted from several sources including Mertens (1942, 1959) based on skull morphology, position of the extra nares and degree of tail compression which proposed that *V. bengalensis* placed in monotytpic subgenus, *Indovaranus* without clarification of its phyletic position in respect to other subgenera in the genus. Besides, these Karyotypes (King and King, 1975), electrophoretic phenotypes (Holmes et al., 1975) data broadly test phylogeny, biogeography and taxonomy and suggested that the African species and Indo-Asian monitors of the *V. salvator* group (including *V. bengalensis*, *V. flavenscens* and *V. rudicollis*) while in the African radiation *V. griseus* was considered to have given rise to *V. niloticus* and *V. exanthematicus* which then radiated southward into Africa. *V. bengalensis* is closely related to *V. olivaceus* suggested by Auffenberg (1988). However, the male intromittent organ morphology (Branch, 1982; Böhme, 1988; Card and Kluge, 1995) and skeletal morphology (Estes and Camp, 1988) suggests that *V. bengalensis* is more closely related to *V. exanthematicus* and *V. niloticus* (African), *V. rudicollics* (Southeast Asia) and *V. flavescens* (India) than previously believed. In addition to these *V. griseus* and *V. dumerili* comprise the Afro-Asian radiation group of the genus and their cladogram shows *V. bengalensis* is closely related to *V. rudicollics* and *V. flavescens* (Bohme, 1988). These studies differ considerably in their conclusions therefore no unanimity phylogeny has emerged. Even use of multiple lines of evidence to attain transparency in phylogenetic relationships, such as DNA sequence data (Baverstock et al., 1993; Ast, 2001; Welton et al., 2010a), historical biogeography (Fuller et al., 1998; Schulte et al., 2003; Vidal et al., 2012) and body size evolution (Pianka, 1995; Collar et al., 2011) resulted in different opinions regarding origin and dispersal of the species. The most recent phylogenomic study on the genus by Brennan et al. (2021) using exon-capture molecular and morphological data sets tried to test the body size variation in the genus *Varanus.* The study

provided a robust phylogeny along with the dynamic biogeographic history to Australian monitors to estimate the relationships among living and extinct Varaniform lizards. However, the sample of *V. bengalensis* used by Brennan et al. (2021) were either museum samples or the captive-bred population that does not show the true picture of the phylogenetic status of the native and wild population of Bengal monitors (Gautam et al., 2023).

The complex and unresolved evolutionary history of the *Varanus* genus highlights the challenges in understanding their phylogenetic relationships. This complexity is not just a scientific dilemma but also has significant conservation implications. This complexity is further exacerbated by the increasing threat of illegal wildlife trade. Despite the formulation of numerous laws, wildlife trafficking continues to escalate. The aim of the study is to identify species and populations from specific geographic regions using a reference genetic database to monitor illegal trade in India. This study will assist enforcement agencies in verifying the authenticity and geographic origin of wildlife products, as well as in tracking their possible trade routes. To effectively combat the escalating threat of illegal wildlife trade, it is crucial to leverage advanced scientific tools that can offer precise species identification and tracking of origin. Therefore, the utility of genetic technology becomes indispensable. While traditional morphological classifications have served as the foundation for taxonomic identification, they often fall short in accuracy, especially in the context of enforcement and conservation.

Figure 1: Image depicting Adult Bengal Monitor Lizard from the vicinity of Dehradun, Uttarakhand

Figure 2: Sub-Adult Bengal Monitor Lizard from Dehradun, Uttarakhand **Pic Credit: Dr. A. Das**

Figure 3: Juvenile Bengal Monitor Lizard from Kaziranga, Assam **Pic Credit: Bitupan Boruah**

Utility of genetic markers in species monitoring

Advanced genetic tools and technology, with their ability to detect even subtle variations at the molecular level, provide a powerful alternative. By analyzing genetic diversity and structure within and between populations, these markers enable more accurate assessments of species, supporting efforts to monitor illegal trade and aiding in the formulation of more effective conservation strategies. Genetic markers play a crucial role in better conservation management of endangered species and are widely used in population estimation, assessment of genetic diversity and to study the gene flow pattern in fragmented habitats (Avise et al., 1994). Historically, morphological classifications were widely used for taxonomic identifications; however, they have limitations, including taxonomic misidentification (Smith and Smith, 1992; Pfenninger et al., 2006). The advent of DNA sequencing has significantly advanced the field by enabling robust statistical analysis of both mitochondrial and nuclear genomes. This evolution

and enhanced computing power have revolutionized the interpretation of genetic variations (Armstrong et al., 2021). Nucleotide variations in DNA sequences can accumulate over time due to evolutionary changes and environmental adaptations. These mutations among nucleotide sequences are used to recognize genetic differentiation between organisms, aiding in estimating genetic divergence and structure (Patwardhan et al., 2014). Neutral genetic markers can assess the adaptive genetic variation within species (Frankham et al., 2002). The selection of molecular markers always depends on the studied questions and adopted methodologies. The prior knowledge of the variability of genetic markers is essential for analyzing population genetic structure and diversity in natural populations (Røed, 1998). Molecular genetic markers have become more widely used to evaluate genetic differentiation among geographically isolated populations, helping to define newly evolved species/subspecies, evolutionary significant units, taxonomic units and management unit for conservation and management purposes and aiding in the reassessment of traditional classifications (Moritz, 1994; Fraser and Bernatchez, 2001).

Molecular markers have revolutionized biological research across diverse disciplines by providing powerful tools for various applications. These markers are widely employed to study phylogenetic relationships, enabling scientists to understand the evolutionary connections between different species. They are essential for assessing genetic variations within and between populations, which helps identify genetic diversity and structure within species.

In evolutionary biology, molecular markers shed light on the processes driving the evolution of species, such as mutation, selection, gene flow, and genetic drift. This understanding is crucial for reconstructing the evolutionary history of species and predicting their future adaptability to changing environments.

Mitochondrial genome

The mitochondrial (mt) genome is a circular structure containing 37 regions and one highly variable control region (non-coding region). The mt genome contains 13 protein-coding genes (PCGs), 22 transfer RNA (tRNA) genes, and 2 ribosomal RNA (rRNA) genes. In reptile, the mitochondrial genome size is around 17.5 kilobases (kb). Mitochondrial DNA (mtDNA) serves as a widely used tool for phylogenetic and phylogeographic studies due to its unique properties. The mtDNA is haploid and maternally inherited also lacks recombination, which ensures that offspring inherit identical mtDNA from their mother. Mitogenome has slower mutation rate compared to non-coding hypervariable regions, such as mtDNA control region and microsatellites. However, when studying purifying selection, the mitogenome exhibits higher mutation rates than other genes. Therefore, mtDNA is particularly suitable for studying divergence events and geographical structuring among populations (Hofreiter and Stewart, 2009). In forensic identifications when the DNA is very limited or highly degraded the mtDNA is useful to obtain the relevant information about the sample identity and origin due to its multiple copies in single cell and property of being maternally inherited (Ma et al., 2018). In the field of wildlife forensics, the Cytochrome *b* (Cyt *b*), Cytochrome c oxidase *I* (CO*I*), 12S rRNA, and 16s rRNA genes of mitochondrial genome are widely utilized. Therefore, development in DNA forensics in the form of Forensically Informative Nucleotide Sequencing (FINS) is a reliable technique to identify the species in traded articles where the morphological identification is not feasible (Bartlett et al., 1992; Guha and Kashyap, 2006).

Microsatellites Markers

Microsatellites consist of short tandem repeat sequences, typically di-, tri-, or tetra-nucleotide units ranging from 1 to 6 base pairs, repeated multiple times, and flanked by unique, non-

repetitive DNA sequences (Tóth et al., 2000). Within a population, microsatellites are multi-allelic, meaning they have several variants, while within an individual they are bi-allelic, possessing two distinct alleles at a given locus. Inheritance follows a co-dominant Mendelian pattern, allowing microsatellites to distinguish between heterozygote's (individuals with two different alleles at a locus) and homozygote's (individuals with two identical alleles at a locus), thereby facilitating the calculation of allele frequencies in a population. Microsatellites exhibit a high mutation rate, approximately ~10^{-4} mutations per locus per replication event, often resulting in the presence of multiple alleles at each locus. These markers are particularly suited for studying recent population genetic events and determining the parentage due to their high polymorphism levels. In forensic analysis, microsatellites or short tandem repeats (STR) played a key role in individual identification because these short tandem repeats are biallelic and co-dominant in nature which can easily differentiate between heterozygote's and homozygote's (Willems et al., 2014; Gymrek, 2017). The geographical reference database of STR markers in the field of wildlife forensic is very crucial for identification of poaching hotspots and trade routes which eventually helps to strategize conservation efforts and management of endangered species.

Justification of the study

The population of *Varanus* continuously decline through their distribution range due to over hunting and destruction of their habitat through anthropogenic activity. Reduction in population size will cause low genetic diversity, which leads to inbreeding rendering the population unfit to survive and pushing it to the brink of extinction. As per the CITES Trade Database during the past decades, heavy exploitation of *Varanus* body parts was recorded. Today, the trade in skins appears of much less concern, compared to the early 1980s however, trade of male intromittant

organ of *V. bengalensis* escalated into global crisis. According to the recent study reported by Sharma et al. (2019), online trading of male intromittant organ or "hemipenis" accelerated at local and international market with spurious name "Hatha Jodi", following which Wildlife Forensics and Conservation Genetic Cell (WFCGC) of Wildlife Institute of India has been regularly receiving seized samples of *Varanus* spp., hemipenis. Due to bad condition of seized samples confirming species level identification based on morphological characteristics alone is not reliable. Hence, genetic analysis to identify a particular species in seizures will give a correct picture of the trade dynamic involving extant *Varanus* spp., in India. This study would be helpful in developing a geographical level genetic database for *Varanus bengalensis* and assist in identification of source location of the seized *Varanus bengalensis* body part. This study would help to identify the hotspots areas for poaching of *Varanus* spp., and its possible trade route which help in advance alertness to the enforcement agencies.

Research Questions

1. Which *Varanus* species is in high trade?
2. What would be the genetic relationship among the seized samples of *Varanus* spp.?
3. Is there any specific population signature among the examined biological samples of *Varanus* spp.?
4. How many distinct populations of *Varanus* existed in Terai Arc Landscape and how they are structured at the genetic level across their distribution ranges?

Objectives

1. To establish the genetic signatures of *Varanus bengalensis* from Terai Arc Landscape and augmenting the genetic database of the species from the opportunistically collected samples from other Indian states.
2. To inventories the *Varanus* biological samples at Wildlife Institute of India for determining their species, phylogenetic status and genetic variation.
3. To determine the population genetic structure of *V. bengalensis* from examined samples.

Chapter-2

To establish the genetic signatures of *Varanus bengalensis* from Terai Arc Landscape and augmenting the genetic database of the species from the opportunistically collected samples from other Indian States

Background

The wildlife trade is as archaic as oldest recorded history, with many evidences of large-scale unsustainable commercial use during the Roman Empire (Hughes, 2003). Early civilizations of Egypt and Greece also documented commercial transactions involving wildlife, a tradition that has persisted without interruption to the present day (van Uhm, 2016). Contemporary awareness towards the large-scale anthropogenic footprints such as urbanization, pollution, human-induced climate change is depleting the biodiversity on earth is increasing (Tittensor et al., 2014). The unsustainable harvesting of flora and fauna around the globe is one of the key threats to biodiversity which is associated with the decline in the abundance of trafficked species. Effective tackling of wildlife trafficking necessitates the integration of socio-economical and practical evidence to comprehensively grasp its drivers and impacts across different timeframes and geographical scales ('t Sas-Rolfes et al., 2019). According to Harfoot et al. (2018), the wildlife products traded internationally between the year 1975 and 2014 were highest for the Whole Organism Equivalents (WOEs) in volume (1.80 billion reported by exporters), followed by reptiles (152 million), invertebrates (79.8 million), birds (24.1 million), mammals (13 million), fish (12.8 million) and amphibians (1.07 million). Trade in reptile is the second highest among animal trades, which make them prone to local extinction, genetic discontinuities, anomalies, influenced speciation and alteration in distributional patterns, majorly caused by natural or induced fluctuation (Gibbons et al., 2000; Harfoot et al., 2018). Monitor lizards are in high demand in the global pet trade and leather industries, making them one of the most sought-after reptile groups (Pernetta, 2009). The growing impact of this trade on specific varanid species is a matter of international concern. Several factors are driving this unsustainable exploitation such as distinctive morphological features of varanids, impressive body size combined with striking

colors and patterns, a unique behavioral ecology and intelligence of these lizards; and their rarity, coupled with national and international protections, which elevate their economic value and demand (Koch et al., 2013). As mentioned earlier, India is home to four species of monitor lizards: Yellow monitor (*V. flavescens*); Water monitor (*V. salvator*); the Desert monitor (*V. koniecznyi*) and the Bengal monitor (*V. bengalensis*). Notably, the distribution range of *V. bengalensis* overlaps with that of *V. flavescens, V. salvator,* and *V. koniecznyi* (Auffenberg, 1994; Böhme et al., 2023; Figure 4). All four extant species of monitor lizard in India are protected under Schedule I of the Wildlife (Protection) Amendment Act, 2022, because of their high demand in the wildlife trade for skin as a leather product and in musical instruments, and eggs and meat for local consumption, traditional medicines, superstitious beliefs and retaliatory killings (Das, 1989; Koch et al., 2013; Bhattacharya and Koch, 2018; TRAFFIC, 2021). Lately, illegal exploitation of monitor lizard genitalia (the "hemipenis") has been rampant under the pseudonym of "Hatha Jodi" in India (Sharma et al., 2019). The trafficked wildlife items may not always be recognizable by its appearance which means morphology will fail to identify the traded species derivatives. The accurate identification of traded species by conservationists is a safeguard measure to understand and mitigate market activities (Gaubert et al., 2015). When morphological identification is not reliable enough, genetics-based species identification aims to match an unknown wildlife species derivative to a verified reference sample whose province or geo-location is known (Baker et al., 2000; Wasser et al., 2004; Baker, 2008; Ghobrial et al., 2010). The lack of authenticated geo-referenced samples of targeted species of interest is a major limitation for the development of reference molecular databases in the field of DNA forensics (Budowle et al., 2005). Bartlett and Davidson, (1992) proposed the concept of FINS which is a technique that combines DNA sequences and phylogenetic analysis to identify the trafficked

samples based on the informative nucleotide sequences. Thereafter, the FINS has since been extensively applied in forensic investigations (Verma and Singh 2003; Baker, 2008; Sahajpal and Goyal, 2009; Rajpoot et al., 2017; Yadav et al., 2021).

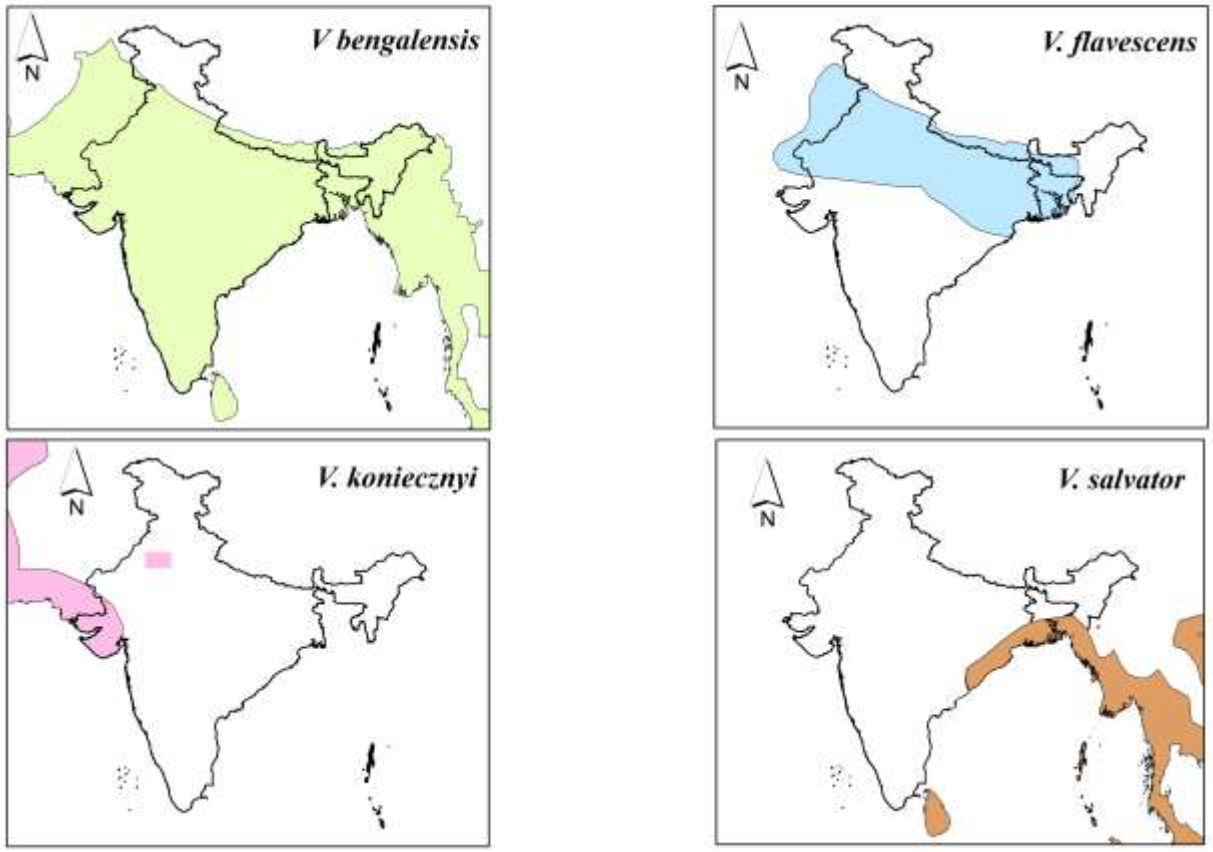

Figure 4: Distribution range of different monitor lizard in India adopted from IUCN and modified

The widespread illegal exploitation of monitor lizards in India poses a significant threat to their survival. Therefore, this chapter aims to investigate the genetic signatures of the Bengal monitor lizard (*V. bengalensis*) and examine its phylogenetic relationships with other monitor lizard species. By analyzing the genetic structure and relationships among these extant species, we can identify significant varanid lineages and provide crucial support for molecular tracking,

conservation strategies, and management planning. This study will aid in differentiating species, enhancing our understanding of their genetic diversity, and informing efforts to protect and manage these vulnerable populations effectively.

STUDY AREA

The Terai Arc Landscape (TAL) in India is a 50-60 km wide and 810 km stretch cradled between the river Yamuna in West and river Bhagmati in east. The stretch is bound by important natural divides: Shiwalik Hills in North, Aravali Hills in South West, Yamuna River in East, southern foothill of Himalayas, Ganga basin, adjoining Bhabhar areas and the Terai flood plains. A major portion of the landscape has a plain like topography with fertile soil. There are nine protected areas present in this landscape, among them four are National parks (NP) and five are Wildlife sanctuaries (WLS). The landscape is mainly spread across the Indian states: Uttarakhand, Uttar Pradesh and Bihar. However, Kalesar WLS of Haryana and Simbalbara WLS of Himachal Pradesh state of India was also included (Johnsingh et al., 2004) (Figure 5).

Methodology

Sample Collection and DNA extraction

The sampling for this study spans across the Himalayan foothills and the Indian region of the Terai Arc Landscape (TAL) (n=37). Additionally, samples were collected from the Ganga basin, regions West and South of the Ganga basin, the Brahmaputra basin, Gujarat, and the Western Ghats (n=47) (Figure 5).

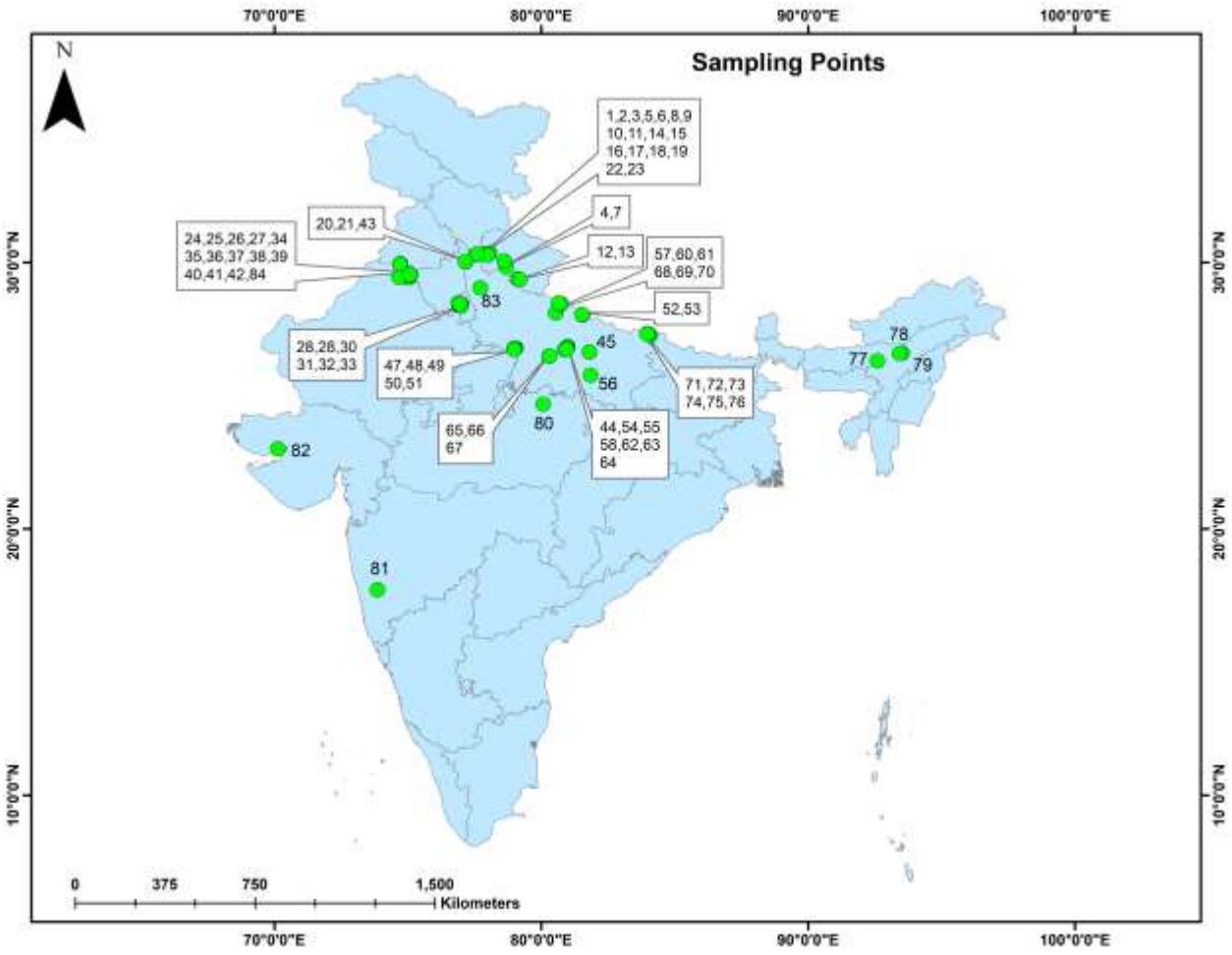

Figure 5: Sampling points of this study

A small piece of tissue from the tail tip up to 2 cm from 84 monitor lizards comprising *V. bengalensis* (n=73), *V. flavescens* (n=9) and *V. griseus* (n=2) was collected. All samples were collected in collaboration with field veterinarians and experts, ensuring proper medical care and adherence to ethical guidelines. Institutional Animal Ethics Committee approved the collection and storage techniques and authors confirm that laboratory works were executed with relevant guidelines and regulations (Letter no: WII/IAEC/2017-18). Detailed information on the origin of the genus *Varanus* included in this study are provided in Table 2. The samples were stored in 70% ethanol at room temperature. The genomic DNA (gDNA) was extracted using the DNeasy Blood Tissue Kit (QIAGEN, Germany) in a final elution volume of 100 µl.

Table 2: Sample origin and Accession number used in this study

S.No.	Tag No.	Species	Latitude	Longitude	Accession No. COI	Accession No. ND5	Accession No. Cyt *b*	Accession No. ND4
1	UKVR1	*V. bengalensis*	30.28389	77.97556	OP117155	OP141886	OP141809	-
2	UKVR2	*V. bengalensis*	30.28361	77.97389	OP117156	OP141887	OP141810	-
3	UKVR3	*V. bengalensis*	30.28611	77.97333	OP117157	OP141888	OP141811	-
4	UKVR5	*V. bengalensis*	29.83806	78.67944	OP117158	OP141889	OP141812	-
5	UKVR6	*V. bengalensis*	30.28083	77.97556	OP117159	OP141890	OP141813	-
6	UKVR7	*V. bengalensis*	30.27556	77.97778	OP117160	OP141891	OP141814	-
7	UKVR8	*V. bengalensis*	30.06067	78.59769	OP117161	OP141892	OP141815	OP141878
8	UKVR9	*V. bengalensis*	30.27722	77.97306	OP117162	OP141893	OP141816	-
9	UKVR10	*V. bengalensis*	30.28167	77.98028	OP117163	OP141894	OP141817	OP141879
10	UKVR11	*V. bengalensis*	30.33278	78.01111	OP117164	OP141895	OP141818	-
11	UKVR12	*V. bengalensis*	30.35167	78.05028	OP117165	OP141896	OP141819	-
12	UKVR13	*V. bengalensis*	29.39167	79.11694	OP117166	OP141897	OP141820	-
13	UKVR15	*V. bengalensis*	29.36491	79.19276	OP117167	OP141898	OP141821	-
14	UKVR18	*V. bengalensis*	30.35167	78.05028	OP117168	OP141899	OP141822	-
15	UKVR19	*V. bengalensis*	30.3196910	77.9307590	-	-	-	-
16	UKVR 20	*V. bengalensis*	30.3022810	77.9559380	-	-	-	-
17	UKVR 21	*V. bengalensis*	30.2929630	77.9537630	-	-	-	-
18	UKVR 22	*V. bengalensis*	30.3063650	77.9523970	-	-	-	-
19	UKVR 23	*V. bengalensis*	30.2795790	77.9730890	-	-	-	-
20	HRVR1	*V. flavescens*	30.03025	77.15409	OP117169	OP141900	OP141823	OP141880
21	HRVR2	*V. bengalensis*	30.03201	77.15384	OP117170	OP141901	OP141824	-
22	HRVR4	*V. bengalensis*	30.29884	77.54257	OP117171	OP141902	OP141825	-
23	HRVR5	*V. bengalensis*	30.31706	77.58087	OP117172	OP141903	OP141826	-
24	HRVR6	*V. griseus*	29.55814	75.05887	OP117173	OP141904	OP141827	OP141881
25	HRVR7	*V. bengalensis*	29.96104	74.70983	OP117174	OP141905	OP141828	-
26	HRVR8	*V. bengalensis*	29.95058	74.69253	OP117175	OP141906	OP141829	-
27	HRVR9	*V. bengalensis*	29.96124	74.71108	OP117176	OP141907	OP141830	-
28	HRVR10	*V. bengalensis*	28.44689	76.98942	OP117177	OP141908	OP141831	-
29	HRVR11	*V. bengalensis*	28.38836	76.90906	OP117178	OP141909	OP141832	-
30	HRVR12	*V. bengalensis*	28.39347	76.9365	OP117179	OP141910	OP141833	-
31	HRVR13	*V. bengalensis*	28.48664	76.88769	OP117180	OP141911	OP141834	-
32	HRVR14	*V. bengalensis*	28.36872	76.98514	OP117181	OP141912	OP141835	-
33	HRVR15	*V. bengalensis*	28.447	76.93731	OP117182	OP141913	OP141836	-
34	HRVR16	*V. bengalensis*	29.55869	75.05951	OP117183	OP141914	OP141837	-
35	HRVR17	*V. bengalensis*	29.53603	75.04934	OP117184	OP141915	OP141838	-
36	HRVR18	*V. bengalensis*	29.52156	75.05326	OP117185	OP141916	OP141839	-
37	HRVR20	*V. bengalensis*	29.41919	75.01047	OP117186	OP141917	OP141840	OP141882

38	HRVR21	*V. bengalensis*	29.51274	75.03675	OP117187	OP141918	OP141841	-
39	HRVR22	*V. bengalensis*	29.53227	75.05969	OP117188	OP141919	OP141842	-
40	HRVR23	*V. bengalensis*	29.44278	74.66769	OP117189	OP141920	OP141843	-
41	HRVR24	*V. bengalensis*	29.52944	75.09206	OP117190	OP141921	OP141844	-
42	HRVR25	*V. bengalensis*	29.5436	75.0491	OP117191	OP141922	OP141845	-
43	HRVR26	*V. bengalensis*	30.03201	77.15384	OP117192	OP141923	OP141846	-
44	UPVR1	*V. bengalensis*	26.8428	80.97205	OP117193	OP141924	OP141847	-
45	UPVR2	*V. bengalensis*	26.64189	81.8028	OP117194	OP141925	OP141848	-
46	UPVR3	*V. bengalensis*	26.64189	81.8028	OP117195	OP141926	OP141849	-
47	UPVR4	*V. flavescens*	26.7891	79.0334	OP117196	OP141927	OP141850	-
48	UPVR5	*V. bengalensis*	26.78612	79.00672	OP117197	OP141928	OP141851	-
49	UPVR6	*V. bengalensis*	26.78305	79.01573	OP117198	OP141929	OP141852	-
50	UPVR7	*V. bengalensis*	26.7957	78.98518	OP117199	OP141930	OP141853	-
51	UPVR8	*V. bengalensis*	26.73375	78.97329	OP117200	OP141931	OP141854	-
52	UPVR9	*V. bengalensis*	28.03693	81.5358	OP117201	OP141932	OP141855	-
53	UPVR10	*V. flavescens*	28.05985	81.52363	OP117202	OP141933	OP141856	OP141884
54	UPVR16	*V. bengalensis*	26.74241	80.86998	OP117203	OP141934	OP141857	-
55	UPVR17	*V. bengalensis*	26.85191	80.98071	OP117204	OP141935	OP141858	-
56	UPVR18	*V. bengalensis*	25.7704	81.84627	OP117205	OP141936	OP141859	-
57	UPVR19	*V. bengalensis*	28.10413	80.53232	OP117206	OP141937	OP141860	-
58	UPVR20	*V. bengalensis*	26.8428	80.97205	OP117207	OP141938	OP141861	OP141883
59	UPVR21	*V. bengalensis*	26.64189	81.8028	OP117208	OP141939	OP141862	-
60	UPVR22	*V. bengalensis*	28.42847	80.70272	OP117209	OP141940	OP141863	-
61	UPVR23	*V. bengalensis*	28.42847	80.70272	OP117210	OP141941	OP141864	-
62	UPVR25	*V. bengalensis*	26.76139	80.96217	OP117211	OP141942	OP141865	-
63	UPVR26	*V. bengalensis*	26.74241	80.86998	OP117212	OP141943	OP141866	-
64	UPVR28	*V. bengalensis*	26.74469	80.94341	OP117213	OP141944	OP141867	-
65	UPVR29	*V. bengalensis*	26.49978	80.29264	OP117214	OP141945	OP141868	-
66	UPVR30	*V. bengalensis*	26.5007	80.30046	OP117215	OP141946	OP141869	-
67	UPVR31	*V. bengalensis*	26.50506	80.30152	OP117216	OP141947	OP141870	-
68	UPVR32	*V. bengalensis*	28.4904770	80.6475140	-	-	-	-
69	UPVR33	*V. bengalensis*	28.4607690	80.7382260	-	-	-	-
70	UPVR34	*V. bengalensis*	28.4893310	80.6469270	-	-	-	-
71	BRVR1	*V. flavescens*	27.3405555	83.9675000	-	-	-	-
72	BRVR2	*V. bengalensis*	27.32003	84.00133	OP117217	OP141948	OP141871	-
73	BRVR3	*V. flavescens*	27.269734	84.088018	-	-	-	-
74	BRVR5	*V. flavescens*	27.3326780	83.9814890	-	-	-	-
75	BRVR6	*V. flavescens*	27.3227640	84.0136750	-	-	-	-
76	BRVR7	*V. flavescens*	27.3208390	83.9642360	-	-	-	-
77	ASVR1	*V. bengalensis*	26.31029	92.58848	OP117218	OP141949	OP141872	-
78	ASVR2	*V. bengalensis*	26.60725	93.52461	OP117219	OP141950	OP141873	-
79	ASVR3	*V. bengalensis*	26.590510	93.426794	-	-	-	-
80	MPVR1	*V. bengalensis*	24.68667	80.06914	OP117220	OP141951	OP141874	-

81	MAVR1	*V. bengalensis*	17.72139	73.85412	OP117221	OP141952	OP141875	-
82	GJVR1	*V. bengalensis*	23.007	70.111	-	-	-	-
83	VF	*V. flavescens*	29.05137	77.70761	OP117222	OP141953	OP141876	-
84	VG	*V. griseus*	29.95401	74.69912	OP117223	OP141954	OP141877	OP141885

PCR amplification and sequencing

To fulfill the objective, I sequenced three mitochondrial regions: CO*I*, 977bp (Kumazawa and Endo, 2004), ND5, 668bp and Cyt *b*, 814bp. For the amplification of ND5 and Cyt *b* gene region, I have designed the forward primer **VAR-ND5F**: 5'-CCATTACTTCAACCTGTTTCAC-3' using the sequence of *V. niloticus* (AB185327), *V. salvator* (AB980995, EU747731 and NC010974) and *V. s. komini* (AB980996) with the combination of mcb869: 5'-CCTCCTAGTTTGTTAGGGATTGATCG-3' (Verma and Singh, 2003).

PCR reactions were performed in 20µl reaction volumes using 1 × PCR buffer (10mM Tris–HCl, pH8.3, and 50mM KCl), 1.5 mM $MgCl_2$, 0.2mM of each dNTPs, 3 pmol of each primer, 0.5 units of DreamTaq DNA Polymerase (Thermo Scientific) and 1 µl (~30 ng) of template DNA. The PCR protocol was used as follows: initial denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 45 sec, annealing at 56°C for 60 sec and extension at 72 °C for 75 sec. The final extension was at 72 °C for 10 min. Negative controls were included in each reaction to check the reliability of the experiment. All PCR amplification was confirmed by electrophoresis on 2% agarose gel stained with Ethidium bromide and visualised under a UV transilluminator. The amplified PCR products were purified with exonuclease-I and shrimp alkaline phosphatase (Thermo Scientific Inc.) at 37°C for 20 min to remove any remaining primer and dNTPs, followed by inactivation of enzymes at 85 °C for 15 min. The purified fragments were sequenced directly in an Applied Biosystems Genetic Analyzer 3500 XL (Applied Biosystems) from the forward and reverse directions using BigDye 3.1 Kit. To check the alignment and the presence of stop codon, all three coding genes were subject to translation into amino acids using Expasy: Translate tool (Gasteiger et al., 2003).

Data Analysis

The amplicons were sequenced for parts of CO*I*, ND5 and Cyt *b* genes for samples of *V. bengalensis* (n=84), *V. flavescens* (n=9) and *V. griseus* (n=2) (OP117155-OP117223; OP141809-OP141877 and OP141886-OP141954). Sequences obtained from the forward and reverse direction of samples for each gene, were edited manually by confirming electropherograms and assembled using SEQUENCHER® 4.9 (Gene Codes Corporation, Ann Arbor, MI, USA). A BLAST (Altschul et al., 1997) search was done with the generated sequences for ND5, Cyt *b* and CO*I* genes for species identification. DnaSP ver 6 (Rozas et al., 2017) was used to compute the number of haplotypes among the generated dataset. The complete mitochondrial genome of *Varanus salvator* (NC010974) was retrieved from NCBI database and aligned with the haplotypes of *V. bengalensis* to identify the specific polymorphic sites or SNPs. We also included published sequences to increase the robustness of the analyses (Table 3). *Heloderma suspectum* was used as an outgroup to reconstruct the phylogenetic relationship between different monitor lizard species of *Varanus* species. Sequences were aligned using the Clustal W program (Thompson, 1994) by BioEdit 7.1.3 (Hall, 1999) with default settings. The alignment was manually optimized and ambiguous regions were excluded for phylogenetic analyses. The data matrix was executed in MEGA X (Kumar et al., 2018) to determine an appropriate model for maximum likelihood analysis.

A Monte Carlo Markov Chain (MCMC) based Bayesian consensus tree was constructed using BEAST 1.7 (Drummond et al., 2012). Bayesian inference analyses were performed using the best-fit model HKY+G+I for CO*I* and ND5, while HKY+I for Cyt *b* genes, using MCMC chains executed for 10 million generations logging every 1000 generations with 10% per run, was discarded as burn-in. The runs were evaluated in Tracer 1.6 and resulting phylogenetic trees were

visualised in FigTree 1.4.0 (http://tree.bio.ed.ac.uk/software/figtree/). The mean distance between groups for all three genes was calculated using the TN93+G model with the lowest BIC score value in MEGA X (Kumar et al., 2018).

Table 3: Sequences information used in this study from National Center for Biotechnology Information (NCBI)

Species	**CO*I***	**ND4**	**ND5**	**CYT *b***
Varanus acanthurus		DQ525104		
Varanus albigularis		KT720981		
Varanus baritji		DQ525105		
Varanus beccarii	KY354294	MK388795		
Varanus bengalensis	MN148451			MG670550
	KF766939			MG670551
	MN239900			MG670552
	MK947910			MG670553
	JN714165			MG670554
				KF766940
Varanus bogerti		MK388793		
Varanus brevicauda		KP076420		
Varanus cerambonensis		KU513445		
Varanus doreanus	KY354298	KU513447		
Varanus douarrha		KY770837		
Varanus eremius		DQ525114		
Varanus exanthematicus		KT720982		
Varanus finschi		KU513443		
Varanus flavescens				MG670555
Varanus giganteus		DQ525159		
Varanus gilleni		DQ631870		
Varanus glauerti		DQ525121		
Varanus glebopalma		DQ525129		
Varanus gouldii		DQ329286		
Varanus indicus	MH274778	KY770825		
	MH274779			
	MH274780			
	MH274781			
Varanus jobiensis		KY770841		
Varanus keithhornei		DQ525168		
Varanus kingorum		DQ525169		
Varanus komodoensis	AB080275	AB080276		
Varanus lirungensis		MT011988		
Varanus melinus		KU513458		
Varanus mertensi		DQ525157		
Varanus mitchelli		DQ525127		
Varanus nebulosus	KY354299			
	EU621818			
Varanus niloticus	MH700865	KT720922	AB185327	AB185327
	MH300911			
	HQ219067			

Species delimitation analysis

Species delimitation uses heuristic methods to constitute groups of individuals either as different populations of a single species or a different species altogether. Uncertainty in morphologies due to a few distinctive traits can be addressed by a semi-automated process of delimitation obtained from genomic data and computer algorithms. The lineage samples of *V. bengalensis* used in this study were inspected through species delimitation tests based on phylogenetic trees of CO*I*, ND5 and Cyt *b* genes. Three different analyses were performed: I- Multi-rate Poisson Tree Processes (mPTP); II- Bayesian Poisson Tree Processes (bPTP); and III- Generalized Mixed Yule-Coalescent (GMYC).

The mPTP analysis was performed using a web server (https://mptp.h-its.org). This model uses a fast approach to estimate the maximum likelihood delimitation from an input tree. On the other hand, the bPTP analysis was performed using a web server (https://species.h-its.org) keeping parameters for MCMC, thinning, burn-in and seed value as default. This model adds Bayesian support (BS) values to delimit species on the input tree. Multiple threshold GMYC models were performed using a web server (https://species.h- its.org). This model uses the likelihood method to delimit species and reconstructed input trees by fitting within and between species branching models.

Results

Genetic analysis of mitochondrial region

In the comprehensive dataset of monitor lizard (n=84), a total of 23 haplotypes were observed in CO*I*, 19 haplotypes were found in ND5 and 26 haplotypes in Cyt *b* (Table 4). The dataset of

CO*I*, ND5 and Cyt *b* gene has been used to analyse genetic diversity. All the sequences are grouped into different genetically identified clusters indicating the maternal relatedness among the Monitor lizard (Table 5). In the COI gene the nucleotide diversity of *V. bengalensis* Lineage-I is π = 0.00108 (s.d. = 0.0002), *V. bengalensis* Lineage-II is π = 0.00034 (s.d. = 0.0001), *V. flavescens* is π = 0.00267 (s.d. = 0.0004) and *V. koniecznyi* is π = 0.00409 (s.d. = 0.0020). The overall nucleotide diversity among *V. bengalensis* is π = 0.01134 (s.d. = 0.0008). Among all the four distinct groups the lowest number of segregating sites were found in *V. koniecznyi* (S = 4), whereas it was high in *V. flavescens* (S = 8). However, in the ND5 gene the nucleotide diversity of *V. bengalensis* Lineage-I is π = 0.00183 (s.d. = 0.0004), *V. bengalensis* Lineage-II is π = 0.00136 (s.d. = 0.0002) and *V. flavescens* is π = 0.00720 (s.d. = 0.0013) and Due to small sequence length (531bp) and less number of sample diversity of *V. koniecznyi* could not be detected. The overall nucleotide diversity among *V. bengalensis* is π = 0.02424 (s.d. = 0.0018). Among all the four distinct groups the lowest number of segregating sites were found in *V. bengalensis* Lineage-I (S = 6), whereas it was high in *V. bengalensis* Lineage-II (S = 11). Moreover, in the Cyt *b* gene, the nucleotide diversity is π = 0.00148 (s.d. = 0.0002) for *V. bengalensis* Lineage-I, π = 0.00155 (s.d. = 0.0002) for *V. bengalensis* Lineage-II, π = 0.00580 (s.d. = 0.0008) for *V. flavescens*, and π = 0.00123 (s.d. = 0.0006) for *V. koniecznyi*. The overall nucleotide diversity among *V. bengalensis* is also π = 0.01597 (s.d. = 0.0011). Among the four distinct groups, the lowest number of segregating sites was found in *V. koniecznyi* (S = 1), while the highest was observed in both *V. bengalensis* Lineage-II and *V. flavescens* (S = 13).

Table 4: Haplotype in dataset from mtDNA gene

Gene	Haplotype No.	No. of Samples	Sample ID
CO*I*	Hap 1	5	UKVR1, UKVR2, UKVR7, UKVR10, UKVR23
	Hap 2	1	UKVR3
	Hap 3	13	UKVR5, UKVR6, UKVR9, UKVR11, UKVR12, UKVR18, UKVR19, UKVR20, UKVR22, UPVR22, UPVR32, UPVR33, UPVR34
	Hap 4	1	UKVR8
	Hap 5	1	UKVR13
	Hap 6	1	UKVR15
	Hap 7	3	ASVR1, ASVR2, ASVR3
	Hap 8	41	UKVR21, HRVR2, HRVR4, HRVR5, HRVR7, HRVR10, HRVR11, HRVR12, HRVR13, HRVR14, HRVR16, HRVR17, HRVR18, HRVR20, HRVR21, HRVR22, HRVR23, HRVR24, HRVR25, HRVR26, MPVR1, UPVR1, UPVR2, UPVR3, UPVR5, UPVR6, UPVR8, UPVR9, UPVR16, UPVR17, UPVR18, UPVR19, UPVR20, UPVR21, UPVR23, UPVR25, UPVR26, UPVR29, UPVR30, UPVR31, BRVR2
	Hap 9	2	HRVR8, HRVR9
	Hap 10	1	HRVR15
	Hap 11	1	MAVR1
	Hap 12	1	UPVR7
	Hap 13	1	UPVR28
	Hap 14	1	GJVR1
	Hap 15	1	VF
	Hap 16	1	HRVR1
	Hap 17	1	UPVR4
	Hap 18	1	UPVR10
	Hap 19	2	BRVR1, BRVR7
	Hap 20	2	BRVR3, BRVR6
	Hap 21	1	BRVR5
	Hap 22	1	VG
	Hap 23	1	HRVR6
ND5	Hap 1	19	UKVR1, UKVR2, UKVR3, UKVR6, UKVR7, UKVR8, UKVR9, UKVR10, UKVR11, UKVR12, UKVR13, UKVR15, UKVR18, UKVR19, UKVR20, UKVR22, ASVR1, ASVR2, ASVR3
	Hap 2	1	UKVR5
	Hap 3	1	UKVR23
	Hap 4	3	UPVR22, UPVR32, UPVR33
	Hap 5	1	UPVR34
	Hap 6	32	UKVR21, HRVR2, HRVR4, HRVR5, HRVR7, HRVR10, HRVR11, HRVR12, HRVR13, HRVR14, HRVR15, HRVR16, HRVR17, HRVR20, HRVR22, HRVR23, HRVR24, HRVR25, MPVR1, UPVR1, UPVR5, UPVR6, UPVR8, UPVR9, UPVR16, UPVR17, UPVR19, UPVR20, UPVR21, UPVR23, UPVR25, BRVR2
	Hap 7	2	HRVR8, HRVR9
	Hap 8	2	HRVR18, HRVR26
	Hap 9	1	HRVR21
	Hap 10	1	MAVR1

	Hap 11	2	UPVR2, UPVR3
	Hap 12	1	UPVR7
	Hap 13	7	UPVR18, UPVR26, UPVR28, UPVR29, UPVR30, UPVR31, GJVR1
	Hap 14	2	VF, UPVR4
	Hap 15	1	HRVR1
	Hap 16	1	UPVR10
	Hap 17	1	BRVR1
	Hap 18	4	BRVR3, BRVR5, BRVR6, BRVR7
	Hap 19	2	VG, HRVR6
Cyt *b*	Hap 1	8	UKVR1, UKVR2, UKVR7, UKVR10, UKVR23, ASVR1, ASVR2, ASVR3
	Hap 2	3	UKVR3, UKVR6, UKVR9
	Hap 3	1	UKVR5
	Hap 4	1	UKVR8
	Hap 5	8	UKVR11, UKVR12, UKVR15, UKVR19, UPVR32, UPVR33, UPVR34, UPVR22
	Hap 6	2	UKVR13, UKVR18
	Hap 7	2	UKVR20, UKVR22
	Hap 8	28	UKVR21, HRVR2, HRVR4, HRVR5, HRVR7, HRVR10, HRVR11, HRVR13, HRVR18, HRVR20, HRVR21, HRVR22, HRVR23, HRVR24, HRVR25, HRVR26, MAVR1, MPVR1, UPVR1, UPVR5, UPVR6, UPVR8, UPVR9, UPVR16, UPVR17, UPVR21, UPVR23, BRVR2
	Hap 9	4	HRVR8, HRVR9, UPVR19, UPVR20
	Hap 10	1	HRVR12
	Hap 11	2	HRVR14, HRVR16
	Hap 12	1	HRVR15
	Hap 13	1	HRVR17
	Hap 14	2	UPVR2, UPVR3
	Hap 15	1	UPVR7
	Hap 16	6	UPVR18, UPVR26, UPVR28, UPVR29, UPVR31, GJVR1
	Hap 17	1	UPVR25
	Hap 18	1	UPVR30
	Hap 19	1	VF
	Hap 20	1	HRVR1
	Hap 21	1	UPVR4
	Hap 22	1	UPVR10
	Hap 23	1	BRVR1
	Hap 24	4	BRVR3, BRVR5, BRVR6, BRVR7
	Hap 25	1	VG
	Hap 26	1	HRVR6

Table 5: Summary of genetic diversity in Monitor lizard population based on mitochondrial DNA

Population	Gene	n	S	h	Hd	π
V. bengalensis (Lineage-I)	**CO*I***	25	6	7	0.697	0.00108
V. bengalensis (Lineage-II)		48	6	7	0.272	0.00034
V. bengalensis (Lineage-I+LineageII)		73	33	14	0.653	0.01134
V. flavescens		9	8	7	0.944	0.00267
V. koniecznyi		2	4	2	1	0.00409
V. bengalensis (Lineage-I)	**ND5**	25	6	9	0.643	0.00183
V. bengalensis (Lineage-II)		48	11	12	0.644	0.00136
V. bengalensis (Lineage-I+LineageII)		73	48	21	0.806	0.02424
V. flavescens		9	10	5	0.806	0.00720
V. koniecznyi		2	-	-	-	-
V. bengalensis (Lineage-I)	**Cyt *b***	25	6	7	0.797	0.00148
V. bengalensis (Lineage-II)		48	13	11	0.645	0.00155
V. bengalensis (Lineage-I+LineageII)		73	40	18	0.824	0.01597
V. flavescens		9	13	6	0.833	0.00580
V. koniecznyi		2	1	2	1	0.00123

Genetic signature

Among the dataset of 84 monitor lizard individuals, the sequences of *V. bengalensis* (n=73) has exclusively included further for the identification of examine the presence of genetic signature within *V. bengalensis*. Interestingly, in *V. bengalensis* I observed two genetic signatures which clearly differentiated the species into two different lineages: Lineage I and Lineage II. The details of SNP variation in both lineages were shown in Table 6. In total, 22 polymorphic sites which consisting 26 parsimoniously informative sites and 7 singleton sites were observed in COI (977bp) gene (Figure 6). In ND5 (668bp) gene, there were 34 polymorphic sites consisting 40 parsimoniously informative sites and 8 singleton sites (Figure 7). However, in Cyt *b* (814bp) gene, there were 23 polymorphic sites consisting 32 Parsimoniously Informative sites and 8 Singleton sites (Figure 8). Frequencies of nucleotide composition of different monitor lizard are represented in (Table 7).

Table 6: Polymorphic site within *V. bengalensis* from different mtDNA genes

Species	Gene	SNP position
*V. bengalensis*_Lineage I	COI	5240, 5285, 5330, 5405, 5474, 5558, 5594, 5861, 5900, 6059, 6089
*V. bengalensis*_ Lineage II		5411, 5738, 5891, 5909, 5945, 5954, 5960, 6002, 6023, 6062, 6110
*V. bengalensis*_Lineage I	ND5	13323, 13363, 13385, 13508, 13559, 13594, 13595, 13598, 13631, 13674, 13727, 13787, 13826, 13878, 13910, 13914, 13931
*V. bengalensis*_ Lineage II		13469, 13520, 13538, 13559, 13586, 13652, 13671, 13709, 13730, 13756, 13766, 13799, 13911, 13914,13917, 13922, 13928
*V. bengalensis*_Lineage I	Cyt *b*	14074, 14089, 14104, 14158, 14185, 14242, 14248, 14338, 14482, 14521, 14636, 14699, 14722
*V. bengalensis*_ Lineage II		14248, 14274, 14282, 14383, 14476, 14551, 14590, 14614, 14681, 14737

Table 7: Nucleotide composition of different monitor lizard species using mtDNA

Common Name	Scientific Name	No. of Sample	Gene	T(U)	C	A	G
Bengal monitor lizard- Lineage I	*V. bengalensis*	25	COI	27.9	30.3	30.1	11.6
			ND5	26.0	33.0	33.2	7.8
			Cyt *b*	27.9	30.3	30.1	11.6
Bengal monitor lizard- Lineage II	*V. bengalensis*	48	COI	27.0	31.3	30.2	11.6
			ND5	25.8	33.3	33.1	7.7
			Cyt *b*	27.0	31.3	30.2	11.6
Yellow monitor lizard	*V. flavescens*	9	COI	27.4	31.1	29.2	12.3
			ND5	27.5	31.8	32.1	8.6
			Cyt *b*	27.4	31.1	29.2	12.3
Desert monitor lizard	*V. koniecznyi*	2	COI	26.0	32.5	29.0	12.5
			ND5	22.6	37.5	31.2	8.6
			Cyt *b*	26.0	32.5	29.0	12.5

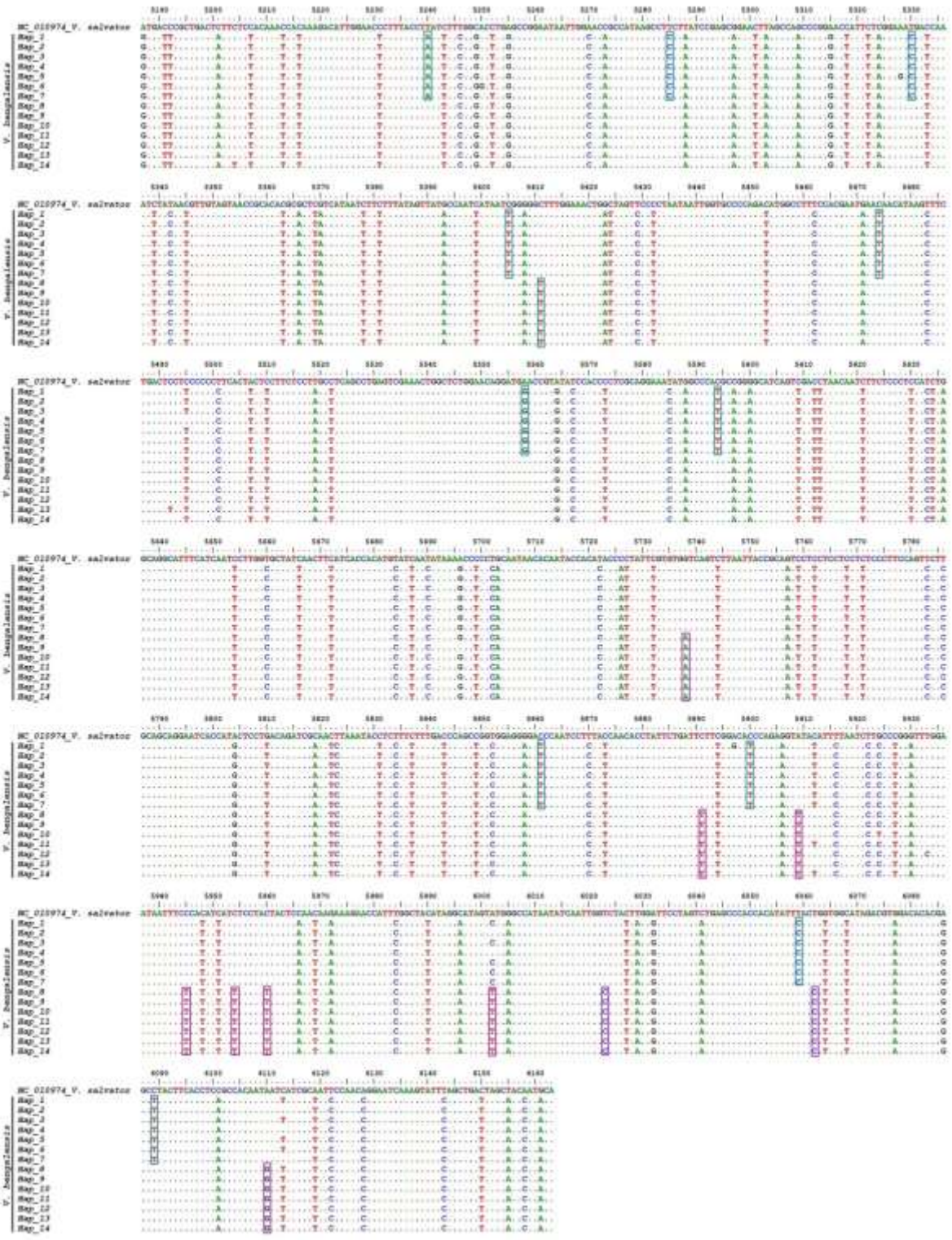

Figure 6: Single Nucleotide Polymorphism (SNPs) positions within *V. bengalensis* in CO*I* gene, nucleotide positions calculated based on the complete mitochondrial genome of *V. salvator* (NC010974)

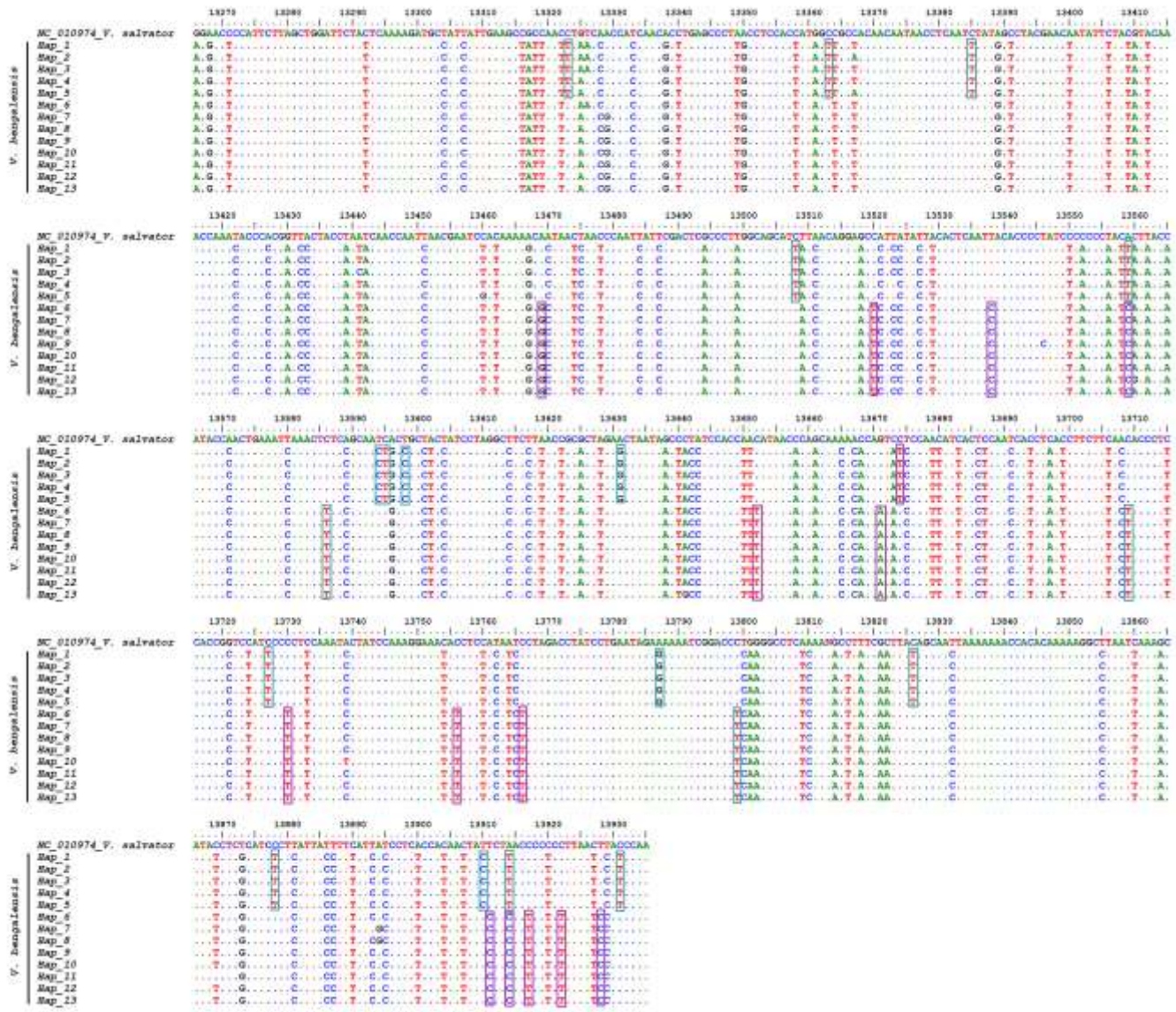

Figure 7: Single Nucleotide Polymorphism (SNPs) within *V. bengalensis* in ND5 gene, nucleotide positions calculated based on the complete mitochondrial genome of *V. salvator* (NC010974)

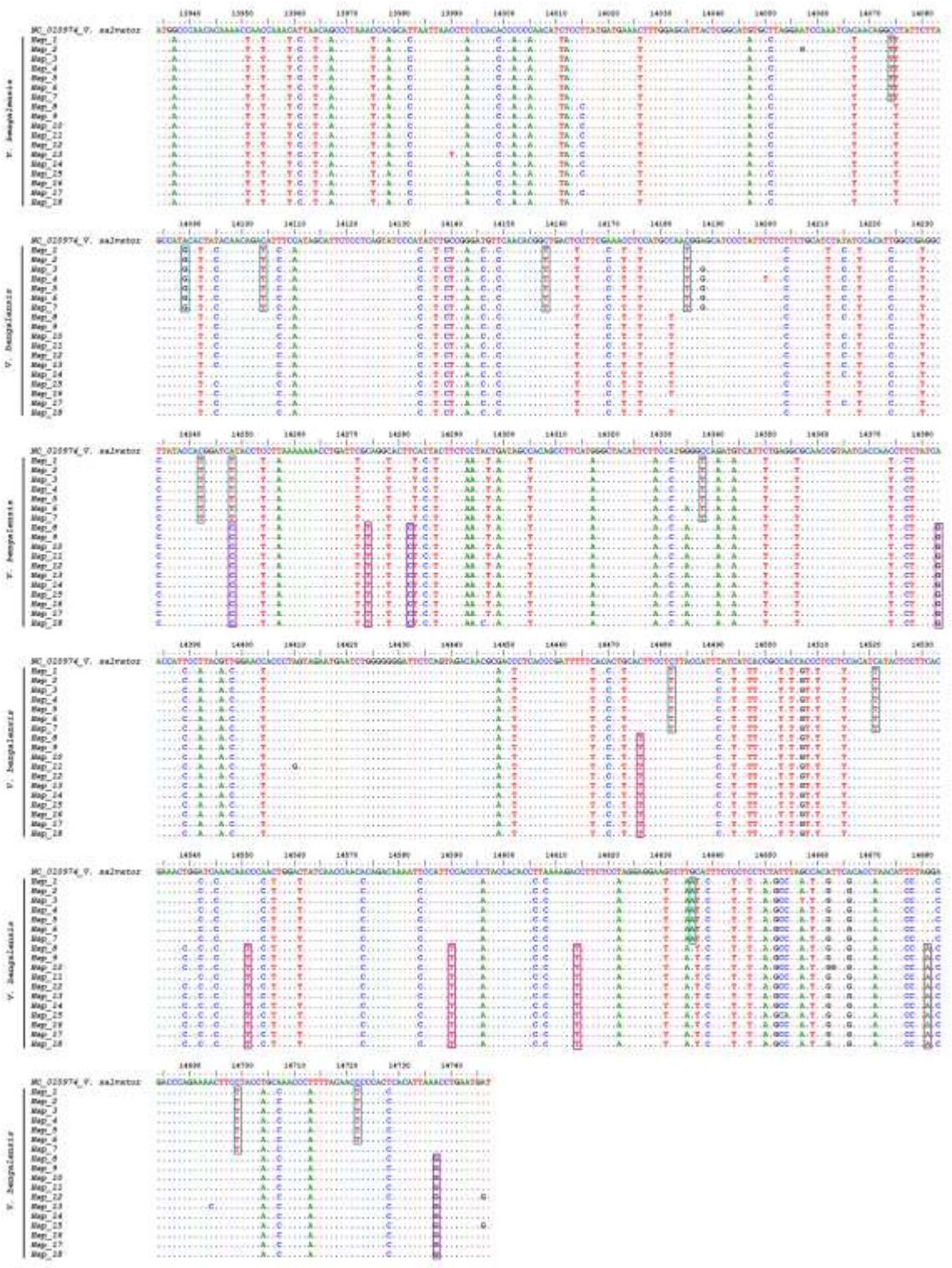

Figure 8: Single Nucleotide Polymorphism (SNPs) within *V. bengalensis* in Cyt *b* gene, nucleotide positions calculated based on the complete mitochondrial genome of *V. salvator* (NC010974)

Phylogenetic analysis

A Bayesian consensus tree of the whole data set was constructed for *Varanus* species, resulting in the same topology as maximum likelihood analysis. Therefore, the Bayesian phylogenetic tree for each gene with posterior probabilities values are presented (Figure 9, Figure 10 and Figure 11).

Phylogenetic trees derived from CO*I*, ND5 and Cyt *b* genes indicated well-supported clades with posterior probability values (>0.6). The resulting tree of ND5 and Cyt *b* genes showed the presence of two well-supported lineages within *V. bengalensis*: Lineage-I and Lineage-II. Conversely, the resultant tree of CO*I* gene showed the presence of three well-supported clades within *V. bengalensis*: Lineage-I and Lineage-II show congruence with the ND5 and Cyt *b* phylotrees, whereas Lineage-III consisted of the sequences from South India taken from NCBI database. Due to the unavailability of ND5 and Cyt *b* sequences from South India, we could not further assess their evolutionary relationships. Our phylogenetic analyses indicate the presence of two distinct clades within *V. bengalensis*. Lineage-I is majorly spread across in the Uttarakhand region in conjunction with some and adjoining areas in Uttar Pradesh, Assam and Arunachal Pradesh. Lineage-II covers the species' distribution in the Indian states of Haryana, Uttar Pradesh, Bihar, Gujrat, Madhya Pradesh and Maharashtra. Lineage-I is restricted to foothills of Himalaya and illustrates a distinct signature. The placement of the *V. bengalensis* sequences of South India at a basal position in CO*I* gene indicates the presence of distinct lineages of *V. bengalensis,* which warrants further extensive pan-Indian research. The other two species, *V. flavescens* and *V. koniecznyi* also shows well supported clades in all three phylotrees. Further, we calculated pairwise genetic differentiation based on analysed three mitochondrial genes. The result suggested clear genetic differentiation between Lineage-I and Lineage-II of *V. bengalensis*

with 1.4%, 6.3% and 3.8% in CO*I*, ND5 and Cyt *b* gene, respectively. Additionally, the genetic differentiation between Lineage-I and Lineage-II with Lineage-III of *V. bengalensis* through CO*I* gene is 2.4% and 2.7%, respectively (Table 8 and Table 9).

Species delimitation

The mPTP, bPTP and GYMC analyses suggested four independent clades in our dataset, namely Lineage-I, Lineage-II, Lineage-III in *V. bengalensis*, whereas *V. flavescens* and *V. koniecznyi* formed their unique clade (Figure 9, Figure 10 and Figure 11). All three analyses delimited the same taxonomic units as inferred from BEAST phylogenetic analysis. The *V. bengalensis* sequence KF766940 and MN148451 in Cyt *b* and CO*I* gene, respectively showed exceptions and delimited as different units. This subdivision is probably caused due to the shorter length of these sequences. The results sustain previously recognized taxonomic divisions and provide new insights corroborated by our analyses.

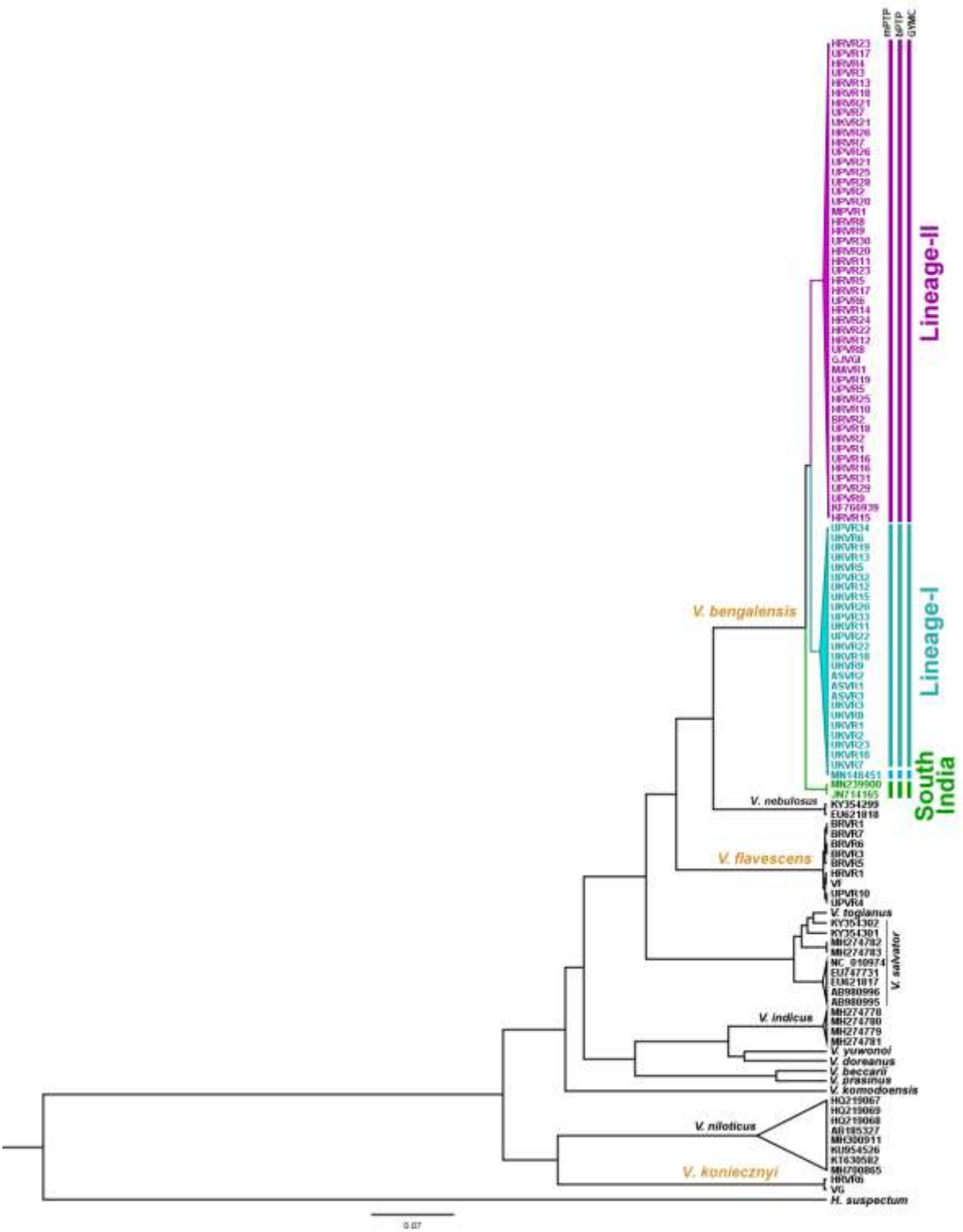

Figure 9: Bayesian inference (BI) of phylogenetic tree for genus *Varanus* based on CO*I* gene. Tree also represents the result of three different molecular species delimitation methods

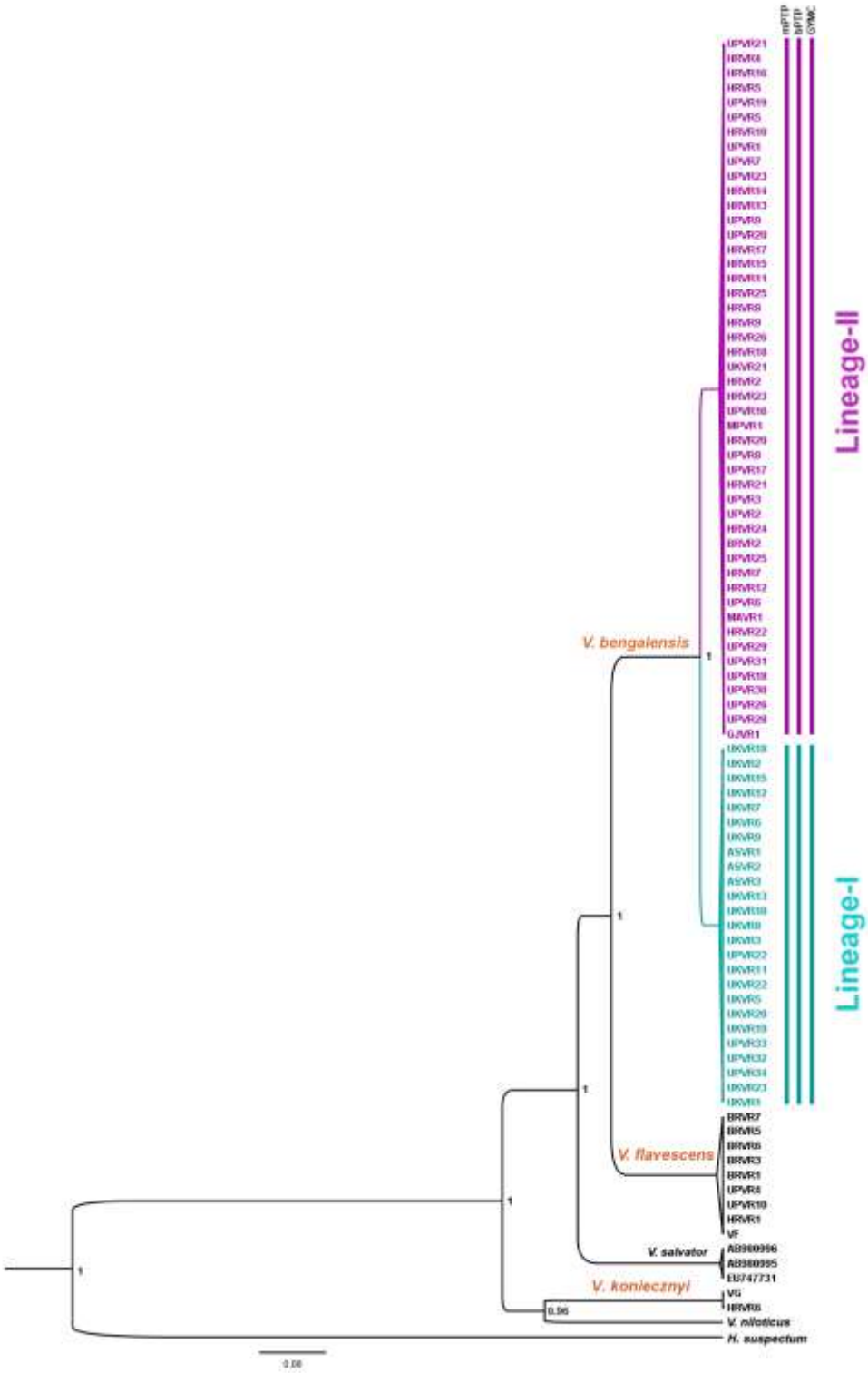

Figure 10: Bayesian inference (BI) of phylogenetic tree for genus *Varanus* based on ND5 gene. Tree also represents the result of three different molecular species delimitation methods

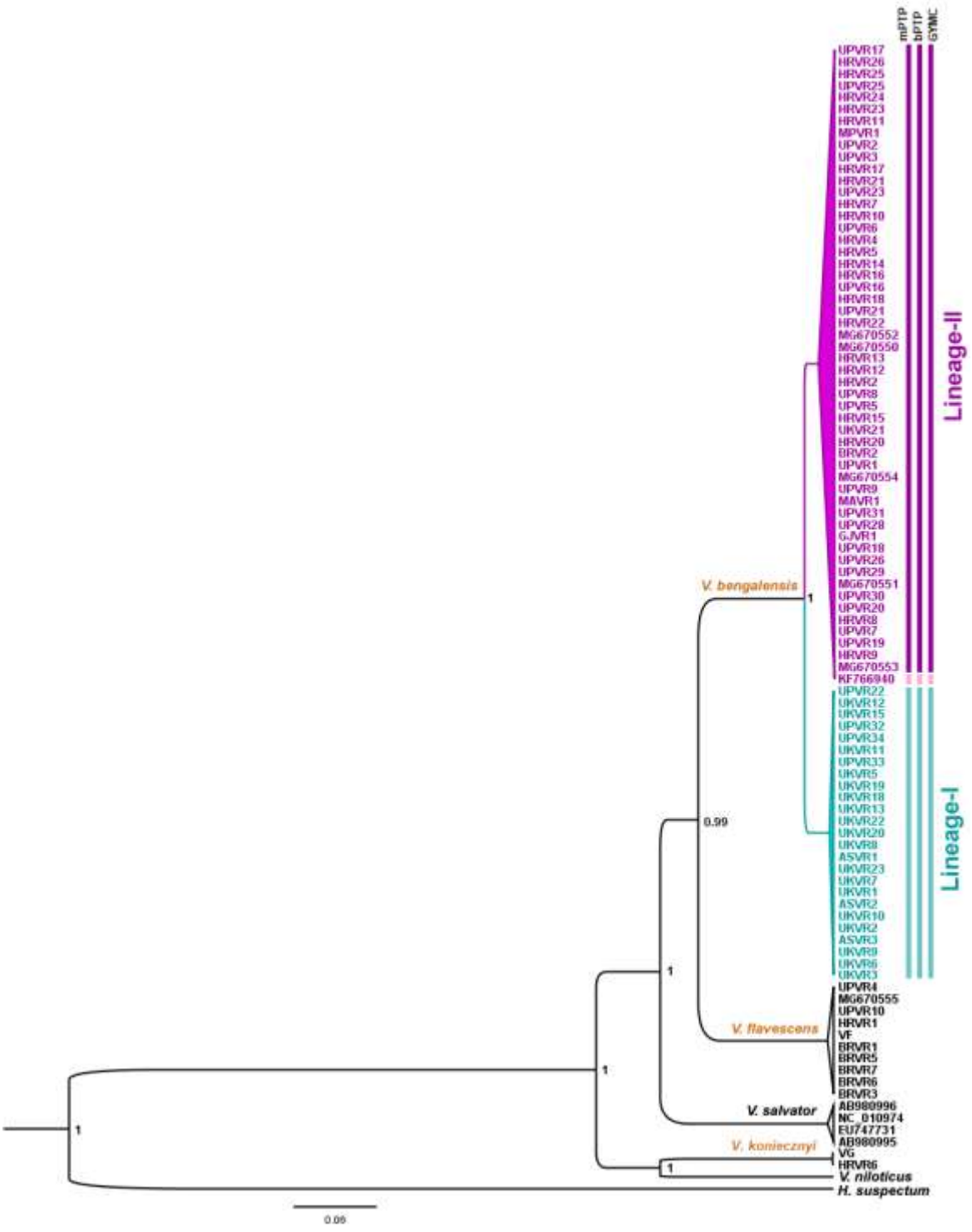

Figure 11: Bayesian inference (BI) of phylogenetic tree for genus *Varanus* based on Cyt *b* gene. Tree also represents the result of three different molecular species delimitation methods

Table 8: Genetic differentiation among *V. bengalensis* and other monitor lizard species from CO*I* gene

Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14
V. bengalensis_lineage-I														
V. bengalensis_lineage-II	**0.014**													
V. bengalensis_lineage-III	**0.024**	0.027												
V. nebulosus	0.147	0.150	0.140											
V. flavescens	0.194	0.169	0.196	0.232										
V. koniecznyi	0.218	0.205	0.209	0.224	0.257									
V. beccarii	0.227	0.209	0.240	0.235	0.235	0.274								
V. doreanus	0.207	0.207	0.204	0.260	0.235	0.291	0.233							
V. indicus	0.262	0.251	0.241	0.284	0.223	0.263	0.205	0.173						
V. komodoensis	0.234	0.234	0.235	0.242	0.225	0.249	0.286	0.266	0.243					
V. niloticus	0.264	0.259	0.253	0.268	0.242	0.236	0.314	0.247	0.218	0.233				
V. prasinus	0.224	0.206	0.228	0.227	0.214	0.269	0.082	0.217	0.212	0.268	0.246			
V. salvator	0.201	0.191	0.191	0.201	0.187	0.200	0.247	0.225	0.235	0.283	0.243	0.229		
V. togianus	0.188	0.180	0.187	0.198	0.178	0.213	0.224	0.239	0.236	0.292	0.257	0.231	0.036	
V. yuwonoi	0.205	0.213	0.187	0.224	0.232	0.261	0.206	0.115	0.138	0.228	0.251	0.186	0.226	0.248

Table 9: Genetic differentiation among *V. bengalensis* and other monitor lizard species from ND5/Cyt *b* gene

Species	1	2	3	4	5
*V. bengalensis_* lineage -I					
*V. bengalensis_* lineage -II	**0.063/0.038**				
V. flavescens	0.356/0.187	0.321/0.202			
V. koniecznyi	0.518/0.286	0.559/0.286	0.544/0.312		
V. niloticus	0.610/0.306	0.627/0.331	0.553/0.378	0.485/0.269	
V. salvator	0.370/0.217	0.382/0.233	0.372/0.242	0.471/0.283	0.499/0.333

Discussion

This chapter generated the baseline novel genetic database of *V. bengalensis*, *V. flavescens* and *V. koniecznyi* from India. The study site for this chapter i.e. Terai Arc landscape, historically has had dense forest harboring a variety of herpetofauna. Extensive land use and habitat change with the extension of agricultural activities have increasingly encroached wild habitats (Semwal, 2005). This habitat degradation and alteration, along with wildlife-human conflicts, has led to wild stock significantly dwindling in the region. However, despite the ongoing population decrease, the region still holds rich biodiversity due to its varied climatic types and landscape features, and the species' natural adaptation to the changing landscape (Johnsingh et. al., 2004). Being highly cosmopolitan, *V. bengalensis* thrives in this heavily human dominated region across multitudes of habitat niches. High anthropogenic pressure and associated conflict between human and *V. bengalensis* in the region has made its local communities the prime stakeholders to efficiently conserve the threatened species. The Terai landscape falls under the administrative states of northern India having good transportation connectivity with railways, roadways and airways making this region a hub for translocation of the wildlife trafficked articles (Mendiratta et al., 2017). The trans-boundary landscape between Nepal and India is also porous (Pers. Obs.). These factors make this region a hub for the translocation of wildlife articles and necessitate the thorough study for tracking of trade which will aid enforcement agencies.

Wildlife crime encompasses two key issues concerning the formulation of legislation. The first pertains to accurately identifying specific species being traded, while the second concerns confidently linking biological material to individual members of those species. This chapter specifically addresses the second issue. Wildlife forensics now encompasses a broad and potent array of technological, methodological, and analytical tools and resources which aims at

identifying traded species and potentially determining and analyzing trace derivatives (Comstock et al., 2003; Gupta et al., 2006). However, there remains a gap in accurately identifying many taxa through genetic approaches, particularly in pinpointing their origins with greater precision (Welton et al., 2013). This particular lacuna in the field of wildlife forensics can be ameliorated with two approaches: firstly, by generating genetic data from natural wild populations across the species' distribution, and secondly, by utilizing the standardized set of mitochondrial and nuclear markers to ensure consistency and accuracy.

In this chapter, I analyzed the genetic signature of *V. bengalensis*. The results revealed distinct single nucleotide polymorphisms (SNPs) that differentiate *V. bengalensis* into three genetically distinct lineages. The lineages within *V. bengalensis* can be identified based on their different genetic signature that present in three mitochondrial genes i.e. COI, ND5 and Cyt *b* (Table 6). The phylogenetic analysis of the dataset (n=84) reveals several distinct clades, including *V. bengalensis*-Lineage-I, *V. bengalensis*-Lineage-II, *V. bengalensis*-Lineage-III, *V. flavescens*, and *V. koniecznyi*, based on COI sequences. However, the analysis of ND5 and Cyt *b* sequences identified only two lineages within *V. bengalensis*, likely due to the unavailability of South Indian sequences in the NCBI database. The level of genetic distinctiveness between the three lineages of *V. bengalensis* found in this study compared with the levels among existing recognized monitor lizard species supports the presence of the distinct lineages. The findings of this chapter established a geo-referenced database for monitor lizards from the Terai arc and associated landscapes in India. This database will assist future analysts in identifying species and lineage assemblages to determine whether traded articles originated from this region or not. Similar studies on African elephants reveals notable variations over small geographic areas, allowing for finer distinctions within wild populations and this dataset used to trace the origin of

the largest ivory seizure (Wasser et al., 2004; Wasser et al., 2007). Ghobrial et al. (2010) generated geo-referenced genetic database of chimpanzee in Cameroon to uncover 'hotspots' of chimpanzee hunting and routes for transporting live animals. Molecular genetic variation and phylogenetic analysis approach has been unitized to determine the origin of ‘whale’ products in retail markets in Japan and the Republic of (South) Korea (Baker et al., 2000). Thus, the geo-referenced genetic dataset established in this chapter will serve as a fundamental resource for wildlife forensic analysis of monitor lizard derivatives.

Chapter-3

To inventories the *Varanus* biological samples at Wildlife Institute of India for determining their species, phylogenetic status and genetic variations

Background

Trade in mammalian species attract the significant concern from the larger public, however in reality the trade in reptiles and amphibians surpasses them due to their smaller size, making them easier to conceal and evade detection (Linacre and Tobe, 2011). Concerns regarding the over-exploitation of reptiles in the pet trade have been a topic of discussion since the late 1960s (Lambert, 1969; Spellerberg, 1976). Recent studies have focused on the unsustainable exploitation of reptile species, highlighting intentional harvesting as the second greatest threat to their survival (Auliya et al., 2016). Popular literature has also shed light on illegal reptile trade activities, detailing its scope and the individuals involved (Böhm et al., 2013).

The international trade of Bengal monitor lizard recorded by CITES highlighted the large volume of traded derivatives reflecting the over-harvesting of the species. Based on the CITES trade database spanning 1975 to 2018, Bengal monitor lizards were primarily traded for their skins, followed by leather products, live individuals, and to a lesser extent, shoes, skeletons, and other body parts (Figure 12). However, it is important to note a limitation of this database that the trade records for Bengal monitor lizards only extend until the year 2014. Meanwhile, a new wildlife product has emerged in both international and national retail and e-commerce markets, known as ‘Hatha Jodi’. The name ‘Hatha Jodi’ is a fusion of two Hindi language words: ‘Hatha’ (arm) and ‘Jodi’ (pair), meaning paired arm. Traditionally, ‘Hatha Jodi’ is believed to be the plant root of *Martynia annua*, also known as baghnakhi or tiger’s claw, which resembles a human arm with clenched fists. However, instead of the root of *Martynia annua*, intromittent organ of *Varanus* spp. (monitor lizard) has been utilized for the trade and identified by DNA sequencing (Sharma et al., 2019). The intromittent organ (hemipenis) of class Reptilia can be

identified by the presence of paired horn on the distal most end of the lobes, which corresponds to the widest region possessing discontinued transverse frill with groove on one side and having nude stalk region continues with body scales attached at the base portion (Gray, 1870; Cope, 1896; Branch, 1982).

Wildlife Crime Control Bureau (WCCB) a statutory multi-disciplinary body established by the Government of India under the Ministry of Environment, Forests and Climate Change (MoEF&CC) has a mandate to combat organized wildlife crime in the country. WCCB organized few Operations namely "WILDNET" (1st May 2017 to 30th June 2017) and LESKNOW II (1st September 2018 to 30th September 2018) where the agency confiscated 42 and 127 'Hatha Jodi' respectively. These seizures along with many other seizures from different enforcement agencies (Custom, Forest department etc.) were sent to Wildlife Forensic and Conservation Genetic Cell (WFCGC), Wildlife Institute of India. The Wildlife Institute of India (WII), an autonomous institute under MoEF&CC, houses the Wildlife Forensic and Conservation Genetics Cell (WFCGC), comprising a specialized team of scientists and researchers. This unit operates state-of-the-art facilities dedicated to conducting research and analysis, producing scientific reports and protocols in the fields of forensics and conservation. The primary functions of the WFCGC include conducting research to improve wildlife forensics protocols, identifying species from diverse wildlife parts and products to aid in enforcement efforts, and establishing a repository for maintaining wildlife reference samples. Therefore, the WFCGC received numerous seizures from various enforcement agencies across the country. Derivatives of monitor lizards include hatha Jodi, claws, cooked meat, skeleton remains, tongues, leather products, and musical instruments (Figure 13). Analyzing each confiscated article of monitor lizard through physical or

morphological characteristics is challenging. Therefore, DNA forensics is crucial for precise identification of the species.

In this chapter, the biological samples derived from monitor lizards were inventoried and identified at the Wildlife Institute of India. This chapter highlights the relatedness of a significant number of seizures to the reference database to track the origin of species. The study involves technique using the mitochondrial Cytochrome *b* gene to determine species identity, phylogenetic status, and genetic variations.

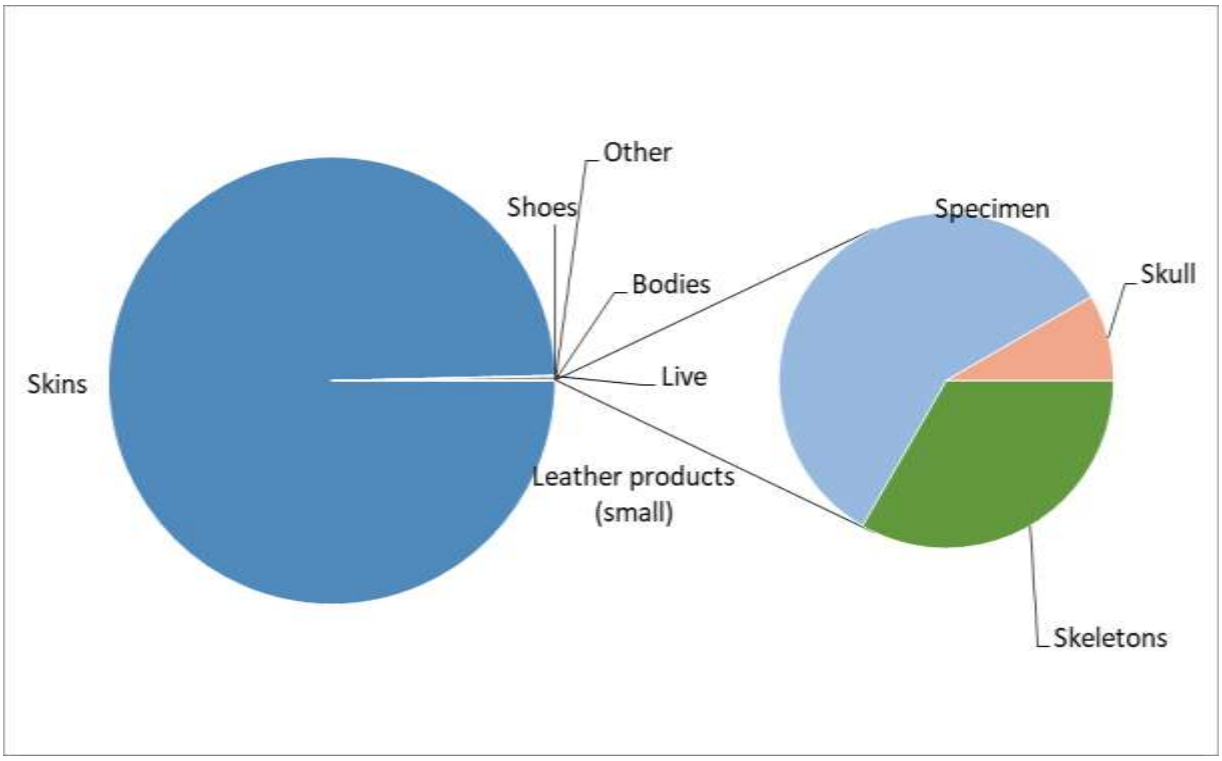

Figure 12: Trade article of Bengal Monitor Lizard (*V. bengalensis*) recorded in CITES (1975-2018)

Figure 13: Confiscated article of monitor lizard received at WFCGC, Wildlife Institute of India (A: Dhol [Drum-Musical instrument], B: Hatha Jodi, C: Musical instrument & D: Purse)

Methodology

Sample collection and DNA extraction

The biological samples seized from different enforcement agencies consists body parts of suspected monitor lizards. The sample type ranges from hemipenis, skin cast, claw, cooked meat etc. The samples (n=114) used in this objective were tagged and details are in Table 10. The gDNA was extracted by using Phenol Chloroform: Isoamyl alcohol extraction method (Sambrook et al., 1989) in a final elution volume of 100 µl.

PCR amplification and sequencing

The Cytochrome *b* region of mitochondrial genome is being targeted and amplified. The universal primer designed by Kocher et al. (1989) is used to amplify the Cyt *b* region. The PCR reactions were performed in 20 µl reaction volumes using 1 × PCR buffer (10mM Tris–HCl, pH8.3, and 50mM KCl), 1.5mM $MgCl_2$, 0.2mM of each dNTPs, 3 pmol of each primer, 0.5 units of DreamTaq DNA Polymerase (Thermo Scientific) and 1 µl (~30 ng) of template DNA. The PCR protocol was used as follows: initial denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 45 sec, annealing at 55°C for 40 sec and extension at 72 °C for 75 sec. The final extension was at 72 °C for 10 min. Each reaction included negative controls to verify the reliability of the experiment. All PCR amplification was confirmed by electrophoresis on 2% agarose gel stained with Ethidium bromide and visualised under a UV transilluminator. The amplified PCR products were purified with exonuclease-I and shrimp alkaline phosphatase (Thermo Scientific Inc.) at 37°C for 20 min to remove any remaining primer and dNTPs, followed by inactivation of enzymes at 85 °C for 15 min. The purified fragments were sequenced

directly in an Applied Biosystems Genetic Analyzer 3500 XL (Applied Biosystems) from the forward and reverse directions using BigDye 3.1 Kit. To check the alignment and the presence of stop codon, generated sequence of Cyt *b* gene were subject to translation into amino acids using Expasy: Translate tool (Gasteiger et al., 2003).

Data Analysis

The amplicons were sequenced for partial region of Cyt *b* gene of mitochondria for confiscated samples (n=114) belongs to the twelve seizures. Sequences obtained were edited manually by confirming electropherograms and assembled using SEQUENCHER® 4.9 (Gene Codes Corporation, Ann Arbor, MI, USA). A BLAST (Altschul et al., 1997) search was done with the generated sequences for Cyt *b* gene for species identification. DnaSP v6 (Rozas et al., 2017) was used to compute the number of haplotypes among the generated dataset. The published dataset of Gautam et al. (2023) was included to increase the robustness of the analyses and to reconstruct the phylogenetic relationships. All the sequences were aligned using the Clustal W program (Thompson, 1994) by BioEdit 7.1.3 (Hall, 1999) with default settings. The alignment was manually optimized, and ambiguous regions were excluded prior to phylogenetic analyses. The data matrix was analyzed in MEGA X (Kumar et al., 2018) to select a suitable model for maximum likelihood analysis. A Monte Carlo Markov Chain (MCMC) based Bayesian consensus tree was generated using BEAST 1.7 (Drummond et al., 2012). Bayesian inference analyses employed the HKY+G+I model for Cyt *b* genes, with MCMC chains running for 10 million generations, sampling every 1000 generations, and discarding the first 10% of runs as burn-in. The analyses were assessed using Tracer 1.6, and resulting phylogenetic trees were visualized using FigTree 1.4.0 (http://tree.bio.ed.ac.uk/software/figtree/). To understand the

spatial distribution of haplotypes from confiscated samples, a haplotype network was constructed using the TCS network method in PopART v 1.7 (Leigh and Bryant, 2015).

Table 10: Details about confiscated samples by enforcement agency

S. No.	Sample ID	Confiscated by enforcement agency of	Year	Haplotype Number
1	VH1	Bhubaneswar, Odisha	2017	4
2	VH2			5
3	VH3			6
4	VH4			4
5	VH5			7
6	VH7			8
7	VH8			9
8	VH12			10
9	VH13			11
10	VH15			12
11	VH25			10
12	VH26			13
13	VH27			14
14	VH28			15
15	VH29			10
16	VH30			9
17	VH33			10
18	VH35			5
19	VH36			5
20	VH37			16
21	VH39			5
22	VH41			10
23	VH42			5
24	VH43			5
25	VH45			10
26	VH46			10
27	VH47			10
28	VH48			10
29	VH49			10
30	VH50			5
31	VH51			5
32	VH52			17
33	VH53			10
34	VH54			10
35	VH55			10
36	VH56			18
37	VH58			19
38	VH59			10
39	VH60			13
40	VH64			4
41	VH86			4
42	VH98			3
43	VH99			4
44	VH100			4
45	VH101			3
46	VH109			3
47	VH214	Kaithal, Haryana	2018	4
48	VH215			4

49	VH217			4
50	VH218			4
51	VH219			4
52	VH220			4
53	VH221			4
54	VH223			3
55	VH224	Ghaziabad, Uttar Pradesh	2018	11
56	VH225			11
57	VH227			3
58	VH228			11
59	VH230			11
60	VH231			1
61	VH233	Dehradun, Uttarakhand	2018	3
62	VH234			2
63	VH235	Udandasta, Bikaner, Rajasthan	2018	3
64	VH238			3
65	VH240			3
66	VH241			3
67	VH242	Amazon	2017	4
68	VH245	Ebay	2017	4
69	VH247	Hansol Nursery, Ahmendabad, Gujarat	2017	4
70	VH248			4
71	VH249	Dausa, Rajasthan	2018	4
72	VH250			4
73	VH251	Jhunjnu, Rajasthan	2018	3
74	VH253	Meerut, Uttar Pradesh	2018	4
75	VH254			20
76	VH258			20
77	VH303	New Delhi	2018	4
78	VH304			11
79	VH305			4
80	VH306			4
81	VH307			3
82	VH333			11
83	VH334			11
84	VH335			4
85	VH336			11
86	VH337			3
87	VH338			11
88	VH339			4
89	VH341			3
90	VH342			3
91	VH343			11
92	VH344			3
93	VH349			11
94	VH350			3
95	VH351			3
96	VH353			4
97	VH354			4
98	VH355			4
99	VH359			4
100	VH360			3
101	VH363			4
102	VH365			11
103	VH369			4
104	VH376			4
105	VH382			11
106	VH384			11
107	VH385			11

108	VH387			3
109	VH388			11
110	VH393			4
111	VH396			3
112	VH397			4
113	VH402			3
114	VH491	Mithila, Bihar	2020	21

Results

Genetic signature

The biological samples were identified *V. bengalensis* and *V. flavescens* in the BLAST tool from NCBI database. Further, a total of 21 haplotypes were identified in the comprehensive dataset of 114 confiscated samples from total twelve seizures (Table 11). The 21 haplotypes sequences of Cyt *b* gene were submitted to NCBI. The two core haplotypes among the twelve seizures are haplotypes 3 and 4. Seizures 1 and 11 are characterized by heavy volumes, with seizure 1 containing 17 different haplotypes, while seizure 11 is grouped into 3 haplotypes. Additionally, there are 13 haplotypes that each is represented by a single sample.

Table 11: Cyt *b* haplotypes and number of samples with each haplotype in twelve confiscations

	Total (n)	H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12	H13	H14	H15	H16	H17	H18	H19	H20	H21
Seizure1	46	-	-	3	6	8	1	1	1	2	14	1	1	2	1	1	1	1	1	1	-	-
Seizure2	8	-	-	1	7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Seizure3	6	1	-	1	-	-	-	-	-	-	-	4	-	-	-	-	-	-	-	-	-	-
Seizure4	2	-	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Seizure5	4	-	-	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Seizure6	2	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Seizure7	2	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Seizure8	2	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Seizure9	1	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Seizure10	3	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-
Seizure11	37	-	-	11	14	-	-	-	-	-	-	12	-	-	-	-	-	-	-	-	-	-
Seizure12	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1

Phylogenetic analysis

The phylogenetic analysis of confiscated samples was performed with the published reference sample (Accession no.: OP141809, OP141846, OP141876 and OP141877) (Gautam et al., 2023). A Bayesian consensus tree of the whole data set was constructed for identification of confiscated articles, resulting in the same topology as maximum likelihood analysis. Therefore, the Bayesian phylogenetic tree for Cyt *b* gene with posterior probabilities value is presented (Figure 14). The seized samples are genetically assigned to *V. bengalensis*-Lineage I, *V. bengalensis*-Lineage II and *V. flavescens*.

The resulting phylogenetic tree showed the matching of confiscated samples with *V. bengalensis* and *V. flavescens*. The clade of *V. bengalensis* is sub-divided into two namely Lineage-I and Lineage-II. Among 114 confiscated samples two samples were clustered with *V. bengalensis* Lineage-I however 111 articles were clustered with *V. bengalensis* Lineage-II. Only one article clubbed with the *V. flavescens*. No article had a matching with *V. koniecznyi*.

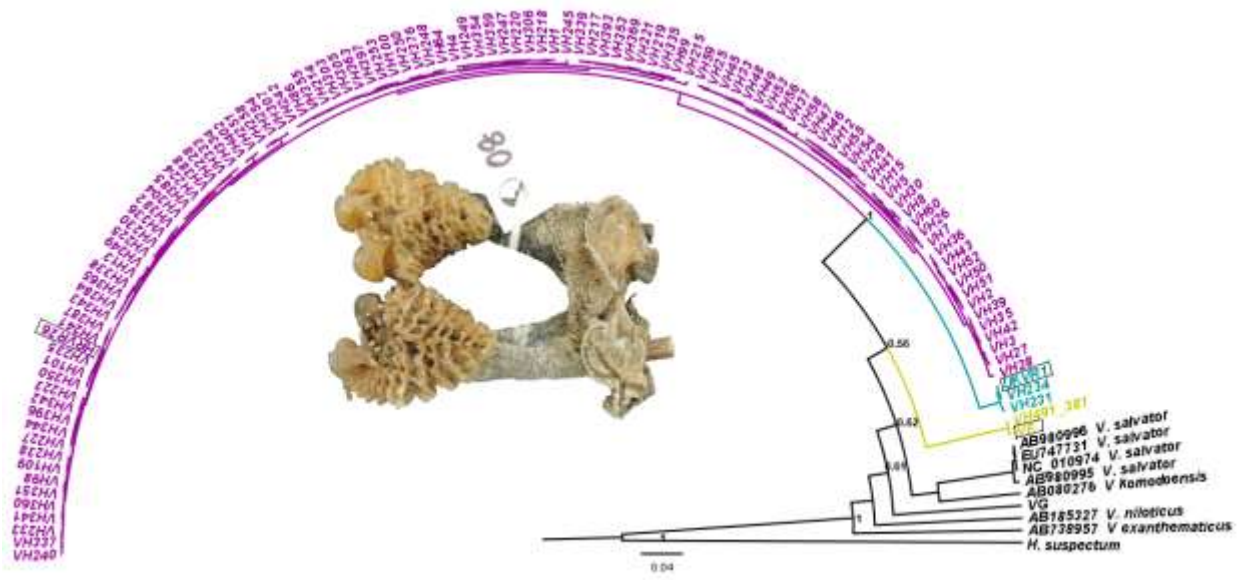

Figure 14: Bayesian inference (BI) of phylogenetic tree for confiscated samples based on Cyt *b* gene. Reference sample of different monitor lizard is in black box

A TCS Network was constructed with the dataset of Cyt *b* gene that comprises references database generated in chapter 2 and dataset of this chapter comprises confiscated samples. Interestingly, the spatial distribution of haplotypes from confiscated samples closely aligned with the phylogenetic relationships observed among the distinct clades of monitor lizards. The TCS network showed two major and one minor cluster corresponding to the phylogenetic clades: *V. bengalensis* Lineage-I, *V. bengalensis* Lineage-II and *V. flavescens*. The *V. bengalensis* Lineage-I has a significant divergence from *V. bengalensis* Lineage-II by ten mutational steps (Figure 15). On the other hand, *V. flavescens* group has noteworthy divergence from *V. bengalensis* group by twenty-two mutational steps. Additionally, there were moderately fewer substitutions separating

sub-groups within the major groups, indicating genetic coherence within groups of *V. bengalensis* Lineage-I, *V. bengalensis* Lineage-II and *V. flavescens*.

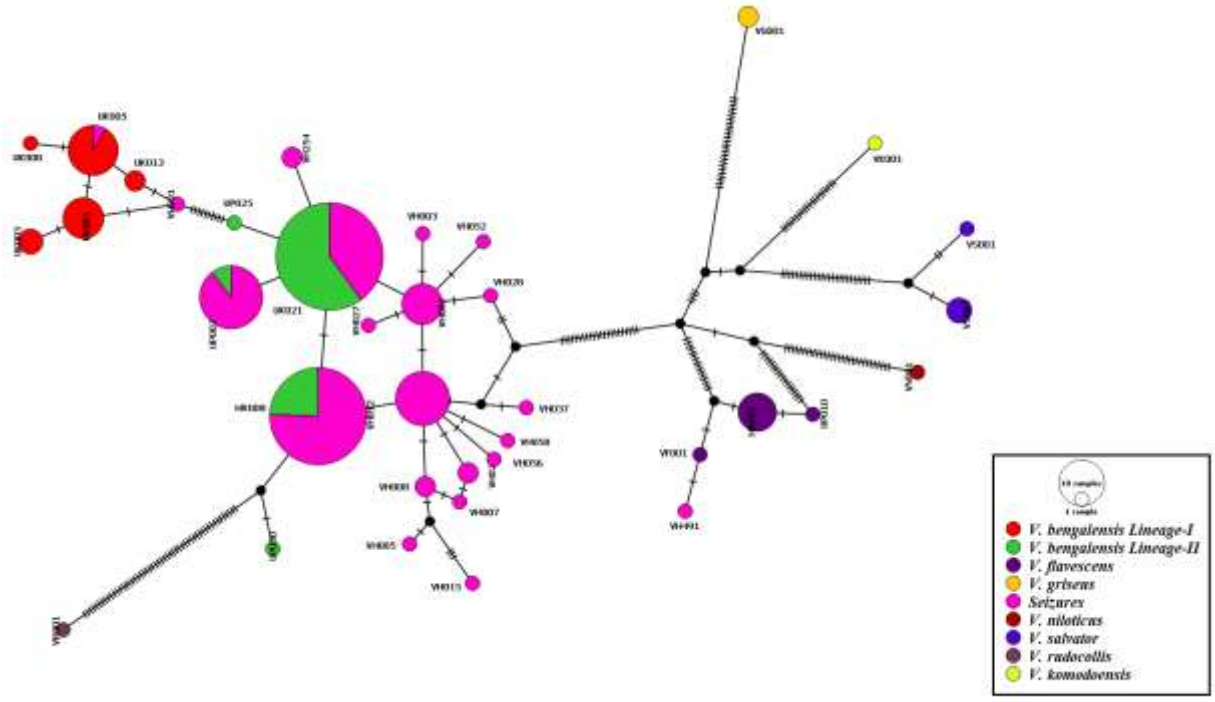

Figure 15: Confiscated and reference haplotype network based on TCS method for the 302 bp mtDNA fragment of Cyt *b*. Circles denote individual haplotypes, with their sizes indicating the relative abundance of each haplotype

Genetic variations

The dataset of confiscated sample from Cyt *b* gene has been used to analyse genetic diversity. All the sequences are grouped into three genetically identified clusters indicating the maternal relatedness among the Monitor lizard namely *V. bengalensis* Lineage-I, *V. bengalensis* Lineage-II and *V. flavescens* (Table 12). According to Cyt *b* gene, the nucleotide diversity of all confiscated samples π = 0.00922 (s.d. = 0.0002). However, when segregating the confiscated samples into cluster the nucleotide diversity in samples showing affinity with *V. bengalensis*

Lineage-I is π = 0.00662 (s.d. = 0.0033) and *V. bengalensis* Lineage-II is π = 0.00543 (s.d. = 0.0004).

Table 12: Summary of genetic diversity in confiscated samples of monitor lizard based on mitochondrial DNA

Population	**Gene**	**n**	**S**	**h**	**Hd**	**π**
All samples	Cyt *b*	114	61	21	0.837	0.00922
Samples matching with *V. bengalensis* (Lineage-I)		2	2	2	1	0.00662
Samples matching with *V. bengalensis* (Lineage-II)		111	18	18	0.828	0.00543

Discussion

The analysis of squamate external genitalia provides useful and necessary information on systematics (Cope, 1896). The hemipeneal morphology revealed different structure of four extant monitor lizard of India (Branch, 1982). Branch, (1982) describes the structure of *V. bengalensis* hemipenis as follows: three "claspers", shallowly forked; the sulcus is undivided and drains into a nude apical region between the horns. This morphological method could be used to identify the species from hemipenis in trade under the false name of ‘Hatha Jodi’ however the traded articles are in poor condition to be identified morphologically. The DNA sequencing method utilized by Sharma et al. (2019) was useful enough to identify the species of monitor lizard involved in trade. Although according to the phylogenetic analysis of reference of geo-tagged individual of monitor lizard revealed that there are two different lineages within Bengal monitor lizard. The discovery of distinct lineages of *V. bengalensis* calls into question the utility of the morphological technique for identifying hemipenis based solely on structure. Hence, to identify the traded article of monitor lizard, the use of DNA sequencing is essential.

The phylogeny of the cytochrome *b* gene demonstrates the genetic distinction between the confiscated seizures. The results are consistent with TCS analysis, indicating that the traded products from twelve seizures correspond to three distinct phylogenetic clades: *V. bengalensis* Lineage-I, *V. bengalensis* Lineage-II, and *V. flavescens*. The water monitor lizard (*V. salvator*), which has a lengthy history of international trade, was not found in any of the seizures investigated in this chapter. The small distribution range of *V. koniecznyi* can be the reason of less trade in market of 'Hatha Jodi'. The trade of *V. flavescens* is considered negligible among the confiscated sample analyzed, as only a single confiscated hemipenis from Mithila, Bihar, was identified as such. The majority of confiscated items are from *V. bengalensis*, which is widely distributed species in South and South East Asia. Within *V. bengalensis*, majority of articles were from lineage-II, however only two articles were identified as Lineage-I. Hence, the grave exploitation of monitor lizards' genitalia "hemipenis" under the pseudonym 'Hatha Jodi' indicated pressure of trade is huge on the *V. bengalensis* lineage-II. All confiscated samples exhibited high genetic diversity. Due to the limited sample size of *V. flavescens* in the dataset, the analysis focused solely on the genetic diversity of *V. bengalensis*. The samples clustering within *V. bengalensis* showed significant genetic diversity. This suggests that the current population of *V. bengalensis* is genetically healthy, with a wide range of genetic variations that can potentially aid in the species' adaptability and resilience to environmental changes. However, the increasing poaching activity poses a serious threat to the wild population of *V. bengalensis*. Poaching can lead to a rapid decline in population size, resulting in a bottleneck effect. This reduces genetic diversity as the gene pool shrinks, making the species more vulnerable to diseases, environmental changes, and reduced reproductive success. If this hunting pressure continues unabated, the *V. bengalensis* population will likely experience a significant loss of

genetic diversity. This decline in genetic variability can lead to inbreeding, further endangering the species and potentially pushing it towards extinction.

The extensive poaching and trade network poses a significant threat to the Bengal monitor lizard population in India. Habitat destruction, coupled with increasing competition and resource scarcity, has led to intensified human-animal encounters, directly threatening the local viability of the species. The Terai Arc Landscape (TAL) has been identified as a major conduit for the illegal wildlife trade, including the trafficking of *Varanus* hemipenis. This area is also a zone of overlap for Bengal monitor lizard and experiences intense human pressure (Alagesan, 2020; WCCB).

The status and impact of hunting on Bengal monitor lizard is poorly understood, and the lack of fundamental data hinders targeted conservation efforts. This underestimation of the true extent of species distribution and exploitation in the landscape prevents the implementation of effective conservation strategies. Understanding the full scope of these threats is crucial for developing informed and effective conservation plans to protect the Bengal Monitor Lizard and ensure its survival amidst growing human pressures.

Chapter-4

To determine the population genetic structure of *V. bengalensis* from examined samples

Background

Robust population genetic research on monitor lizards, which are the largest lizards found in the Indian subcontinent and belong to the genus *Varanus*, has not yet been undertaken despite their widespread distribution across the old-world tropics and subtropics (Auliya and Koch, 2020). The Indian subcontinent, with its rich landscape, provides an ideal opportunity to explore how monitor lizards have responded to climatic fluctuations and the impact of the interplay between geological changes and recent human activities, although this aspect has remained poorly documented.

V. bengalensis is widely distributed in nature, thrives in diverse and heavily human-dominated landscapes across its range. Initially, *V. bengalensis* was considered a polytypic species with three subspecies: *V. b. bengalensis* distributed in South and Southeast Asia, *V. b. nebulosus* in Southeast Asia and *V. b. irrawadicus* in Yunnan province, China (Pianka and King, 2004; Yang and Li, 1987). Currently, *V. b. nebulosus* and *V. b. irrawadicus* has been elevated to species status, but resolving the taxonomic issues related to *V. bengalensis* requires a thorough investigation (Koch et al., 2013; Wang et al., 2022). Phenotypic characterization may help clarify its taxonomic classification, infer evolutionary relationships, and validate its species status (Merrell, 1981). However, relying solely on morphological traits can sometimes obscure underlying patterns, and advanced genetic tools can provide a more comprehensive understanding of a species' biogeography (Avise, 2000). Identifying taxonomic and population units within species is crucial for conserving biodiversity (Allendorf et al., 2007). Recent advances in genetics have played a pivotal role in delineating lineages and accurately tracing evolutionary history (Rissler and Apodaca, 2007; Welton et al., 2010a; Welton et al., 2010b;

Brown et al., 2012). Molecular studies using a multi-locus approach have yielded valuable insights into various geographic regions, such as African (Portik and Papenfuss, 2012), Indonesian (Welton et al., 2010a; Weijola et al., 2016; Weijola et al., 2017; Weijola et al., 2019; Weijola et al., 2020), Australian (Smissen et al., 2013), and Asian (Vidal et al., 2012) territories, shedding light on the separation of divergent assemblages of monitor lizard species.

The widespread illegal exploitation poses a significant threat to monitor lizards in India. The confiscated samples in seizure of monitor lizard from the different areas did not elaborate the ground zero of poaching activities due to their tertiary/quaternary location of crime. The major seizure received by WII is from north India (Figure 16).

Hence, in this chapter, I aimed to investigate the genetic structure of confiscated articles and their relationships among the extant monitor lizard species which could help in identifying evolutionarily significant varanid lineages and support molecular tracking, conservation strategies, and management planning. This will also provide improvement in understanding the evolutionary relationships of *V. bengalensis* in comparison to other monitor lizard species, with a specific focus on the Terai arc landscape to gain insights into how landscape structure and geological and climatic processes have influenced species differentiation.

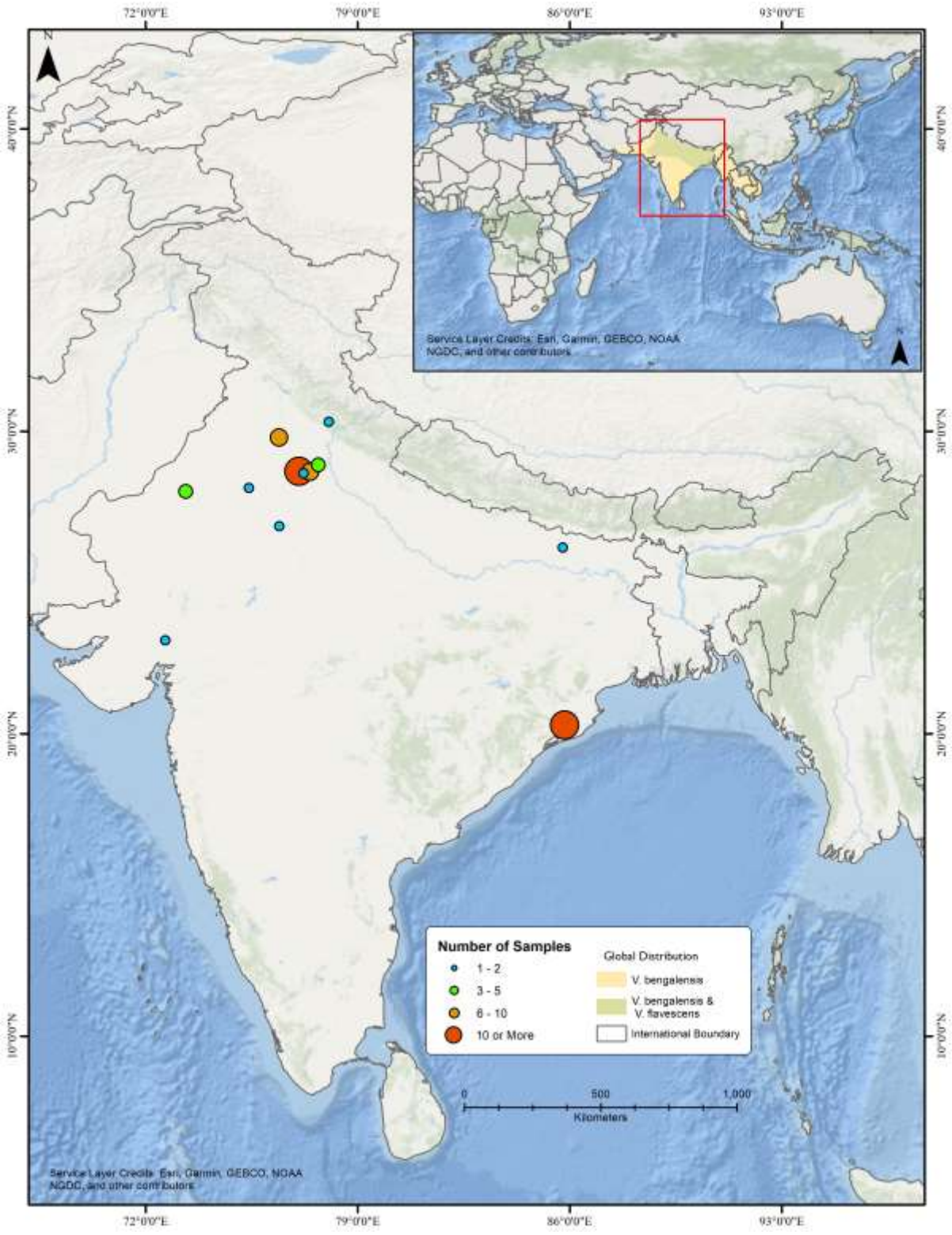

Figure 16: **Confiscation points of monitor lizards from different enforcement agencies received in Wildlife Institute of India**

Methodology

Sample Collection and DNA extraction

Genomic DNA (gDNA) was extracted from stored reference samples of 84 monitor lizards, comprising *V. bengalensis* (n=73), *V. flavescens* (n=9), and *V. griseus* (n=2), using the DNeasy Blood & Tissue Kit (QIAGEN, Germany) with a final elution volume of 100 µl. Additionally, gDNA was extracted from 114 biological samples, suspected to be monitor lizards, seized by enforcement agencies, using the Phenol Chloroform: Isoamyl alcohol extraction method (Sambrook et al., 1989) with a final elution volume of 100 µl. Consequently, the sample matrix for this chapter includes reference geo-tagged individual monitor lizards from the Terai Arc Landscape, opportunistically collected samples, and confiscated samples sent to WFCGC, WII by enforcement agencies.

PCR amplification and sequencing for divergence estimation

Here, I sequenced the NADH dehydrogenase subunit 4 (ND4, 600 bp) region of the mitochondrial gene (Kumazawa and Endo, 2004). The region of ND4 gene is used to estimate the divergence times, hence I sequenced *V. bengalensis* (n=4), *V. flavescens* (n = 2) and *V. koniecznyi* (n = 2).

PCR reactions for each primer were performed in 20 µl reaction volumes using 1 × PCR buffer (10mM Tris–HCl, pH8.3, and 50mM KCl), 1.5mM $MgCl_2$, 0.2mM of each dNTPs, 3 pmol of each primer, 0.5 units of DreamTaq DNA Polymerase (Thermo Scientific) and 1 µl (~30 ng) of template DNA. The PCR protocol was used as follows: initial denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 45 sec, annealing at 56°C for 60 sec and

extension at 72 °C for 75 sec. The final extension was at 72 °C for 10 min. Negative controls were included in each reaction to ensure the experiment's reliability. PCR amplification was confirmed by electrophoresis on a 2% agarose gel stained with ethidium bromide and visualized under a UV transilluminator. The amplified PCR products were purified using exonuclease-I and shrimp alkaline phosphatase (Thermo Scientific Inc.) at 37°C for 20 minutes to remove any remaining primers and dNTPs, followed by enzyme inactivation at 85°C for 15 minutes. The purified fragments were sequenced directly in an Applied Biosystems Genetic Analyzer 3500 XL (Applied Biosystems) from both the forward and reverse directions using the BigDye 3.1 Kit. To check the alignment and the presence of stop codons, generated sequences were translated into amino acids using the Expasy: Translate tool (Gasteiger et al., 2003).

Microsatellite amplification and genotyping

Initially, eleven microsatellite loci were screened for *V. bengalensis* (n=73), *V. flavescens* (n=9), *V. koniecznyi* (n=2), and twelve seizures (n=114) out of which eight were successfully amplified. Two of the eight loci were monomorphic and hence excluded from further analysis. Further, the analysis was performed with six microsatellite loci: Varsa10; Varsa07 (Fu et al., 2011), VA74 (Fitch et al., 2005), K15; K11; K22 (Ciofi et al., 2011) (Table 13). The forward primer of each locus was connected with one of the 5′ universal sequence tail: T7 (5′-TAATACGACTCACTATAGGG), M13 (5′-TGTAAAACGACGGCCAGT) and M13R (5′-CAGGAAACAGCTATGACC) (Ge et al., 2014). The universal primer M13, M13R and T7 were labeled with fluorescent dye FAM, HEX and TET, respectively. Multiplexing was carried out in 10µl PCR reaction volumes according to basepair size and dye pattern, containing 5µl of QIAGEN Multiplex PCR Buffer Mix (QIAGEN Inc.), 0.5µl of Q-solution, 0.2µM labeled forward primer (Applied Biosystems), 0.2µM unlabeled reverse primer, and 80–100 ng of the

template DNA. The PCR cycle was performed under the touchdown conditions, initial denaturation at 95°C for 15 min, followed by 8 cycles of denaturation at 95 °C for 45 sec, annealing at 58°C for 60 sec and extension at 72 °C for 75 sec, again followed by 15 cycles of denaturation at 95 °C for 45 sec, annealing at 58 to 50°C for 60 sec and extension at 72 °C for 75 sec which followed by 12 cycles of denaturation at 95 °C for 45 sec, annealing at 52°C for 60 sec and extension at 72 °C for 75 sec with final extension of 60°C for 30 min. Positive and negative controls were included in each reaction to monitor the reliability of the reaction. The alleles were determined using the Liz-500 Size Standard (Applied Biosystems) in an ABI 3500XL Genetic Analyzer (Applied Biosystems) and further analyzed using GeneMarker 2.7.4 (Applied Biosystems).

Table 13: Details of microsatellite loci (*) shows monomorphic locus

S. No.	Locus	Sequences (5`-3`)	Citation
1	K7*	F: TCACAATGACTTCAGTGCTATCCTG R: AACCAACTGTGCTACGCCCTC	Ciofi and Bruford, 1998
2	VA38*	F: CTCTTTGGTTCTGGTAGTCC R: GCTTCTGATTTAATGTCTGAC	Fitch et al., 2005
3	VA74	F: AAATAAACATGCCTCTGATTG R: GACCAGAAAGATTGCCTTC	Fitch et al., 2005
4	K15	F: ATGTCCTTCTCAGTTGTAAG R: CTGATTCTCCCCTATCGT	Ciofi et al., 2011
5	K11	F: CCGTAGCTCCTTGTGAAGC R: GTGGTGATGAACTGGTGCTC	Ciofi et al., 2011
6	K22	F: TGTCCTGCTTATTTTCATGCTTT R: CTTGTTTTCATGCTGCCTCA	Ciofi et al., 2011
7	Varsa10	F: CACCAGCCTTGTGAAGAA R: TAGCCTCCAGGTAAACCA	Fu et al., 2011
8	Varsa07	F: CTATCGCCAATCTCAAAC R: ACACTTGGGATACTCTGC	Fu et al., 2011

Data Analysis

Divergence estimation

The amplicons were sequenced for parts of ND4 gene for samples of *V. bengalensis* (n=4), *V. flavescens* (n=2) and *V. koniecznyi* (n=2) (OP141878-OP141885). Published sequences were also included to increase the robustness of the analyses (Table 3). Sequences obtained from the

forward and reverse direction of samples for each gene, were edited manually by confirming electropherograms and assembled using SEQUENCHER® 4.9 (Gene Codes Corporation, Ann Arbor, MI, USA). A BLAST (Altschul et al., 1997) search was done with the generated sequences for ND4 gene for species identification. Sequences were aligned using the Clustal W program (Thompson, 1994) by BioEdit 7.1.3 (Hall, 1999) with default settings. The alignment was manually optimized and ambiguous regions were excluded for phylogenetic analyses.

A strict molecular clock method was implemented in BEAST 1.7 (Drummond et al., 2012) to infer lineage divergence times of genus *Varanus*. Divergence times of phylogenetic clades were calibrated at two points with normal distribution: one prior point was at 28 Mya (SD=2), which indicates the African *Varanus* fossils (Holmes et al., 2010), while the other prior point was at 20 Mya (SD=1) which is supported by *Varanus* fossils from Australia (Molnar, 2004). Further, the Yule speciation model and HKY substitution rate model were used for tree construction. The analysis included two independent runs, using MCMC lengths of 60 million generations and logging every 6000 generations. Tracer 1.6 was used to evaluate all the runs. As burn-in, the first 10% per run was discarded. We used TreeAnnotator (implemented in BEAST 1.7 Package) to acquire maximum credibility trees. The final phylogenetic tree was visualized in FigTree 1.4.0 (http://tree.bio.ed.ac.uk/software/figtree/).

Microsatellite analysis

For microsatellite analysis, a total of six polymorphic loci were selected and genotyped for *V. bengalensis* (n=73), *V. flavescens* (n=9), *V. koniecznyi* (n=2) and twelve seizures (n=114). Alleles were identified and scored in GeneMarker 2.7.4 (Applied Biosystems). The genotyping error caused by null alleles was checked in MICROCHECKER 2.2.3 (Oosterhout et al., 2003).

All the loci were examined for Hardy Weinberg Equilibrium in GenAlEx 6.5 (Peakall and Smouse, 2006).

To examine genetic partitioning within *V. bengalensis*, a Bayesian clustering analysis was performed using STRUCTURE 2.3.4 (Pritchard et al., 2000). We used a correlated allele frequency model with admixture to analyse the data with a burn-in 60,000 followed by 600,000 MCMC (Markov chain Monte Carlo) replications. Ten runs were carried out for each cluster set (K) from 1 to 10 and visualised in web server ClumpaK (http://clumpak.tau.ac.il) (Kopelman et al., 2015). The optimal number of clusters (*K*) was estimated by examining ΔK with web server-based program STRUCTURE HARVESTER (Earl and VonHoldt, 2012). Additionally, to identify the number of genetic clusters, a multivariate and model-free approach, the Discriminant Analysis of Principal Components (DAPC) was implemented using the ADEGENET package in R to strengthen the data (Jombart et al., 2010). We used CONVERT 1.31 software to obtain the required input file format (Glaubitz, 2004). The pairwise F_{ST} values among the different monitor lizard *Varanus* populations were calculated using GenAlEx 6.5 (Peakall et al., 2006).

Results

Divergence dating

Our divergence results show congruence with the recent timeline provided by Brennan et al. (2021) for modern monitor lizard species. Resultant diversification through partial fragment of ND4 gene also suggests a crown age in the early to mid-Oligocene. When we focus on the extant monitor species in the Indian subcontinent, the split between desert monitor (*V. koniecznyi*) of the subgenus *Psammosaurus* and the subgenus *Polydaedalus* including Nile monitor (*V. niloticus*) occurred in Late Oligocene-Miocene, around 24.7 mya ($CI_{95\%}$: 21.57-27.82). The split

between the subgenus *Empagusia* including yellow monitor (*V. flavescens*) and Bengal monitor (*V. bengalensis*) occurred in the mid-Miocene, around 12.45 mya ($CI_{95\%}$: 9.97-15.11). This analysis indicated that the split between *V. bengalensis* (Lineage-I) and (Lineage-II) had taken place in mid-Pliocene around 3.06 mya ($CI_{95\%}$: 1.98-4.33) (Figure 17). The divergence analysis is in congruence with phylogenetic analyses which indicated the presence of two distinct clades within *V. bengalensis*. Lineage-I is spread across the Uttarakhand region and adjoining areas in Uttar Pradesh, Assam and Arunachal Pradesh. Lineage-II covers the species' distribution in the Indian states of Haryana, Uttar Pradesh, Bihar, Madhya Pradesh, Gujarat and Maharashtra (considered as the remainder of the mainland populations). Hence it is clearly established by phylogenetic and divergence analysis that Lineage-I of *V. bengalensis* is spread across foothills of the Himalaya and possess a distinct signature with divergence date of 3.06 mya.

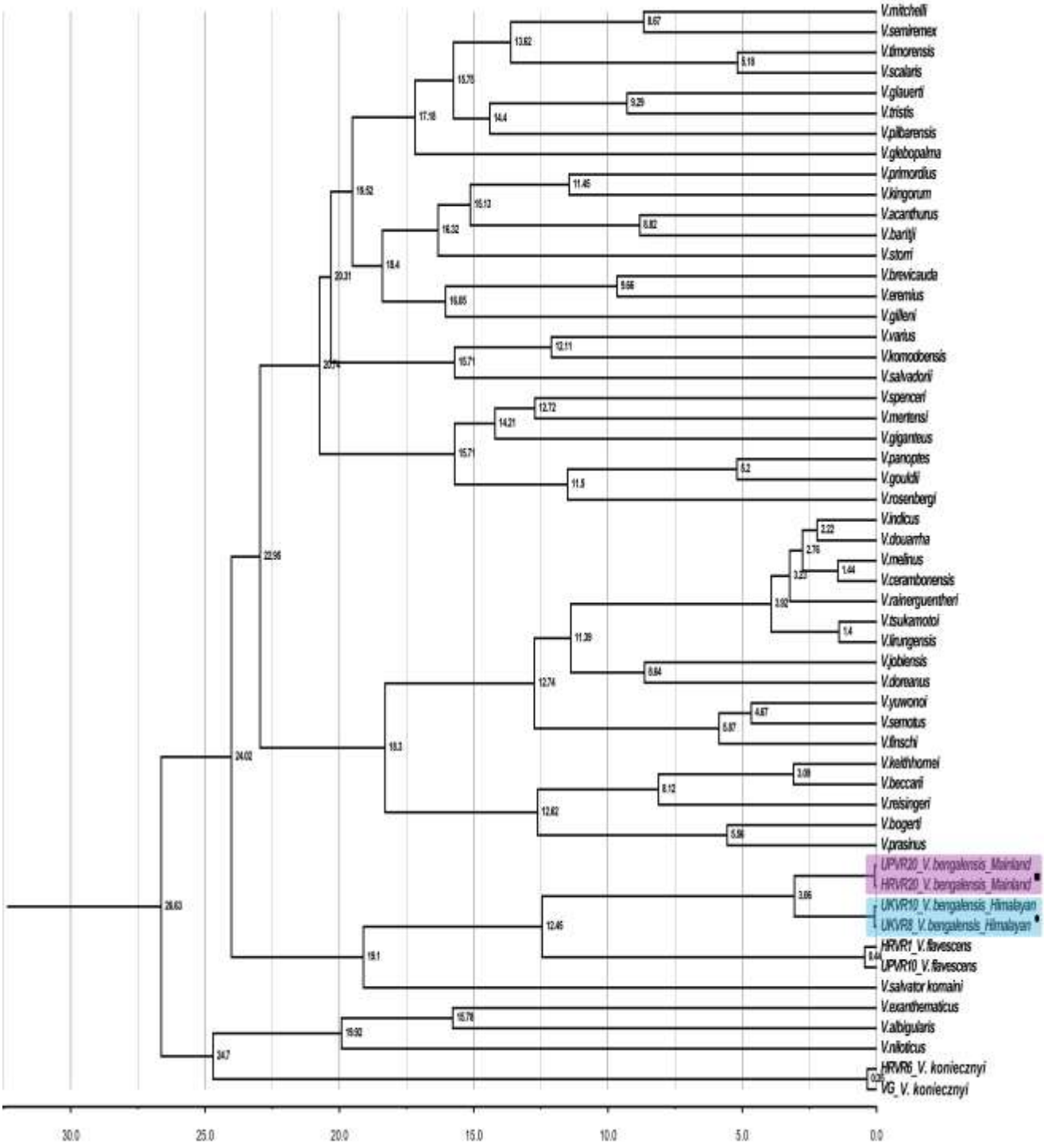

Figure 17: Divergence time estimation based on maximum credibility tree using ND4 gene generated from BEAST analysis. X-axis denotes million years ago (mya). The square denotes *V. bengalensis* from the remainder of the mainland India, while the circle denotes *V. bengalensis* from the Himalayan foothills

Population genetic structure and genetic differentiation

In this chapter, the microsatellite (STR) database of 84 individual monitor lizard were generated which were geo-tagged reference samples comprising *V. bengalensis* (n=73), *V. flavescens* (n=9) and *V. koniecznyi* (n=2) from six loci. These same six loci were used to generate STRs for confiscated samples (n=114). Further analysis was done by combining the dataset of reference sample and confiscated sample. The Bayesian clustering analysis using the admixture model identified the highest ΔK when K was at 4 (Mean LnP(K)= -5484.33; ΔK=151.188). So, to depict the genetic structure the value of K=4 was adopted, where individuals from our dataset fall into four different clusters *V. flavescens*, *V. koniecznyi* and *V. bengalensis* (Lineage-I) and *V. bengalensis* (Lineage-II). The clustering of *V. bengalensis* differentiated in two distinct groups one from Himalayan foothills and other from remainder of mainland group (Figure 18, Figure 19 and Figure 20). The samples of *V. bengalensis* from Himalayan foothills showed a genetically distinct cluster compared to the (ROM) remainder of mainland *V. bengalensis*. The confiscated sample showed a similarity of genetic structure with three group of monitor lizard. The major confiscated sample (n=111) matched with the cluster of *V. bengalensis* remainder of mainland population, whereas only two sample has an affinity with *V. bengalensis* from Himalayan foothills population. Lastly, there was one single sample which falls in the cluster of *V. flavescens*. The multivariate DAPC corroborated the Bayesian clustering analysis that differentiated the populations into four genetic clusters and clustering of seizures with *V. bengalensis* (Himalayan foothills), *V. bengalensis* (ROM) and *V. flavescens* (Figure 21). A parallel pattern between genetic cluster assignment and phylogenetic clades was observed. We found evidence of genetic differentiation between Lineage-I Himalayan foothills and Lineage-II ROM, the remainder of mainland population of *V. bengalensis* of 5.5%. We further observed

well-supported distinctiveness between other monitor lizard species. *V. flavescens* is 21.7% and 13.5% distinct from Lineage-I and Lineage-II of *V. bengalensis,* respectively. Likewise, *V. griseus* is 27.5% and 26% genetically different from *V. bengalensis* Lineage-I and Lineage-II, respectively. Moreover, the genetic differentiation between *V. koniecznyi* and *V. flavescens* is 41.1% (Table 14).

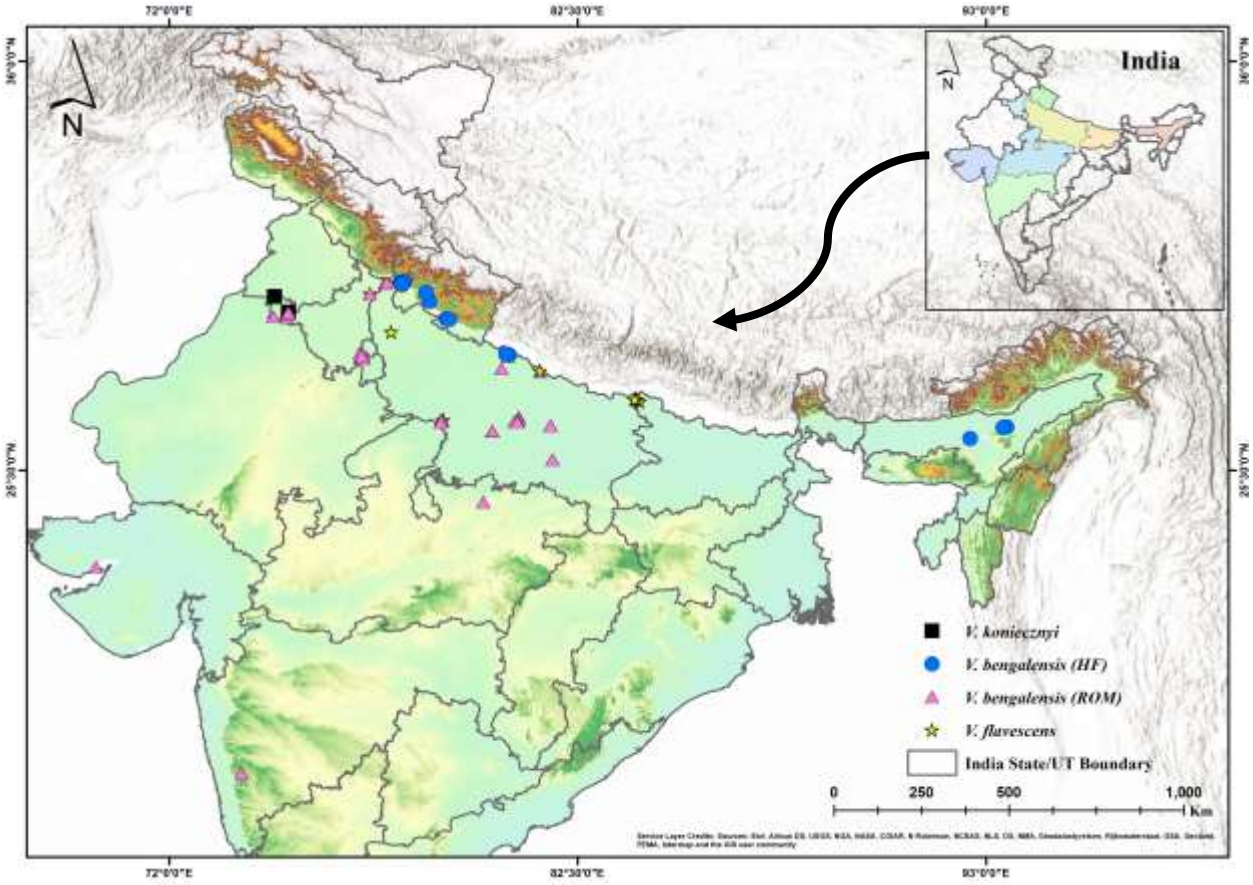

Figure 18: **Reference Geo-tagged sampling points with the identity of specific species cluster**

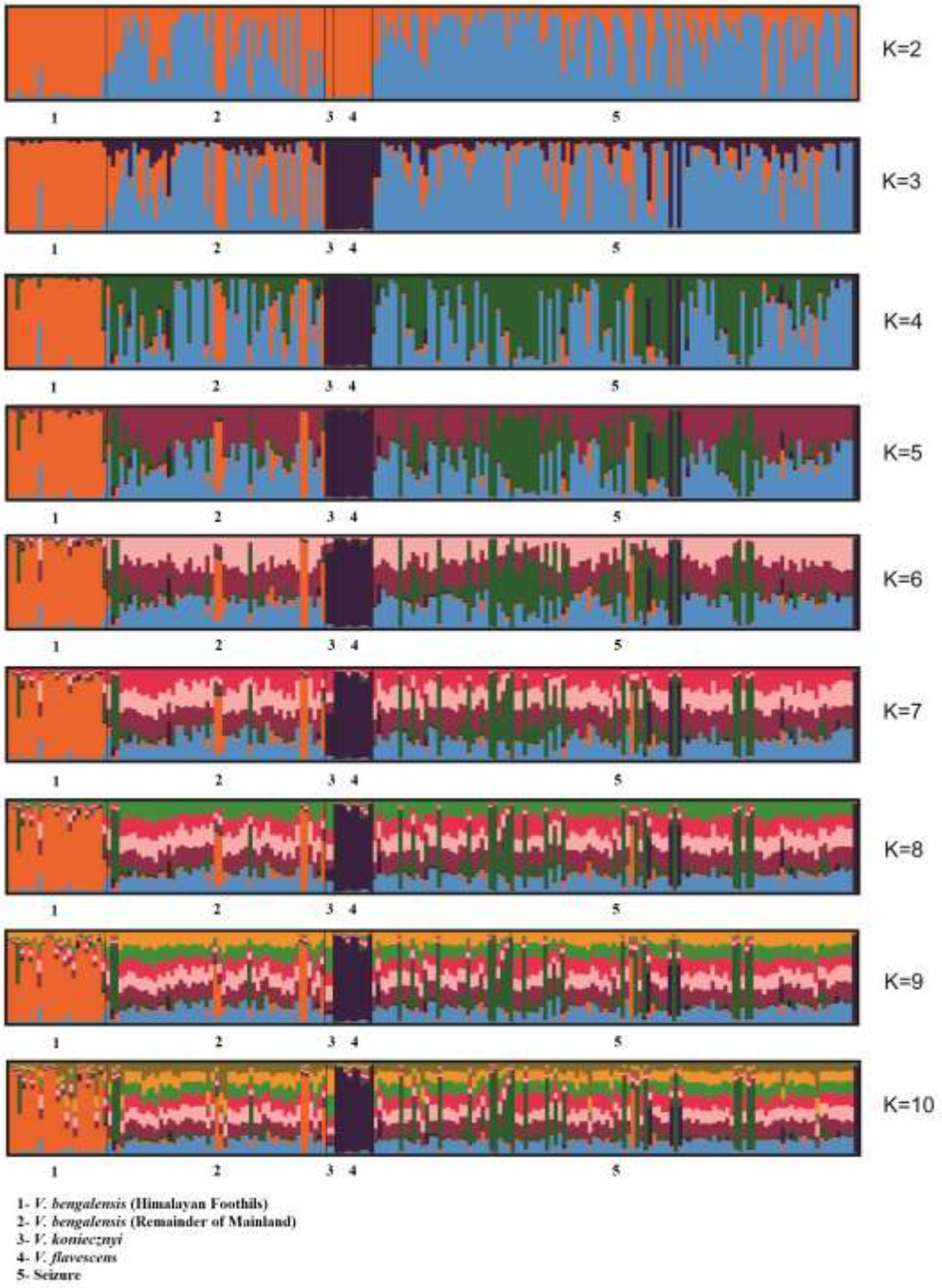

Figure 19: Barplot indicating the genetic structure at K=1 to K=10, showing the population assignments for each individual of *V. bengalensis* (Himalayan foothills), *V. bengalensis* (remainder of mainland), *V. flavescens* and *V. koniecznyi*. The Y axis is depicting the proportion derived from each cluster

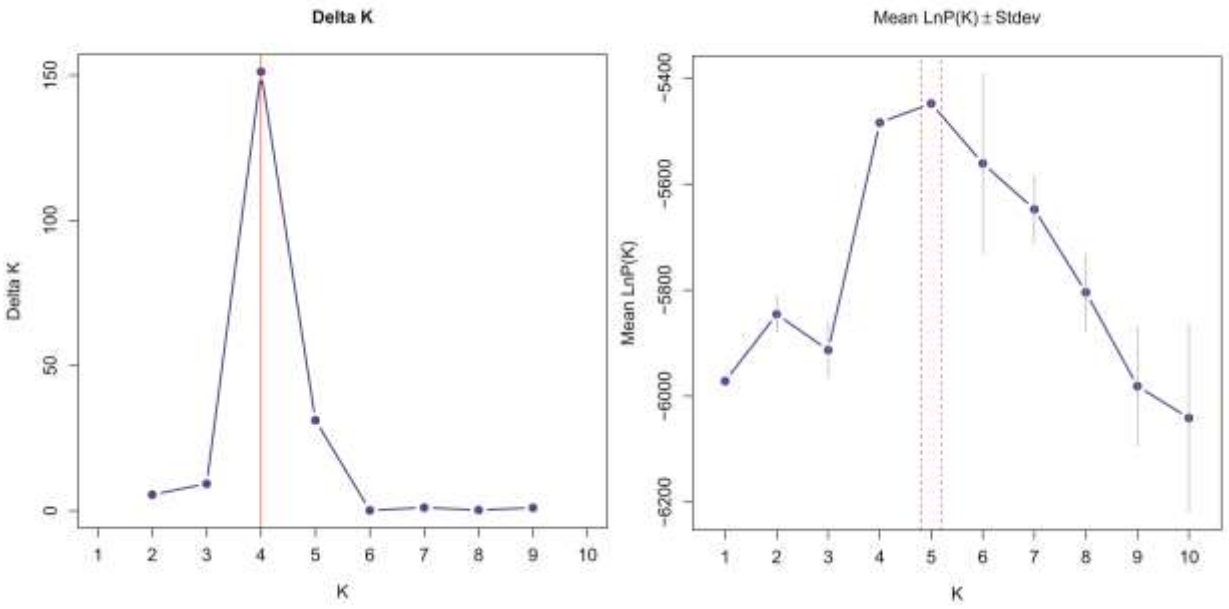

Figure 20: Results of genetic clusters inferred from Structure 2.3.4. a) Delta K values with respect to K. b) Mean of estimates Ln probability of data with respect to K

Table 14: Pairwise FST value based on Microsatellite loci

Species	**1**	**2**	**3**	**4**
V. bengalensis_ lineage -I				
V. bengalensis_ lineage -II	**0.055**			
V. koniecznyi	0.275	0.260		
V. flavescens	0.217	0.135	0.411	0.0

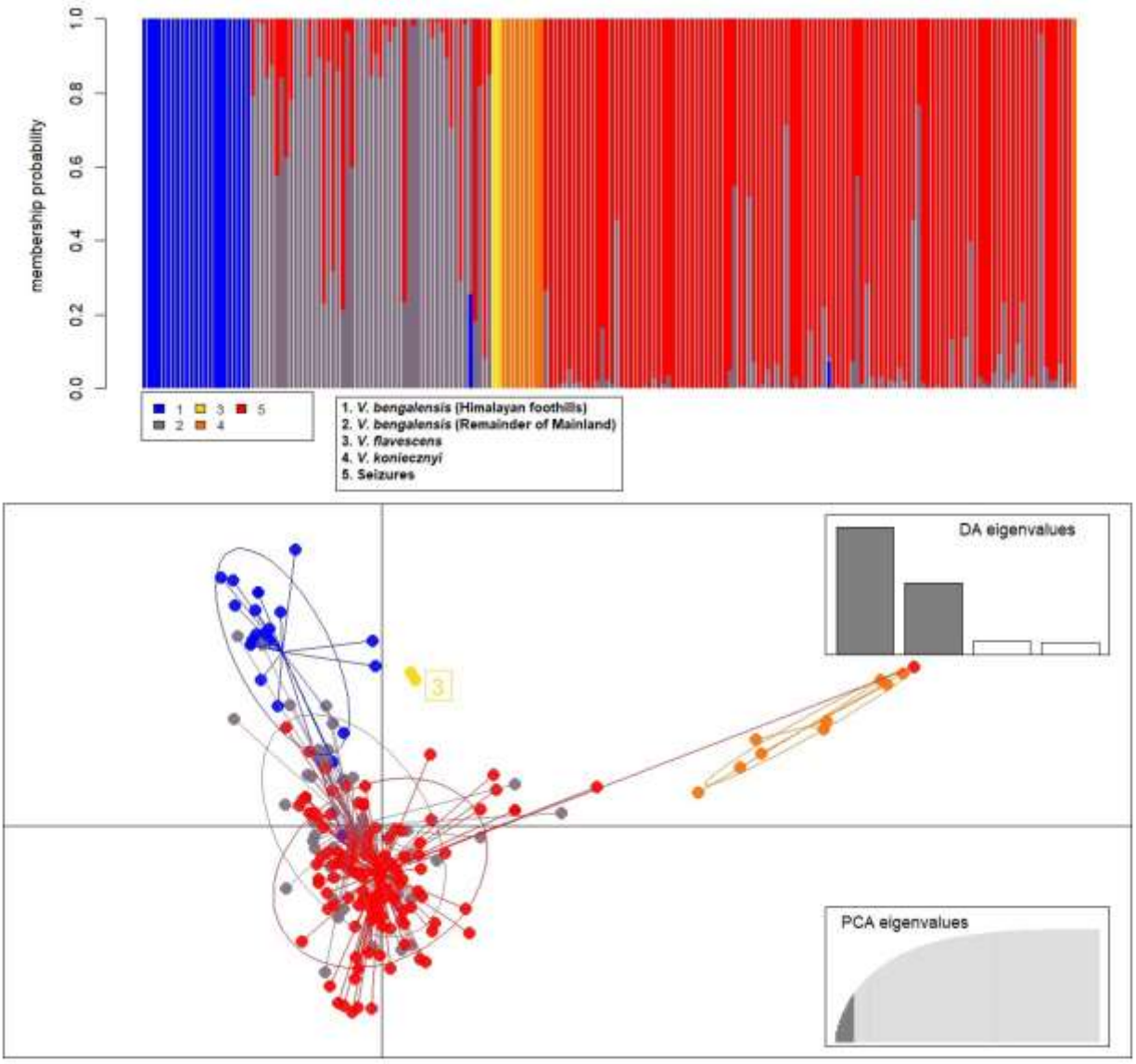

Figure 21: The bar plot results show genetic clustering implemented in Discriminant Analysis of Principal Components (DAPC) (a). Each column along the X-axis represents one *Varanus* individual. The Y-axis represents the assignment of the membership probability of each individual. Scatter plots of DAPC using a hierarchical islands model and shown by different colors and inertia ellipses (b). The DA and PCA eigenvalues of the analyses are displayed in the inset

Discussion

The Himalayan region encompasses noteworthy species diversity and endemism. Temporal record of biological processes reveals high-resolution information about the Himalayan history and environmental changes. The herpetofauna of the Himalayas is a vital component of the mountain ecosystem, with origins tracing back to the early Paleocene. Notably, the diversification of many groups significantly accelerated during the Miocene (Xu et al., 2021). Reptiles are particularly vulnerable to local extinctions, genetic disruptions, anomalies, speciation events, and shifts in distribution patterns, often driven by natural fluctuations or human-induced changes (Gibbons et al., 2000). Climatic variations and human-caused habitat destruction have notably impacted the distribution patterns of numerous reptilian species. The Indian subcontinent offers an ideal landscape for studying how *Varanus* species have responded to climatic changes and the complex interactions between mountain formation and human activities over time, a topic that remains underexplored. The widely distributed *V. bengalensis* was earlier known with *V. b. bengalensis*, *V. b. irrawadicus*, and *V. b. nebulosus* as its subspecies (Ziegler and Böhme, 1997; Böhme, 2003; de Lisle, 2009). However, with the elevation of *V. b. nebulosus* and *V. b. irrawadicus* as a species status without much genetic evidence raise doubts. Therefore, this chapter analyzed the genetic structure of *V. bengalensis* from India and revealed two genetically distinct lineages, one from the Himalayan foothills and the other from southward of foothills i.e., remainder of mainland (ROM). As the name suggests, the Himalayan foothills lineage of *V. bengalensis* inhabits the foothills of Indian Himalayan states of Uttarakhand, Uttar Pradesh, and Assam to Arunachal Pradesh. The two genetic clusters were also confirmed by DAPC and Structure analyses. The level of genetic distinctiveness revealed by the two lineages of *V. bengalensis* compared in this chapter against the levels among existing recognized monitor

lizard species supports the presence of the distinct Himalayan foothills and remainder of mainland lineages. Interestingly, the genetic signature found in this study within *V. bengalensis* is not affected by major rivers systems such as the Ganga and Brahmanputra, similarly observed in small bodied lizards e.g. *Sitana* and *Cyrtodactylus* (Agarwal et al., 2017; Deepak and Karanth, 2018). The consequential divergence between *V. bengalensis* in Himalayan foothills and remainder of mainland suggest that some barriers or factors might have played a role in intraspecific differentiation. The diversification within *Cyrtodactylus* explored the India-Asia collision and provided insights into Himalayan biogeography (Agarwal et al., 2014). Another example of intraspecific differentiation is in the wide-ranging king cobra with four independently evolving genetic lineages but with low level of morphological divergence (Shankar et al., 2021). Few more phylogenetic studies on birds (Johansson et al., 2007; Martens et al., 2011), insects (Schmidt et al., 2012), amphibian (Hofmann et al., 2017) and reptiles (Xu et al., 2021) provide information and understanding regarding divergence in the Himalayan biota.

The baseline genetic database of reference samples has established as distinct signature for monitor lizard species from Terai Arc landscape and associated landscape (Gautam et al., 2023). The reference microsatellite dataset of this chapter is negating the limitation of misidentification of species. Such geo-tagged reference is a requirement to assign the harvest localities with accuracy (Welton et al., 2013). This chapter proposes a framework for the recognition of monitor lizard species from the confiscated samples which would facilitate the implementation of standardized protocol for molecular based species identification tool that help wildlife forensics to track the origin of species and poaching hotspots.

To assist in the field of wildlife forensics effectively, evidence must be carefully preserved and documented in its original state to ensure justice is served (Saferstein, 2009). Failing to secure a

crime scene adequately can lead to evidence being destroyed or altered, potentially misleading investigators and complicating efforts to identify the perpetrator accurately. Conversely, in wildlife poaching activities, finding an intact crime scene is often an unattainable task for investigators. Locating the primary crime scene, where the initial illegal act occurred in wildlife poaching or hunting cases, is challenging. Law enforcement agencies primarily monitor wildlife trafficking activities at the tertiary crime scene, where animals are transformed into articles or derivatives and transported between locations. The quaternary crime scene involves places such as shops and e-commerce sites where confiscations may occur (Figure 22). Therefore, in wildlife illicit activities, identifying the exact crime scene is challenging. Hence, studying the distribution range of traded species becomes crucial because these species can only be traded from within their natural habitat. By pinpointing these vulnerable areas, enforcement agencies can implement monitoring strategies to intercept hunting activities at their source.

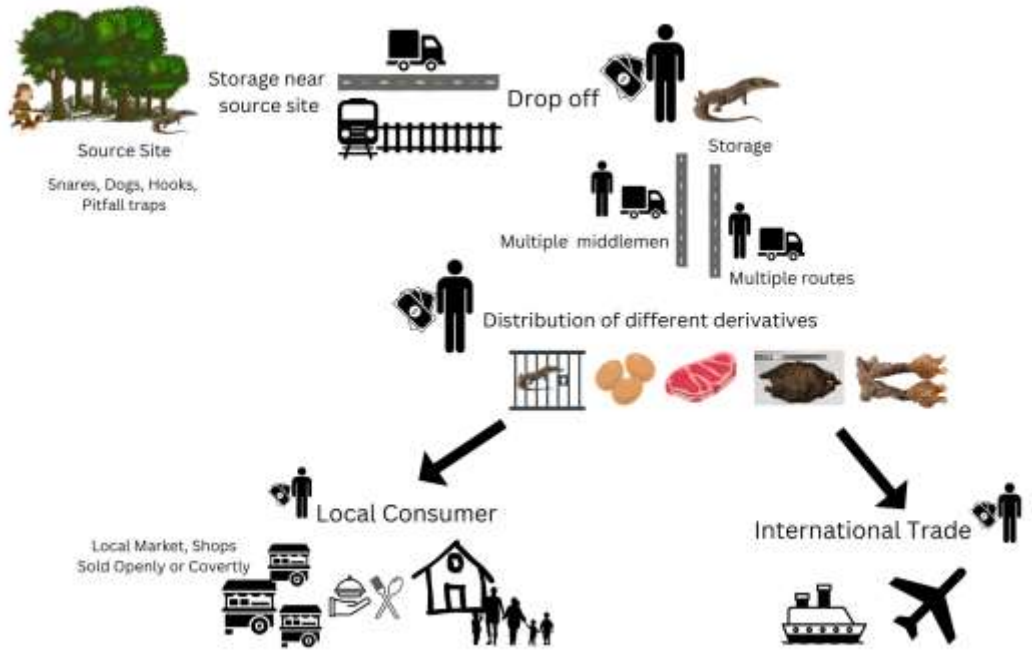

Figure22: Illustration of Wildlife poaching activity

One such vulnerable location in India is Terai arc landscape comprising major transportation routes and eco-sensitive Himalayan foothills. The confiscated samples (n=114) from twelve seizures are majorly from the north Indian state such as Haryana, Uttar Pradesh, Uttarakhand, Rajasthan, New Delhi, Gujarat and Bihar except one which was from Bhubaneswar, Odisha. Among these twelve seizures, heavy volume of samples was confiscated from Odisha and New Delhi. The microsatellite generated in this chapter when combines with reference sample resulted into interesting clustering pattern. Around 97% of confiscation clustered with the *V. bengalensis* (ROM) lineage on the other hand 2% samples were clustered with *V. bengalensis* (Himalayan foothills) lineage. Only one article which makes 1% of total seizure falls into the genetic cluster of *V. flavescens*. The clustering pattern of seizures with the reference database suggests that the pressure of poaching is heavy on remainder of mainland lineage of *V. bengalensis.* The confiscated article of seizure from Ghaziabad, Uttar Pradesh and Dehradun, Uttarakhand possessed one sample each which has the affinity with *V. bengalensis* (Himalayan foothills) lineage. Hence, the poaching happening in the fringes of the Himalayan foothills is making this newly identified distinct Evolutionary Significant Unit (ESUs) on a podium of threat.

Chapter-5

CONCLUSION

Summary

The molecular database generated in this work provides novel baseline information for the monitor lizards of India. It compiles extensive analyses of mitochondrial and microsatellite loci to understand the population genetic structure. The study also highlights geological events which may have influenced the demographic pattern of *V. bengalensis* resulting in speciation. The phylogeny of this study is aligned with the recent phylogenetic assessment of monitor lizards suggested by Brennan et al. (2021), supporting the monophyly of the genus *Varanus,* which delimited the African, Indo-Asian and Indo-Australian clades and placed *V. bengalensis, V. flavescens* in the Indo-Asian group, while *V. griseus* in the African group. Distinctive findings within *V. bengalensis* were supported by species delimitation methods, corroborating the divergence between *V. bengalensis* from the Himalayan foothills and *V. bengalensis* from the remainder of the mainland as separate evolutionary significant units (ESUs).

The findings of the study was compared with previous morphological data of Auffenberg's monograph on *V. bengalensis* where 495 specimens were observed for scale counts and did not reveal any explicit pattern of geographical variation. Two populations of *V. bengalensis* were recognized on the basis of scalation, one west of Myanmar i.e. *V. b. bengalensis*, while the other in east of Myanmar i.e. *V. b. nebulosus*, with an intermediate zone from lower West Bengal through Bangladesh to the eastern highland boarder area of Myanmar and Thailand. Within these two major populations of *V. bengalensis* recognized by Auffenberg, (1994) several subsets were also observed. However, CO*I* gene indicated more than 15% differentiation between *V. b. nebulosus* and the three *V. bengalensis* lineages, surpassing sub-species level and confirming the status elevation by Koch et al. (2013) as *V. nebulosus*. Hence, previously reported morphology data is ambiguous and the present study focuses more on the genetics of *V. bengalensis*.

The intraspecific differentiation observed in this chapter is also seen in other sister monitor lizard species. Smissen et al. (2013) revealed three geographically separated clades from ND4 gene and microsatellite data for understanding the *V. varius* biogeography. Range-wide multilocus assessment of *V. niloticus* group has exhibited genetic differentiation above that of typically found between sister monitor lizard species (Dowell et al., 2016). However, new monitor lizard species were had also been recorded highlighting both local and regional endemism with 2.4-13.5% and 2.3-0.6% pairwise distances in ND4 and 16S rRNA gene, respectively (Weijola et al., 2017). Welton et al. (2014) has reported distinct species within *V. salvator* complex by 1.00-3.5% based on ND1 and ND2 genes. Among Indo-Australian varanids, Fitch et al. (2006) reported the genetic differentiated ranging from 3.4-27.7 % using ND4 region.

This study is focused in understanding the genetic structure and evolutionary history of *V. bengalensis* in India, with a special emphasis on the populations in the Himalayan foothills. India separated from Madagascar around 85-90 mya and advanced northwards until about 20-30 mya (Acton, 1999). Collision of India with the Neotethyan intraoceanic arc is considered as 57 Ma event and afterward sudden drop in northward speed was due to the joining of India-Asia by 55-50 mya (Huang et al., 2000; Valdiya, 2002). The formation of the Himalaya due to the northward movement of the Indian plate in a phased manner started around 45 Mya and major upliftment occurred 8-6 mya at the eastern edge (mostly Tibetan plateau) (Favre et al., 2015; Spicer, 2017). The divergence split between the subgenus *Soterosaurus* including *V. salvator* group and *V. flavescens* of the subgenus *Empagusia* was recorded in the Early Miocene ~19.1 mya ($CI_{95\%}$: 15.95-22.21). The split of *V. bengalensis* from *V. flavescens* was observed around Mid-Miocene ~12.45 mya ($CI_{95\%}$: 9.97-15.11). The study by Brennan et al. (2021) using nuclear exon dataset suggested the divergence of *V. bengalensis* and *V. flavescens* around 9.5 mya, also supported our

study where the divergence age estimated by mtDNA falls within the confidence internal ($CI_{95\%}$). The slight variation in the divergent date is likely due to the different evolutionary rates observed in mtDNA and nuclear genes. The study shows concurrence with Laurasian origin and corresponds with Hugall and Lee, (2004), Sweet and Pianka, (2007), Amer and Kumazawa, (2008) and Vidal et al. (2012), thus countering the Gondwanan hypotheses as proposed by Schulte et al. (2003). The divergence within *V. bengalensis* illustrates the split between Himalayan foothills and remainder of mainland lineages in the mid-Pliocene ~3.06 mya ($CI_{95\%}$: 1.98-4.33), exemplifying that the newly identified lineage has been formed during Shiwalik broadening. The Shiwalik Basin spans from Sindh region in the West to Tripura, Cachar, and Arunachal Pradesh regions in the East (Valdiya, 2002). The upliftment of Himalayan foothills caused Shiwalik succession at 18.3 mya, 11 mya, 5.3 mya and 0.22 mya, respectively, due to accelerated erosion following the deposition of detritus (Valdiya, 2002). The Himalayan upliftment supplements variable environmental conditions. The rise in elevation of Himalaya supports the intensification of South Asian Monsoon, which may have facilitated climatic gradient along the range and disconnected the landmasses of India, Sino-Japanese and South East Asia (Pandit, 2017). The uplifted Himalayan range with the strengthened monsoon may have provided diverse climatic niches, and new habitats, and dispersal barriers may have influenced evolutionary opportunities to accelerate speciation rate (Xu et al., 2021). Repeated climatic changes in mountainous regions and stochastic inter-annual weather fluctuations may have led to a high rate of speciation facilitated by repeated isolation, *in situ* diversification and remixing of gene pools during favorable conditions. Recent range extension of *V. bengalensis* at 3000 asl indicated species movement to higher elevation either under global climate change pressure or lack of robust field studies (Singh et al., 2020).

The eastern Himalayas are older than the western Himalayas and hence, most Himalayan reptiles are largely Indo-Chinese and Malayan derivatives e.g., *Japalura tricarinata, Japalura kumaonensis, Paraxenodermus, Tropidophorus assamensis* (Wang et al., 2019; Deepak et al., 2021; Lalremsanga et al., 2022). The Himalayan upliftment has helped in the evolution and diversification of Asiatic ancestral stock into endemic high-altitude elements (Mani, 1974). An increase in the number of species by rapid speciation and isolation on highlands manifested due to evolutionary changes during Pliocene (Valdiya, 2002). Furthermore, Pleistocene glaciations and associated climatic factors may have played a role in the evolution of Pliocene endemic forms and given rise to numerous subspecies and species (Mani, 1974). Late Pliocene Shiwalik fauna comprised diverse mammalian mega-fauna (Dennell et al., 2006). During Plio-Pleistocene, warm and moist climatic conditions became the precursor to survival and proliferation of species.

Interestingly, fossils of the genus *Varanus* have been reported from India, such as Pleistocene deposits of *V. bengalensis* from Billa Surga and Kurnool Caves, areas that are still inhabited by the species (Lydekker, 1886; Lydekker, 1888; de Fejervary, 1918; 1935; Prasad and Yadagiri, 1986). A rare large-bodied fossil known from only two vertebrae and a part of the humerus from Pliocene rocks of Shiwalik Hills was recorded and named *V. sivalensis* (Falconer, 1868). The two vertebrae align with *V. salvator* fossil, while the humerus is morphologically different from *V. komodoensis* even though it is of similar size (Hocknull et al., 2009; Conrad et al., 2012). Hocknull et al. (2009) suggested the rarity or extinction of *V. sivalensis* due to the absence of records from younger deposits in the same region. Considering the fossil record of *V. sivalensis* from Pliocene with respect to the split time of Himalayan foothills lineage of *V. bengalensis*, we speculate affinity between these two since no studies have compared *V. sivalensis* with *V.*

bengalensis for a reliable investigation of the theoretical affinity. Hence, future confirmation warrants further resolution in Bengal monitor lizard at the population, clades and species level since robust recognition is the foundation for efficient conservation.

This study offers extensive data on wild monitor lizard populations, revealing previously unknown geographic signature that can aid in monitoring trafficking and trade in these species. India is home to the largest natural population of Bengal monitor lizards, making it a significant source for poaching and increasingly becoming a global hotspot for seizures of these animals. Replicating the methodology used in this thesis will enable enforcement agencies to develop guidelines for highly trafficked Bengal monitor lizard. This includes defining the native ranges, trade routes, and destination states where seizures occur for different species. Furthermore, the mitochondrial and microsatellite datasets of geo-referenced monitor lizards have significantly filled a gap in the availability of genetic data on NCBI (National Center for Biotechnology Information). The discovery of lineages within *V. bengalensis* suggests that poaching pressure for hemipenis affects the mainland population more significantly than the Himalayan or South Indian populations. This study will aid in curbing poaching activities by pinpointing areas requiring heightened vigilance for enforcement agencies. The current genetic database, derived from confiscated samples, will serve as a critical reference for developing effective conservation plans. By understanding the genetic makeup and diversity of the population, conservationists can identify and prioritize genetically important individuals and populations for breeding programs and other conservation efforts. This genetic information will be crucial for implementing strategies aimed at maintaining or enhancing genetic diversity, such as habitat protection, anti-poaching measures, and possibly reintroduction or translocation programs. Ensuring the genetic

health of *V. bengalensis* will be vital for the long-term survival and recovery of the species in the wild.

Recommendation

- There is a vital need for an extensive study using robust genetic markers similar to those employed in this research, focusing on the Yellow monitor lizard, Desert monitor lizard, and Water monitor lizard.
- Replication of this study across different landscapes in India would be highly beneficial in the field of wildlife forensics, aiding in the effort to combat the trade of monitor lizards because this study is pioneering in tracking the trade of the Bengal monitor lizard, and it will assist enforcement agencies in maintaining vigilance and formulating science-based policies.
- Raising awareness about the inappropriate use of hemipenis as "Hatha jodi" due to superstitious beliefs will ultimately decrease demand, thereby disrupting the supply chain as well.
- Furthermore, awareness among local communities and farmers about the ecological importance of monitor lizards will be crucial in reducing unsustainable harvesting from wild populations.
- Sensitization efforts can help mitigate poaching and hunting activities by fostering a deeper understanding of the role these species play in the ecosystem.
- In the near future, a comprehensive taxonomic investigation, facilitated by robust sampling and morphology data, can establish conclusive evidence regarding intraspecific

differentiation within the Bengal monitor lizard (*V. bengalensis*) as a distinct Evolutionarily Significant Unit (ESU) in the Indian subcontinent.

Bibliography

1. ’t Sas-Rolfes, M., Challender, D. W., Hinsley, A., Veríssimo, D., & Milner-Gulland, E. J. (2019). Illegal wildlife trade: Scale, processes, and governance. *Annual Review of Environment and Resources, 44*, 201–228.
2. Acton, G. D. (1999). Apparent polar wander of India since the Cretaceous with implications for regional tectonics and true polar wander. *Memoir Geological Society of India, 44*, 129-175.
3. Agarwal, I., & Ramakrishnan, U. (2017). A phylogeny of open-habitat lizards (Squamata: Lacertidae: *Ophisops*) supports the antiquity of Indian grassy biomes. *Journal of Biogeography, 44*, 2021-2032.
4. Agarwal, I., Bauer, A. M., Jackman, T. R., & Karanth, K. P. (2014). Insights into Himalayan biogeography from geckos: A molecular phylogeny of *Cyrtodactylus* (Squamata: Gekkonidae). *Molecular Phylogenetics and Evolution, 80*, 145-155.
5. Alagesan, S. P. (2020). Wildlife crime data integration from multiple sources: A way forward to ensure public access to information and data analytics. *CrimRxiv*.
6. Allendorf, F. W., Luikart, G., & Aitken, S. N. (2007). Conservation and genetics of populations. *Mammalia, 2007*, 189–197.
7. Altschul, S. F., Madden, T. L., Schäffer, A. A., Zhang, J., Zhang, Z., Miller, W., & Lipman, D. J. (1997). Gapped BLAST and PSIBLAST: A new generation of protein database search programs. *Nucleic Acids Research, 25*, 3389–3402.
8. Amer, S. A. M., & Kumazawa, Y. (2008). Timing of a mtDNA gene rearrangement and intercontinental dispersal of varanid lizards. *Genes & Genetic Systems, 83*, 275–280.
9. Aplin, K. P., Fitch, A. J., & King, D. J. (2006). A new species of *Varanus* Merrem (Squamata: Varanidae) from the Pilbara region of Western Australia, with observations on sexual dimorphism in closely related species. *Zootaxa, 1313*(1313), 1-38.
10. Armstrong, E. E., Khan, A., Taylor, R. W., Gouy, A., Greenbaum, G., Thiéry, A., ... & Ramakrishnan, U. (2021). Recent evolutionary history of tigers highlights contrasting roles of genetic drift and selection. *Molecular Biology and Evolution, 38*(6), 2366-2379.
11. Ast, J. C. (2001). Mitochondrial DNA evidence and evolution in Varanoidea (Squamata). *Cladistics, 17*(3), 211-226.
12. Auffenberg, W. (1988). *Gray's monitor lizard*. Univ Press of Florida, Gainesville.
13. Auffenberg, W. (1994). *The Bengal Monitor*. Univ Press of Florida, Gainesville.
14. Auliya, M., & Koch, A. (2020). *Visual Identification Guide to the Monitor Lizard Species of the World (*Genus *Varanus)*. Bundesamt für Naturschutz, Bonn, Germany.
15. Auliya, M., Altherr, S., Ariano-Sanchez, D., Baard, E.H., Brown, C., Brown, R.M., Ziegler, T. (2016). Trade in live reptiles, its impact on wild populations, and the role of the European market. *Biological Conservation, 204*, 103–119.
16. Avise, J. C. (1994). *Molecular markers, natural history and evolution*. Chapman and Hall, New York.
17. Avise, J. C. (2000). *Phylogeography: The History and Formation of Species*. Harvard University Press, London, England.
18. Baker, C. S. (2008). A truer measure of the market: the molecular ecology of fisheries and wildlife trade. *Molecular Ecology, 17*(18), 3985–3998.

19. Baker, C. S., Lento, G. M., Cipriano, F., & Palumbi, S. R. (2000). Predicted decline of protected whales based on molecular genetic monitoring of Japanese and Korean markets. *Proceedings of the Royal Society B: Biological Sciences, 267*, 1191–1199.
20. Bartlett, S. E., & Davidson, W. S. (1992). FINS (forensically informative nucleotide sequencing): a procedure for identifying the animal origin of biological specimens. *Biotechniques, 12*, 408–411.
21. Baverstock, P. R., King, D., King, M., Birrell, J., & Krieg, M. (1993 Ast). The evolution of species of the Varanidae: Microcomplement fixation analysis of serum albumins. *Australian Journal of Zoology, 41*, 621–638.
22. Bennett, D. (1995). *A little book of monitor lizards: A guide to the monitor lizards of the world and their care in captivity* (pp. 60-63). Viper Press.
23. Bhattacharya, S., & Koch, A. (2018). Effects of traditional beliefs leading to conservation of water monitor lizards (*Varanus Salvator*) and threatened marshlands in West Bengal, India. *Herpetological Conservation and Biology, 13*, 408–414.
24. Böhm, M., Collen, B., Baillie, J. E., Bowles, P., Chanson, J., et al. (2013). The conservation status of the world's reptiles. *Biological Conservation, 157*, 372–385.
25. Böhme, W. (1988). Der Arguswaran (*Varanus panoptes* STORR, 1980) auf Neuguinea: *V. panoptes horni ssp. n. Salamandra, 24*(2-3), 87-101.
26. Böhme, W. (2003). Checklist of the living monitor lizards of the world (family Varanidae). *Zoologische Verhandelingen, 341*, 4–43.
27. Böhme, W., & Jacobs, H. J. (2001). *Varanus macraei* sp. n., eine neue Waranart der *V. prasinus*-Gruppe aus West Irian, Indonesien. *Herpetofauna, 23*(133), 5-10.
28. Böhme, W., & Ziegler, T. (2005). A new monitor lizard from Halmahera, Moluccas, Indonesia (Reptilia: Squamata: Varanidae). *SALAMANDRA-BONN, 41*(1/2), 51.
29. Böhme, W., Ahmed, S. H., Al-Sheikhly, O. F., Ararat, K., Auer, M., Khudur, F. A., & Langner, C. (2023). Desert Monitor Lizards (Squamata: Varanidae: *Varanus*: *Psammosaurus*) from the Middle East: Further Records of Nesterov's Desert Monitor, *Varanus* (*P.*) *nesterovi* Böhme, Ehrlich, Milto, Orlov et Scholz, 2015, from Iraq, and Adjacent Localities of *Varanus* (*P.*) *g. griseus* (Daudin, 1803) and *Varanus* (*P.*) *g. caspius* (Eichwald, 1831), with Comments on Biogeography and Taxonomy. *Russian Journal of Herpetology, 30*(6), 518-528.
30. Böhme, W., Ehrlich, K., Milto, K., Orlov, N., & Scholz, S. (2015). A new species of desert monitor lizard (Varanidae: *Varanus*: *Psammosaurus*) from the western Zagros region (Iraq, Iran). *Russian Journal of Herpetology, 22*(1).
31. Böhme, W., Jacobs, H. J., Koppetsch, T., & Schmitz, A. (2019). The Kei Islands monitor lizard (Squamata: Varanidae: *Varanus*: *Euprepiosaurus*) as a distinct morphological, taxonomic, and conservation unit. *Russian Journal of Herpetology, 26*(5).
32. Böhme, W., Philipp, K., & Ziegler, T. (2002). Another new member of the *Varanus* (*Euprepiosaurus*) *indicus* group (Sauria, Varanidae): An undescribed species from Rennell Island, Solomon Islands. *SALAMANDRA-BONN, 38*(1), 15-26.
33. Branch, W. R. (1982). Hemipeneal morphology of platynotan lizards. *Journal of Herpetology, 16*, 16–38.
34. Brennan, I. G., Lemmon, A. R., Lemmon, E. M., Portik, D. M., Weijola, V., Welton, L., Donnellan, S. C., & Keogh, J. S. (2021). Phylogenomics of monitor lizards and the role of competition in dictating body size disparity. *Systematic Biology, 70*, 120–132.

35. Brown, R. M., Siler, C. D., Grismer, L. L., Das, I., & McGuire, J. A. (2012). Phylogeny and cryptic diversification in southeast Asian flying geckos. *Molecular Phylogenetics and Evolution, 65*, 351–361.
36. Brygoo, E. R. (1987). Les types de varanidés (reptiles, sauriens) du Muséum National d'Histoire Naturelle: catalogue critique. *Bulletin du Muséum national d'histoire naturelle. Section A, Zoologie, biologie et écologie animales*, *9*(2), 21-38.
37. Budowle, B., Garofano, P., Hellman, A., Ketchum, M., Kanthaswamy, S., Parson, W., van Haeringen, W., Fain, S., & Broad, T. (2005). Recommendations for animal DNA forensic and identity testing. *International Journal of Legal Medicine, 119*, 295–302.
38. Card, W., & Kluge, A. G. (1995). Hemipeneal skeleton and varanid lizard systematics. *Journal of Herpetology, 29*(2), 275–280.
39. Ciofi, C., & Bruford, M. W. (1998). Isolation and characterization of microsatellite loci in the Komodo dragon *Varanus komodoensis*. *Molecular Ecology*, 7, 134-136.
40. Ciofi, C., Tzika, A. C., Natali, C., Watts, P. C., Sulandari, S., Zein, M. S., & Milinkovitch, M. C. (2011). Development of a multiplex PCR assay for fine-scale population genetic analysis of the Komodo monitor *Varanus komodoensis* based on 18 polymorphic microsatellite loci. *Molecular Ecology Resources, 11*, 550–556.
41. Collar, D. C., Schulte II, J. A., & Losos, J. B. (2011). Evolution of extreme body size disparity in monitor lizards (*Varanus*). *Evolution: International Journal of Organic Evolution, 65*(9), 2664–2680.
42. Comstock, K. E., Ostrander, E. A., & Wasser, S. K. (2003). Amplifying nuclear and mitochondrial DNA from African elephant ivory: A tool for monitoring the ivory trade. *Conservation Biology, 17*(6), 1840–1843.
43. Conrad, J. L., Balcarcel, A. M., & Mehling, C. M. (2012). Earliest example of a giant monitor lizard (*Varanus*, Varanidae, Squamata). *PLoS ONE, 7*, e41767.
44. Cope, E. D. (1896). On the hemipenes of the Sauria. *Proceedings of the Academy of Natural Sciences of Philadelphia, 48*, 461–467.
45. Cota, M., Stuart, B. L., Grismer, L., Quah, E., Panitvong, N., Neang, T., Nguyen, N. S., Wogan, G., Lwin, K., Srinivasulu, C., Srinivasulu, B., Vijayakumar, S. P., Ramesh, M., Ganesan, S. R., Madala, M., Sreekar, R., Rao, D.-Q., Thakur, S., Mohapatra, P., & Vyas, R. (2021). *Varanus bengalensis*. The IUCN Red List of Threatened Species 2021: e.T164579A1058949. https://dx.doi.org/10.2305/IUCN.UK.2021-2.RLTS.T164579A1058949.en. Accessed on 22 August 2024.
46. Das, A., Hasan, M. K., Bhattarai, S., Wangyal, J., & Mohapatra, P. (2021). *Varanus flavescens*. The IUCN Red List of Threatened Species 2021: e. T22872A127899602. https://dx.doi.org/10.2305/IUCN.UK.2021-2.RLTS.T22872A127899602.en. Accessed on 23 August 2024.
47. Das, I. (1989). Indian monitor lizards: A review of human utilization patterns. *Hamadryad, 14*, 16–19.
48. Daudin, F. M. (1802). *Histoire Naturelle, générale et particulière des reptiles*, ouvrage faisant suite, à l'histoire naturelle, générale et particulière composée par LECLERC DE BUFFON, et rédigée par C. S. SONNINI, vol. 3. F. Dufart, Paris.
49. de Fejervary, G. J. (1918). Contributions to a monography on fossil Varanidae and on Megalanidae. *Annales Musei Nationalis Hungarici, Pars Zoologica, 16*, 341-467.

50. de Fejervary, G. J. (1935). Further contributions to a monograph of Megalanidae and fossil Varanidae - With notes on recent varanians. *Annales Musei Nationalis Hungarici, Pars Zoologica, 29*, 1-130.
51. de Lisle, H. F. (2009). Catalog of the genus *Varanus* (Reptilia: Squamata: Varanidae) with new designations of a neotype and a lectotype. *ZooNova, 1*, 8–32.
52. Deepak, V., & Karanth, P. (2018). Aridification driven diversification of fan-throated lizards from the Indian subcontinent. *Molecular Phylogenetics and Evolution, 120*, 53-62.
53. Deepak, V., Lalronunga, S., Lalhmingliani, E., Das, A., Narayanan, S., Das, I., & Gower, D. J. (2021). Phylogenetic relationships of xenodermid snakes (Squamata: Serpentes: Xenodermidae), with the description of a new genus. *Vertebrate Zoology, 71*, 747-762.
54. Dennell, R., Coard, R., & Turner, A. (2006). The biostratigraphy and magnetic polarity zonation of the Pabbi Hills, northern Pakistan: An upper Siwalik (Pinjor stage) upper Pliocene–Lower Pleistocene fluvial sequence. *Palaeogeography, Palaeoclimatology, Palaeoecology, 234*, 168-185.
55. Doughty, P., Kealley, L., Fitch, A., & Donnellan, S. C. (2014). A new diminutive species of *Varanus* from the Dampier Peninsula, western Kimberley region, Western Australia. *Records of the Western Australian Museum, 29*(Part 2), 128-140.
56. Dowell, S. A., Portik, D. M., de Buffrénil, V., Ineich, I., Greenbaum, E., Kolokotronis, S. O., & Hekkala, E. R. (2016). Molecular data from contemporary and historical collections reveal a complex story of cryptic diversification in the *Varanus* (*Polydaedalus*) *niloticus* species group. *Molecular Phylogenetics and Evolution, 94*, 591-604.
57. Drummond, A. J., Suchard, M. A., Xie, D., & Rambaut, A. (2012). Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution, 29*, 1969–1973.
58. Earl, D. A., & VonHoldt, B. M. (2012). STRUCTURE HARVESTER: A website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources, 4*, 359–361.
59. Eidenmüller, B., & Wicker, R. (2005). Eine weitere neue Waranart aus dem *Varanus prasinus*-Komplex von der Insel Misol, Indonesien. *Sauria, 27*(1), 3-8.
60. Esri, 2018. ArcGIS Desktop: Release 10.6.1. Environmental Systems. Research Institute, Redlands, CA.
61. Estes, R., & Camp, C. L. (Eds.). (1988). *Phylogenetic Relationships of the Lizard Families: Essays Commemorating Charles L. Camp*. Stanford University Press.
62. Falconer, H. P. (1868). *Paleontological Memoirs and Notes of the Late Hugh Falconer*. Robert Hardwicke Publishers.
63. Favre, A., Päckert, M., Pauls, S. U., Jähnig, S. C., Uhl, D., Michalak, I., & Muellner-Riehl, A. N. (2015). The role of the uplift of the Qinghai-Tibetan Plateau for the evolution of Tibetan biotas. *Biology, 90*, 236-253.
64. Fitch, A. J., Goodman, A. E., & Donnellan, S. C. (2005). Isolation and characterisation of microsatellite markers for the Australian monitor lizard, *Varanus acanthurus* (Squamata: Varanidae) and their utility in other selected varanid species. *Molecular Ecology Notes, 5*, 521–523.
65. Fitch, A. J., Goodman, A. E., & Donnellan, S. C. (2006). A molecular phylogeny of the Australian monitor lizards (Squamata: Varanidae) inferred from mitochondrial DNA sequences. *Australian Journal of Zoology, 54*, 253-269.

66. Frankham, R., Briscoe, D. A., & Ballou, J. D. (2002). *Introduction to conservation genetics*. Cambridge University Press.
67. Fraser, D. J., & Bernatchez, L. (2001). Adaptive evolutionary conservation: Towards a unified concept for defining conservation units. *Molecular Ecology, 10*(12), 2741-2752.
68. Fu, M., Yu, D., Peng, J., Wang, Y., Gao, S., Wang, L., Liu, X., Hu, S., & Gao, L. (2011). Isolation and characterization of novel microsatellite markers in water monitor (*Varanus salvator*). *Conservation Genetics Resources, 3*, 777–779.
69. Fuller, S., Baverstock, P., & King, D. (1998). Biogeographic origins of goannas (Varanidae): A molecular perspective. *Molecular Phylogenetics and Evolution, 9*, 294–307.
70. Gasteiger, E., Gattiker, A., Hoogland, C., Ivanyi, I., Appel, R. D., & Bairoch, A. (2003). ExPASy: The proteomics server for in depth protein knowledge and analysis. *Nucleic Acids Research, 31*, 3784–3788.
71. Gaubert, P., Njiokou, F., Olayemi, A., Pagani, P., Dufour, S., Danquah, E., ... Dernat, R. (2015). Bushmeat genetics: Setting up a reference framework for the DNA typing of African forest bushmeat. *Molecular Ecology Resources, 15*(3), 633-651.
72. Gaulke, M., & Curio, E. (2001). A new monitor lizard from Panay Island, Philippines (Reptilia, Sauria, Varanidae). *Spixiana, 24*(3), 275-286.
73. Gautam, K. B., Kumar, A., Das, A., & Gupta, S. K. (2023). Himalayan upliftment and Shiwalik succession act as a cradle for divergence in Bengal monitor lizard *Varanus bengalensis* (Reptilia: Varanidae) in India. *Cladistics, 39*(5), 382-397.
74. Ge, C., Cui, Y. N., Jing, P. Y., & Hong, X. Y. (2014). An alternative suite of universal primers for genotyping in multiplex PCR. *PLoS One, 9*, e92826.
75. Ghobrial, L., Lankester, F., Kiyang, J. A., Akih, A. E., De Vries, S., Fotso, R., ... Gonder, M. K. (2010). Tracing the origins of rescued chimpanzees reveals widespread chimpanzee hunting in Cameroon. *BMC Ecology, 10*, 1-15.
76. Gibbons, J. W., Scott, D. E., Ryan, T. J., Buhlmann, K. A., Tuberville, T. D., Metts, B. S., ... Winne, C. T. (2000). The global decline of reptiles, deja-vu amphibians. *Bioscience, 50*, 653–667.
77. Glaubitz, J. C. (2004). Convert: A user-friendly program to reformat diploid genotypic data for commonly used population genetic software packages. *Molecular Ecology Notes, 4*, 309–310.
78. Gray, J. E. (1831). *A synopsis of the species of the class Reptilia*. Whittaker, Treacher, and Company.
79. Gray, J. E. (1870). On the claspers of male lizards. *Annals and Magazine of Natural History, 7*, 283.
80. Guha, S., & Kashyap, V. K. (2006). Molecular identification of lizard by RAPD and FINS of mitochondrial 16s rRNA gene. *Legal Medicine (Tokyo), 8*, 5-10.
81. Guibé, J. (1954). Catalogue des types de lézards du Muséum National d'Histoire Naturelle. Muséum national d'histoire naturelle.
82. Gupta, S. K., Thangaraj, K., & Singh, L. (2006). A simple and inexpensive molecular method for sexing and identification of the forensic samples of elephant origin. *Journal of Forensic Sciences, 51*(4), 805-807.
83. Gymrek, M. (2017). A genomic view of short tandem repeats. In *Molecular and Genetic Bases of Disease* (pp. 9–16).

84. 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.
85. Hardwicke, T., & Gray, J. E. (1828). A Synopsis of the Species of Saurian Reptiles: Collected in India by Major-General Hardwicke. W. Phillips.
86. Harfoot, M., Glaser, S. A., Tittensor, D. P., Britten, G. L., McLardy, C., Malsch, K., & Burgess, N. D. (2018). Unveiling the patterns and trends in 40 years of global trade in CITES-listed wildlife. *Biological Conservation, 223*, 47-57.
87. Hocknull, S. A., Piper, P. J., van den Bergh, G. D., Due, R. A., Morwood, M. J., & Kurniawan, I. (2009). Dragon's paradise lost: Palaeobiogeography, evolution and extinction of the largest-ever terrestrial lizards (Varanidae). *PLoS One*, *4*, e7241.
88. Hofmann, S., Stöck, M., Zheng, Y., Ficetola, F. G., Li, J. T., Scheidt, U., & Schmidt, J. (2017). Molecular phylogenies indicate a Paleo-Tibetan origin of Himalayan lazy toads (*Scutiger*). *Scientific Reports, 7*, 1-12.
89. Hofreiter, M., & Stewart, J. (2009). Ecological change, range fluctuations and population dynamics during the Pleistocene. *Current Biology, 19*(14), R584-R594.
90. Holmes, R. B., Murray, A. M., Attia, Y. S., Simons, E. L., & Chatrath, P. (2010). Oldest known *Varanus* (Squamata: Varanidae) from the upper Eocene and lower Oligocene of Egypt: Support for an African origin of the genus. *Palaeontology, 53*, 1099-1110.
91. Holmes, R. S., King, M., & King, D. (1975). Phenetic relationships among varanid lizards based upon comparative electrophoretic data and karyotypic analyses. *Biochemical Systematics and Ecology, 3*(4), 257-263.
92. Huang, C. Y., Yuan, P. B., Lin, C. W., Wang, T. K., & Chang, C. P. (2000). Geodynamic processes of Taiwan arc–continent collision and comparison with analogs in Timor, Papua New Guinea, Urals and Corsica. *Tectonophysics*, *325*, 1-21.
93. Hugall, A. F., & Lee, M. S. Y. (2004). Molecular claims of Gondwanan age for Australian agamid lizards are untenable. *Molecular Biology and Evolution, 21*, 2102-2110.
94. Hughes, J. D. (2003). Europe as consumer of exotic biodiversity: Greek and Roman times. *Landscape Research, 28*, 21-31.
95. Inskipp, T. (1981). *Indian trade in reptile skins* (pp. 2).
96. Jacobs, H. J. (2003). A further new emerald tree monitor lizard of the *Varanus prasinus* species group from Waigeo, West Irian (Squamata: Sauria: Varanidae). *SALAMANDRA-BONN*, *39*(2), 65-74.
97. Johansson, U. S., Alström, P., Olsson, U., Ericson, P. G., Sundberg, P., & Price, T. D. (2007). Build-up of the Himalayan avifauna through immigration: A biogeographical analysis of the *Phylloscopus* and *Seicercus* warblers. *Evolution, 61*, 324-333.
98. Johnsingh, A. J. T., Qureshi, Q., Goyal, S. P., Rawat, G. S., Ramesh, K., David, A., Rajapandian, K., & Prasad, S. (2004). Conservation status of Tiger and associated species in the Terai Arc Landscape, India. Wildlife Institute of India, Dehradun, RR-04/001, Pp.viii + 110.
99. Jombart, T., Devillard, S., & Balloux, F. (2010). Discriminant analysis of principal components: A new method for the analysis of genetically structured populations. *BMC Genetics, 11*, 1-15.
100. King, M., & King, D. (1975). Chromosomal evolution in the lizard genus *Varanus* (Reptilia). *Australian Journal of Biological Sciences, 28*(1), 89-108.

101. Koch, A., Arida, E., Schmitz, A., Böhme, W., & Ziegler, T. (2009). Refining the polytypic species concept of mangrove monitors (Squamata: *Varanus indicus* group): A new cryptic species from the Talaud Islands, Indonesia, reveals the underestimated diversity of Indo-Australian monitor lizards. *Australian Journal of Zoology, 57*(1), 29-40.
102. Koch, A., Gaulke, M., & Böhme, W. (2010). Unravelling the underestimated diversity of Philippine water monitor lizards (Squamata: *Varanus salvator* complex), with the description of two new species and a new subspecies. *Zootaxa, 2446*(1), 1-54.
103. Koch, A., Ziegler, T., Boehme, W., Arida, E., & Auliya, M. (2013). Pressing problems: Distribution, threats, and conservation status of the monitor lizards (Varanidae: *Varanus* spp.) of Southeast Asia and the Indo-Australian archipelago. *Herpetological Conservation and Biology, 8*, 1-62.
104. Kocher, T. D., Thomas, W. K., Meyer, A., Edwards, S. V., Pääbo, S., Villablanca, F. X., & Wilson, A. C. (1989). Dynamics of mitochondrial DNA evolution in animals: Amplification and sequencing with conserved primers. *Proceedings of the National Academy of Sciences, 86*(16), 6196-6200.
105. Kopelman, N. M., Mayzel, J., Jakobsson, M., Rosenberg, N. A., & Mayrose, I. (2015). Clumpak: A program for identifying clustering modes and packaging population structure inferences across K. *Molecular Ecology Resources, 15*, 1179-1191.
106. Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K. (2018). MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution, 35*, 1547-1549.
107. Kumazawa, Y., & Endo, H. (2004). Mitochondrial genome of the Komodo dragon: Efficient sequencing method with reptile oriented primers and novel gene rearrangements. *DNA Research, 11*, 115-125.
108. Lalremsanga, H. T., Decemson, H., Vabeiryureilai, M., Malsawmdawngliana, F., Lalhlimpuia, V., Muansanga, L., & Biakzuala, L. (2022). Phylogenetic position of Tropidophorus assamensis Annandale, 1912 with updated morphological data and distributional records. *Journal of Herpetology, 32*.
109. Lambert, M. R. K. (1969). Tortoise drain in Morocco. *Oryx, 10*(3), 161-166.
110. Laurenti (1768). Specimen medicum exhibens synopsis reptilium emendatam cum experiments circa venea et antidota reptilium Austriacorum. In Viennae.
111. Leigh, J. W., & Bryant, D. (2015). POPART: Full-feature software for haplotype network construction. *Methods in Ecology and Evolution, 6*(9), 1110-1116.
112. Linacre, A., & Tobe, S. S. (2011). An overview to the investigative approach to species testing in wildlife forensic science. *Investigative Genetics, 2*, 1-9.
113. Lydekker, R. (1886). Fauna of the Karnul caves. *Palaeontologica Indica, 10*, 1-58.
114. Lydekker, R. (1888). Catalogue of the fossil Reptilia and Amphibia in the British Museum (Natural History), Cromwell Road, S.W. Pt.1. The Orders Ornithosauria, Crocodilia, Dinosauria, Squamata, Rhynchocephalia, and Preterosauria. London: The Trustees. 245.
115. Ma, K., Zhao, X., Li, H., Cao, Y., Li, W., Ouyang, J., ... & Liu, W. (2018). Massive parallel sequencing of mitochondrial DNA genomes from mother-child pairs using the ion torrent personal genome machine (PGM). *Forensic Science International: Genetics, 32*, 88-93.
116. Mani, M. S. (1974). Ecology and biogeography in India. The Hague: Dr. W. Junk. Publishers.

117. Martens, J., Tietze, D. T., & Päckert, M. (2011). Phylogeny, biodiversity, and species limits of passerine birds in the Sino-Himalayan region—a critical review. *Ornithological Monographs, 70*, 64-94.
118. Maryan, B., Oliver, P. M., Fitch, A. J., & O'Connell, M. (2014). Molecular and morphological assessment of *Varanus pilbarensis* (Squamata: Varanidae), with a description of a new species from the southern Pilbara, Western Australia. *Zootaxa, 3768*(2), 139-158.
119. Mendiratta, U., Sheel, V., & Singh, S. (2017). Enforcement seizures reveal large-scale illegal trade in India's tortoises and freshwater turtles. *Biological Conservation, 207*, 100-105.
120. Merrell, D. J. (1981). *Ecological Genetics*. University of Minnesota Press, Minneapolis, MN.
121. Mertens, R. (1937). Reptilien und Amphibien aus dem südlichen Inner-Afrika. *Abhandlungen der Senckenbergischen Naturforschenden Gesellschaft (Frankfurt), 435*, 1-23.
122. Mertens, R. (1942). Die Familie der Warane (Varanidae), 3. Teil: Taxonomie. *Abhandlungen der Senckenbergischen Naturforschenden Gesellschaft*, 466, 235-391.
123. Mertens, R. (1959). Liste der Warane Asiens und der indo-australischen Inselwelt mit systematischen Bemerkungen. *Senckenbergiana Biologica, 40*, 221-240.
124. Mertens, R (1963). *Helodermatide, Varanidae, Lanthanotide*. Das Tierreich, Lief. 79, Gruyter ed
125. Molnar, R. E. (2004). *Dragons in the dust: The paleobiology of the giant monitor lizard Megalania*. Indiana University Press, Bloomington, Indiana.
126. Moritz, C. (1994). Applications of mitochondrial DNA analysis in conservation: A critical review. *Molecular Ecology, 3*(4), 401-411.
127. Oosterhout, C. V., Hutchinson, W. F., Wills, D. P. M., & Shipley, P. F. (2003). Micro-checker version 2.2.3. Department of Biological Sciences and Department of Computer Science, University of Hull, Hull, UK.
128. Pandit, M. K. (2017). *Life in the Himalaya: An ecosystem at risk*. Harvard University Press.
129. Park, L. K., & Moran, P. (1994). Developments in molecular genetic techniques in fisheries. *Reviews in Fish Biology and Fisheries, 4*, 272-299.
130. Patwardhan, A., Ray, S., & Roy, A. (2014). Molecular markers in phylogenetic studies-a review. *Journal of Phylogenetics & Evolutionary Biology, 2*(2), 131.
131. Pavón-Vázquez, C. J., Esquerré, D., Fitch, A. J., Maryan, B., Doughty, P., Donnellan, S. C., & Keogh, J. S. (2022). Between a rock and a dry place: Phylogenomics, biogeography, and systematics of ridge-tailed monitors (Squamata: Varanidae: *Varanus acanthurus* complex). *Molecular Phylogenetics and Evolution, 173*, 107516.
132. Peakall, R. O. D., & Smouse, P. E. (2006). GENALEX 6: Genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes, 6*, 288-295.
133. Pernetta, A. P. (2009). Monitoring the trade: Using the CITES database to examine the global trade in live monitor lizards (*Varanus spp.*). *Biawak, 3*, 37-45.
134. Pfenninger, M., Cordellier, M., & Streit, B. (2006). Comparing the efficacy of morphologic and DNA-based taxonomy in the freshwater gastropod genus Radix (*Basommatophora, Pulmonata*). *BMC Evolutionary Biology, 6*, 1-14.

135. Pianka, E. R. (1995). Evolution of body size: Varanid lizards as a model system. *The American Naturalist, 146*(3), 398-414.
136. Pianka, E., & King, D. (2004). *Varanoid lizards of the world*. Indiana University Press, Bloomington, Indiana.
137. Portik, D. M., & Papenfuss, T. J. (2012). Monitors cross the Red Sea: The biogeographic history of *Varanus yemenensis*. *Molecular Phylogenetics and Evolution, 62*, 561-565.
138. Prasad, K. N., & Yadagiri, P. (1986). Pleistocene cave fauna, Karnool District, Andhra Pradesh. *Rec. Geol. Surv. India, 115*, 71-77.
139. Pritchard, J. K., Stephens, M., & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics, 155*, 945-959.
140. Quah, E., Lwin, K., Cota, M., Grismer, L., Neang, T., Wogan, G., ... & Koch, A. (2021). *Varanus salvator*. The IUCN Red List of Threatened Species 2021: e. T178214A113138439. https://dx.doi.org/10.2305/IUCN.UK.2021-2.RLTS.T178214A113138439.en. Accessed on 23 August 2024
141. Rajpoot, A., Kumar, V. P., Bahuguna, A., & Kumar, D. (2017). Forensically informative nucleotide sequencing (FINS) for the first time authentication of Indian *Varanus* species: Implication in wildlife forensics and conservation. *Mitochondrial DNA Part A, 28*(6), 892-900.
142. Rissler, L. J., & Apodaca, J. J. (2007). Adding more ecology into species delimitation: Ecological niche models and phylogeography help define cryptic species in the black salamander (*Aneides flavipunctatus*). *Systematic Biology, 56*, 924-942.
143. Røed, K. H. (1998). Microsatellite variation in Scandinavian Cervidae using primers derived from Bovidae. *Hereditas, 129*(1), 19-25.
144. Rozas, J., Ferrer-Mata, A., Sánchez-DelBarrio, J. C., Guirao-Rico, S., Librado, P., Ramos-Onsins, S. E., & Sánchez-Gracia, A. (2017). DnaSP 6: DNA sequence polymorphism analysis of large data sets. *Molecular Biology and Evolution, 34*(12), 3299-3302.
145. Saferstein, R. (2009). *Forensic Science: From the Crime Scene to the Crime Lab*. Pearson Prentice Hall. Retrieved from https://books.google.co.in/books?id=TFglHwAACAAJ
146. Sahajpal, V., & Goyal, S. P. (2009). Identification of a forensic case using microscopy and forensically informative nucleotide sequencing (FINS): A case study of small Indian civet (*Viverricula indica*). *Science & Justice, 50*, 94-97.
147. Sambrook, J., Fritsch, E. F., & Maniatis, T. (1989). *Molecular Cloning: A Laboratory Manual* (2nd ed.). Cold Spring Harbor Laboratory Press.
148. Schlaepfer, M. A., Hoover, C., & Dodd, C. K. (2005). Challenges in evaluating the impact of the trade in amphibians and reptiles on wild populations. *AIBS Bulletin, 55*(3), 256-264.
149. Schmidt, J., Opgenoorth, L., Hoell, S., & Bastrop, R. (2012). Into the Himalayan exile: The phylogeography of the ground beetle Ethira clade supports the Tibetan origin of forest-dwelling Himalayan species groups. *PLoS One, 7*(9), e45482.
150. Schulte, J. A., Melville, J., & Larson, A. (2003). Molecular phylogenetic evidence for ancient divergence of lizard taxa on either side of Wallace's Line. *Proceedings of the Royal Society of London B: Biological Sciences, 270*(1515), 597-603.
151. Semwal, R. L. (2005). *The Terai Arc Landscape in India, Securing Protected Areas in the Face of Global Change*. WWF-India, New Delhi.

152. Shankar, P. G., Swamy, P., Williams, R. C., Ganesh, S. R., Moss, M., Höglund, J., ... Wüster, W. (2021). King or royal family? Testing for species boundaries in the King Cobra, *Ophiophagus hannah* (Cantor, 1836), using morphology and multilocus DNA analyses. *Molecular Phylogenetics and Evolution*, *165*, 107300.
153. Sharma, C. P., Kumar, A., Sharma, V., Singh, B., Kumar, G. C., & Gupta, S. K. (2019). Online selling of wildlife part with spurious name: A serious challenge for wildlife crime enforcement. *International Journal of Legal Medicine*, *133*, 65-69.
154. Singh, A., Rana, B. S., Thakur, M., Tripathy, B., Sharma, L. K., & Chandra, K. (2020). Range extension of the Bengal monitor (*Varanus bengalensis*) for the Trans-Himalayan Region with an altitude record for monitor lizards. *Zootaxa*, *4732*, 4732.
155. Smissen, P. J., Melville, J., Sumner, J., & Jessop, T. S. (2013). Mountain barriers and river conduits: Phylogeographical structure in a large, mobile lizard (Varanidae: *Varanus varius*) from eastern Australia. *Journal of Biogeography*, *40*, 1729-1740.
156. Smith, J. S. C., & Smith, O. S. (1992). Fingerprinting crop varieties. *Advances in Agronomy*, *47*, 85-140.
157. Soorae, P., Eid, E.K.A., Behbehani, S.J.Y., Al Johany, A.M.H., Amr, Z.S.S., Egan, D.M., Els, J., Baha El Din, S., Böhme, W., Orlov, N.L., Wilkinson, J., Tuniyev, B., Lymberakis, P., Aghasyan, A., Cogălniceanu, D., Ananjeva, N.B., Disi, A.M., Mateo, J.A., Sattorov, T., Nuridjanov, D. & Chirikova, M. 2021. *Varanus griseus*. The IUCN Red List of Threatened Species 2021: e.T62252A3110663. https://dx.doi.org/10.2305/IUCN.UK.2021-2.RLTS.T62252A3110663.en. Accessed on 23 August 2024.
158. Spellerberg, I. F. (1976). The amphibian and reptile trade with particular reference to collecting Europe. *Biological Conservation*, *10*, 221-232.
159. Spicer, R. A. (2017). Tibet, the Himalaya, Asian monsoons and biodiversity – In what ways are they related? *Plant Diversity*, *39*, 233-244.
160. Sweet, S. S., & Pianka, E. R. (2007). Monitors, mammals and Wallace's Line. *Mertensiella*, *16*, 79-99.
161. Thompson, J. D., Higgins, D. G., & Gibson, T. J. (1994). CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research*, *22*, 4673-4680.
162. Tikader, B. K. (1983). *Threatened animals of India*. Threatened animals of India. pp 241-247
163. Tittensor, D. P., Walpole, M., Hill, S., Boyce, D., Britten, G. L., Burgess, N., ... Teh, L. S. L. (2014). A mid-term analysis of progress towards international biodiversity targets. *Science*, *346*, 241-244.
164. Tóth, G., Gáspári, Z., & Jurka, J. (2000). Microsatellites in different eukaryotic genomes: Survey and analysis. *Genome Research*, *10*(7), 967-981.
165. TRAFFIC. (2021). *Monitor lizard factsheet*. WWF India. Retrieved from https://join.wwfindia.org/illegal-wildlife-trafficking/MonitorLizardFactsheet2021.pdf
166. Uetz, P., Freed, P., Aguilar, R., Reyes, F., Kudera, J., & Hošek, J. (Eds.). (2023). *The Reptile Database*. http://www.reptile-database.org. Accessed on 22 August 2024.
167. Valdiya, K. S. (2002). Emergence and evolution of Himalaya: reconstructing history in the light of recent studies. *Progress in Physical Geography*, *26*, 360-399.

168. van Uhm, D. P. (2016). *The Illegal Wildlife Trade: Inside the World of Poachers, Smugglers and Traders*. Cham, Switzerland: Springer International Publishing.
169. Verma, S. K., & Singh, L. (2003). Novel universal primers establish identity of an enormous number of animal species for forensic application. *Molecular Ecology Notes, 3*, 28-31.
170. Vidal, N., Marin, J., Sassi, J., Battistuzzi, F. U., Donnellan, S., Fitch, A. J., ... Hedges, S. B. (2012). Molecular evidence for an Asian origin of monitor lizards followed by tertiary dispersals to Africa and Australasia. *Biology Letters, 8*, 853-855.
171. Wang, K., Che, J., Lin, S., Deepak, V., Aniruddha, D. R., Jiang, K., ... Siler, C. D. (2019). Multilocus phylogeny and revised classification for mountain dragons of the genus *Japalura sl.* (Reptilia: Agamidae: Draconinae) from Asia. *Zoological Journal of the Linnean Society, 185*, 246-267.
172. Wang, K., Lyu, Z., Wang, J., Qi, S., & Che, J. (2022). The updated checklist and zoogeographic division of the reptilian fauna of Yunnan Province, China. *Biodiversity Science, 30*(4), 21326.
173. Wasser, S. K., Shedlock, A. M., Comstock, K., Ostrander, E. A., Mutayoba, B., & Stephens, M. (2004). Assigning African elephant DNA to geographic region of origin: applications to the ivory trade. *Proceedings of the National Academy of Sciences, 101*(41), 14847-14852.
174. Wasser, S. K., Mailand, C., Booth, R., Mutayoba, B., Kisamo, E., Clark, B., & Stephens, M. (2007). Using DNA to track the origin of the largest ivory seizure since the 1989 trade ban. *Proceedings of the National Academy of Sciences, 104*(10), 4228-4233.
175. Weijola, V. S. Å., & Sweet, S. S. (2010). A new melanistic species of monitor lizard (Reptilia: Squamata: Varanidae) from Sanana Island, Indonesia. *Zootaxa, 2434*(1), 17-32.
176. Weijola, V., Donnellan, S. C., & Lindqvist, C. (2016). A new blue-tailed monitor lizard (Reptilia, Squamata, *Varanus*) of the *Varanus indicus* group from Mussau Island, Papua New Guinea. *ZooKeys* (568), 129.
177. Weijola, V., Kraus, F., Vahtera, V., Lindqvist, C., & Donnellan, S. C. (2017). Reinstatement of *Varanus douarrha* Lesson, 1830 as a valid species with comments on the zoogeography of monitor lizards (Squamata: Varanidae) in the Bismarck archipelago, Papua New Guinea. *Australian Journal of Zoology, 64*, 434–451.
178. Weijola, V., Vahtera, V., Lindqvist, C., & Kraus, F. (2019). A molecular phylogeny for the Pacific monitor lizards (*Varanus* subgenus *Euprepiosaurus*) reveals a recent and rapid radiation with high levels of cryptic diversity. *Zoological Journal of the Linnean Society, 186*, 1053–1066.
179. Weijola, V., Vahtera, V., Koch, A., Schmitz, A., & Kraus, F. (2020). Taxonomy of Micronesian monitors (Reptilia: Squamata: *Varanus*): Endemic status of new species argues for caution in pursuing eradication plans. *Royal Society Open Science, 7*, 200092. https://doi.org/10.1098/rsos.200092
180. Weijola, V., & Kraus, F. (2023). Two new species of monitor lizards (Squamata: *Varanus*) endemic to the Louisiade and Tanimbar Archipelagos with a key to the subgenus *Euprepiosaurus*. *Journal of Natural History, 57*(13-16), 947-975.
181. Welton, L. J., Siler, C. D., Bennett, D., Diesmos, A., Duya, M. R., Dugay, R., ... Brown, R. M. (2010a). A spectacular new Philippine monitor lizard reveals a hidden biogeographic boundary and a novel flagship species for conservation. *Biology Letters, 6*, 654–658.

182. Welton, L. J., Siler, C. D., Linkem, C. W., Diesmos, A. C., & Brown, R. M. (2010b). Philippine bent-toed geckos of the *Cyrtodactylus agusanensis* complex: multilocus phylogeny, morphological diversity, and descriptions of three new species. *Herpetological Monographs, 24*, 55–85.
183. Welton, L. J., Siler, C. D., Linkem, C. W., Diesmos, A. C., Diesmos, M. L., Sy, E., & Brown, R. M. (2013). Dragons in our midst: Phyloforensics of illegally traded Southeast Asian monitor lizards. *Biological Conservation, 159*, 7-15.
184. Welton, L. J., Travers, S. L., Siler, C. D., & Brown, R. M. (2014). Integrative taxonomy and phylogeny-based species delimitation of Philippine water monitor lizards (*Varanus salvator* Complex) with descriptions of two new cryptic species. *Zootaxa*, *3881*(3), 201-227.
185. Willems, T., Gymrek, M., Highnam, G., Mittelman, D., Erlich, Y., & 1000 Genomes Project Consortium. (2014). The landscape of human STR variation. Genome research, 24(11), 1894-1904.
186. Xu, W., Dong, W. J., Fu, T. T., Gao, W., Lu, C. Q., Yan, F., Wu, Y. H., Jiang, K., Jin, J. Q., Chen, H. M., & Zhang, Y. P. (2021). Herpetological phylogeographic analyses support a Miocene focal point of Himalayan uplift and biological diversification. *Naturel Sciences Review, 8*, 263.
187. Yadav, P., Kumar, A., Sahoo, S., Yadav, N., Hussain, S. A., & Gupta, S. K. (2021). Identification of Gangetic turtles based on species-specific variations on mitochondrial cyt b and nuclear Cmos genes. *Forensic Science International: Animals and Environments, 1*, 100035. https://doi.org/10.1016/j.fsiae.2021.100035
188. Yang, D., & Li, S. (1987). A new species of *Varanus* from Yunnan, with morphological comparison between it and six other species from Southeast Asia. *Chinese Herpetological Research, 1987*, 60-63.
189. Ziegler, T., & Böhme, W. (1997). On the synonymy and taxonomy of the Bengal monitor lizard, *Varanus bengalensis* (Daudin, 1802) complex (Sauria: Varanidae). *Amphibia-Reptilia*, *18*(3), 207-211.
190. Ziegler, T., Böhme, W., & Schmitz, A. (2007). A new species of the *Varanus indicus* group (Squamata, Varanidae) from Halmahera Island, Moluccas: Morphological and molecular evidence. *Zoosystematics and Evolution*, 83(S1), 109-119.

PUBLICATION

Peer Reviewed Articles

1. **Gautam, K. B**., Kumar, A., Das, A., & Gupta, S. K. (2023). Himalayan upliftment and Shiwalik succession act as a cradle for divergence in Bengal monitor lizard *Varanus bengalensis* (Reptilia: Varanidae) in India. *Cladistics, 39*(5), 382-397.

Conferences and Workshops

1. **Gautam, K. B**., Kumar, A., Das, A., & Gupta, S. K. "*Himalayan Upliftment and Shiwalik succession driven diversification of Bengal monitor lizard, Varanus bengalensis in India"* 17th Uttarakhand State Science & Technology (UCOST) Rural Science Congress – 2023
2. **Gautam, K. B**., Kumar, A., Das, A., & Gupta, S. K. "*Linking the origin of hemipenis': A molecular approach for identifying the trade route of Monitor Lizard*". Student Conference on Conservation Science (SCCS) Cambrige-2023
3. **Gautam, K. B**., Kumar, A., Das, A., & Gupta, S. K. *Influence of Himalayan upliftment on diversification of Bengal monitor lizard*". Student Conference on Conservation Science (SCCS)- Bengaluru-2023
4. **Gautam, K. B.,** Kumar, A., Das, A., & Gupta, S. K. *Genetic approach to identify the origin of monitor lizard hemipenis*". Commemorating 50 years of Tiger Project & First Indian Conservation Conference, Mysore, India – 2023
5. **Gautam, K. B.,** From evidence to insights: Practicing forensic science in monitoring the Monitor". National Conferece on Emerging Trends and Techniques in Forensic Investigation. University of Ladakh and NFSU-2024
6. **Gautam, K. B.,** Kumar, A., Das, A., & Gupta, S. K. *When Biogeography meets Forensics: Case study of Monitor Lizard (Varanus bengalensis)*. 10th World Congress of herpetology, Borneo, Sarawak, Malyasia-2024

Grants

1. Titled Project "Leveraging community and technology to combat illegal trade of Bengal Monitor lizard in Terai Arc, India" from The Rufford Small Grant Foundation
2. International Travel Support Scheme, Science and Engineering Research Board (SERB)

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Cladistics

Cladistics (2023) 1–16

doi:10.1111/cla.12542

Himalayan upliftment and Shiwalik succession act as a cradle for divergence in Bengal monitor lizard *Varanus bengalensis* (Reptilia: Varanidae) in India

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Received 3 November 2022; Revised 1 April 2023; Accepted 5 April 2023

Abstract

The Himalayan foothills and associated environment are well-known for driving the rapid diversification of many species and the formation of biodiversity hotspots. The effects of environmental change since the Miocene have accelerated species diversification, and hence are useful for studying population genetic structure, and evolutionary relationships via genetic approaches. To date, the effects of climatic fluctuations on the biogeography of large-bodied lizards have not been assessed comprehensively. Herein, we examine the diversification of *Varanus bengalensis*, focusing on its genetic structure to provide insights into how landscape structure and climatic fluctuations have shaped species differentiation. We confirm the existence of two distinct lineages within *V. bengalensis* distributed across the Himalayan foothills and the remainder of mainland India. Divergence analyses revealed the split between the Himalayan foothills and the remainder of the mainland lineages of *V. bengalensis* in the mid-Pliocene ~3.06 Ma, potentially as a consequence of the Siwalik broadening and climatic fluctuations across the Himalayan foothills. The results suggest recognition of a new lineage of *V. bengalensis* from the Himalayan foothills as a distinctive evolutionarily significant unit.

Introduction

The Himalayan herpetofauna is an integral part of mountain ecosystem dating back to the early Palaeocene, with drastic acceleration in diversification of most groups in the Miocene (Klaus et al., 2016; Xu et al., 2021). Reptiles are prone to local extinction, genetic discontinuities, speciation and alteration in distributional patterns, caused primarily by natural or human-induced fluctuations (Gibbons et al., 2000). Climatic oscillation and anthropogenic habitat destruction have affected the distribution patterns of many reptile species. Extensive diversification caused by climatic fluctuations in the Indian subcontinent has been recorded in many small reptiles, including *Ophisops, Sitana* and *Hemidactylus* (Agarwal and Ramakrishnan, 2017; Deepak and Karanth, 2018; Lajmi and Karanth, 2020). However, no robust phylogenetic studies have been conducted on monitor lizards, which are widespread and the largest lizards found in the Indian subcontinent. Monitor lizards are categorized under the genus *Varanus* and are widely distributed throughout the Old World tropics and subtropics (Auliya and Koch, 2020). The Indian subcontinent is a model landscape in which to examine the temporal response to climatic fluctuations and the effect of dynamic interplay between orogeny and recent anthropogenic activities on monitor lizards, which has been poorly documented so far. India harbours four species of monitor lizards: the Yellow monitor (*V. flavescens*) is recorded from Terai landscape, and western and eastern Indian floodplains; the Water monitor *V. salvator macromaculatus* and *V. salvator andamanensis* is found on the eastern coast and north-eastern India, and the Andaman Islands, respectively;

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the Desert monitor (*V. griseus koniecznyi*) is limited to Indian deserts; and the Bengal monitor (*V. bengalensis*) is widely distributed throughout India. The distribution range of *V. bengalensis* overlaps with those of *V. flavescens*, *V. salvator* and *V. griseus* (Auffenberg, 1994; Fig. 1).

All four extant monitor lizard species in India are protected under Schedule I of the Wildlife Protection Act, 1972, because of their high demand in the wildlife trade for skin as a leather product and in musical instruments, and eggs and meat for local consumption, traditional medicines, superstitious beliefs and retaliatory killings (Das, 1989; Koch et al., 2013; Bhattacharya and Koch, 2018; TRAFFIC, 2021). Lately, illegal exploitation of monitor lizard genitalia (the "hemipenis") has been rampant under the pseudonym of "Hatha jodi" in India (Sharma et al., 2019). In the last 4 years, the Wildlife Forensic and Conservation Genetics Cell at the Wildlife Institute of India has received more than 500 confiscated hemipenes of monitor lizards through different enforcement agencies with unknown geographical origin. This scale of

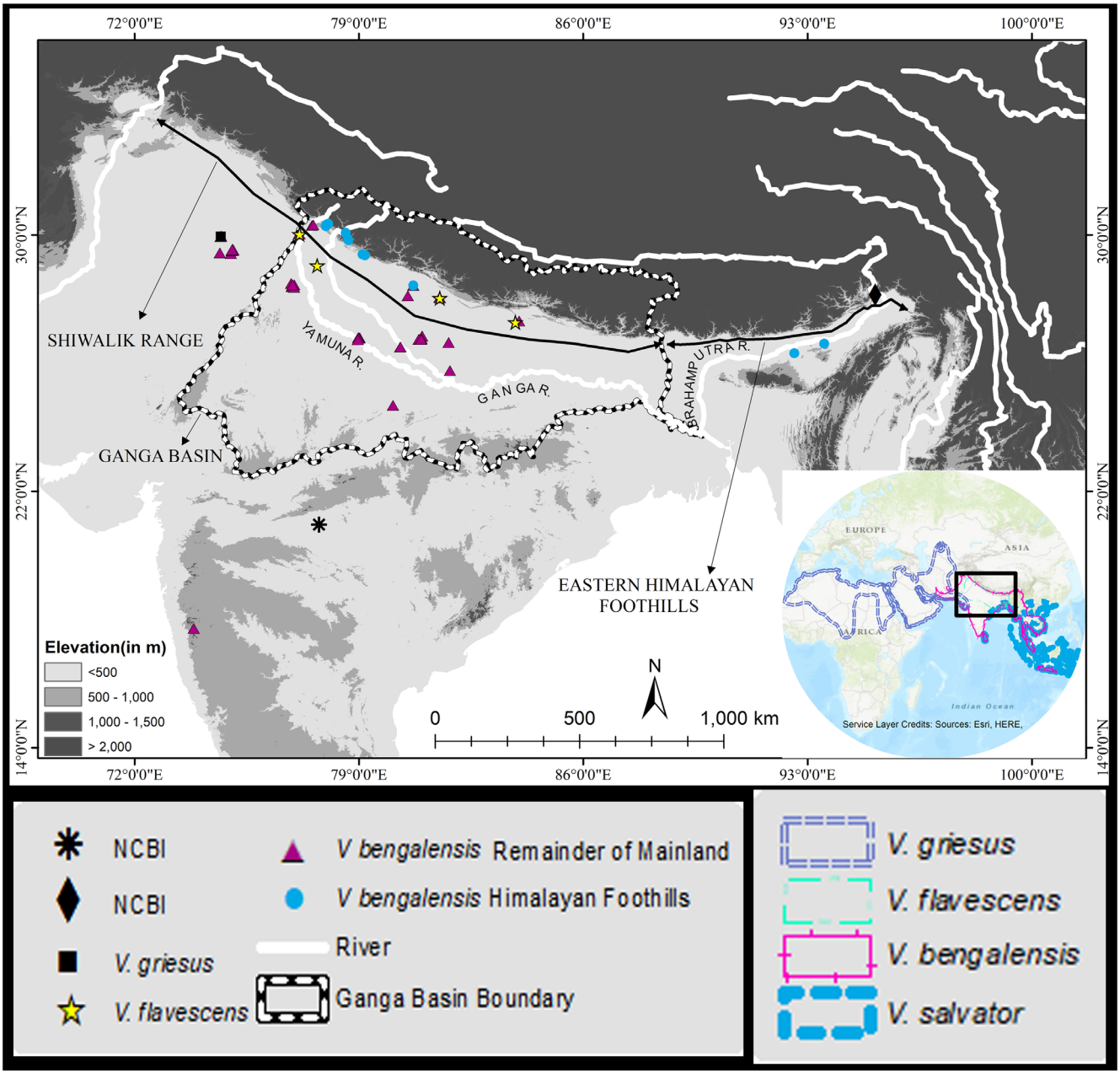

Fig. 1. Map of sampling sites of this study and a representative distribution range of *V. bengalensis*, *V. flavescens*, *V. griseus* and *V. salvator* in India in inset. Layers adopted from Farr et al., 2007; Lehner et al., 2008; Lehner and Grill, 2013; Esri, 2018; World Bank, 2022.

extensive illegal exploitation may be a major threat for monitor lizards in India. Information on genetic structure and the relationships among extant monitor lizard species could assist in recognizing evolutionarily significant varanid lineages and aid molecular tracking, conservation strategies and management planning.

Highly cosmopolitan, *V. bengalensis* thrives in diverse and heavily human-dominated regions across landscapes. Formerly *V. bengalensis* was listed as a polytypic species with two subspecies, namely *V. b. bengalensis* distributed in South and Southeast Asia, and *V. b. nebulosus* distributed in Southeast Asia (Pianka and King, 2004). Currently the subspecies *V. b. nebulosus* holds species status but requires a robust investigation to resolve the taxonomic issue related to *V. bengalensis* (Koch et al., 2013). Phenotypic characterization may clarify taxonomic classification, evolutionary inferences and species validation (Merrell, 1981). Nevertheless, exclusive dependency on morphological characters can conceal underlying patterns, wherein advanced genetic tools can provide an in-depth understanding of species' biogeography (Avise, 2000). Identifying taxonomic and population units within species to understand their evolutionary relationships is vital for conserving biological diversity (Allendorf et al., 2007). Recent advancements in genetics have become paramount to delimiting lineages and accurately inferring evolutionary history (Rissler and Apodaca, 2007; Welton et al., 2010a, b; Brown et al., 2012). Genetic tools have effectively unravelled cryptic lineages, identified new species of monitor lizards and helped in unfolding complex phylogenetic relationships (Smith et al., 2007; Smissen et al., 2013; Maryan et al., 2014; Dowell et al., 2016). Molecular studies with multilocus approaches have revealed immense amounts of information helping in the separation of divergent assemblages of monitor lizard species across African (Portik and Papenfuss, 2012), Indonesian (Welton et al., 2010a; Weijola et al., 2016, 2017, 2019, 2020), Australian (Smissen et al., 2013) and Asian (Vidal et al., 2012) geographies. In this study, we investigated the genetic structure and evolutionary relationship of *V. bengalensis* with other monitor lizard species, focusing on the Himalayan foothills, a biodiversity hotspot, to provide insight into how landscape structure, and geological and climatic processes have shaped species differentiation.

Methods

Study area

The present study site spans across the Himalayan foothills and covers the Indian region of the Terai Arc Landscape, which features the Shiwalik Hills in the north, the Aravali Hills in the south-west, the Yamuna River in the east, the southern foothills of the Himalayas, the Ganga river basin, adjoining Bhabhar areas, the eastern Himalayan foothills and the Terai flood plains. Additionally, several samples were included from south of the Ganga basin, the Brahmaputra basin and the Western Ghats (Fig. 1).

Sample collection and DNA extraction

A small 2 cm piece of tissue from the tail tip of 69 monitor lizards comprising *V. bengalensis* (n = 63), *V. flavescens* (n = 4) and *V. griseus* (n = 2) was collected. All samples were collected with the help of field veterinarians and experts with proper medical care. The Institutional Animal Ethics Committee approved the collection and storage techniques, and the authors confirm that laboratory works were executed within relevant guidelines and regulations (letter no: WII/IAEC/2017-18). Details of the origin and sample type of the genus *Varanus* included in this study are provided in Table S1. All samples were stored in 70% ethanol at room temperature. The genomic DNA was extracted using the DNeasy Blood Tissue Kit (QIAGEN, Hilden, Germany) in a final elution volume of 100 μL.

Mitochondrial DNA

We sequenced three mitochondrial regions: CO*I* (977 bp; Kumazawa and Endo, 2004), ND5 (668 bp) and Cyt *b* (814 bp). For amplification of ND5 and Cyt *b* gene regions, we designed the forward primer VAR-ND5F: 5′-CCATTACTTCAACCTGTTTCAC-3′ using the sequence of *V. niloticus* (AB185327), *V. salvator* (AB980995, EU747731 and NC010974) and *V. s. komini* (AB980996), and used it in combination with mcb869: 5′-CCTCCTAGTTTGTTAGGGATTGATCG-3′ (Verma and Singh, 2003). In addition, to estimate divergence times, we sequenced *V. bengalensis* (n = 4), *V. flavescens* (n = 2) and *V. griseus* (n = 2) using the fragment of NADH dehydrogenase subunit 4 (ND4, 600 bp) region (Kumazawa and Endo, 2004).

PCR reactions for each primer were performed in 20-μL reaction volumes using 1× PCR buffer (10 mM Tris–HCl, pH 8.3, and 50 mM KCl), 1.5 mM $MgCl_2$, 0.2 mM of each dNTPs, 3 pmol of each primer, 0.5 units of DreamTaq DNA Polymerase (Thermo Fisher Scientific, Waltham, MA, USA) and 1 μL (~30 ng) of template DNA. The PCR protocol used was as follows: initial denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 45 s, annealing at 56 °C for 60 s and extension at 72 °C for 75 s. The final extension was at 72 °C for 10 min. Negative controls were included in each reaction to check the reliability of the experiment. All PCR amplification was confirmed by electrophoresis on 2% agarose gel stained with Firefly dye and visualized under a UV transilluminator. The amplified PCR products were purified with exonuclease-I and shrimp alkaline phosphatase (Thermo Fisher Scientific) at 37 °C for 20 min to remove any remaining primer and dNTPs, followed by inactivation of enzymes at 85 °C for 15 min. The purified fragments were sequenced directly in a Genetic Analyser 3500 XL (Applied Biosystems/Thermo Fisher Scientific, Waltham, MA, USA) from the forward and reverse directions using a BigDye 3.1 kit. To check the alignment and the presence of stop codons, all four coding genes were subject to translation into amino acids using the Expasy: Translate tool (Gasteiger et al., 2003).

Microsatellite genotyping

Initially, 11 microsatellite loci were screened for *V. bengalensis* (n = 63), *V. flavescens* (n = 4) and *V. griseus* (n = 2), of which eight were successfully amplified. Two of the eight loci were monomorphic and excluded from further analysis. Further analysis was thus performed with six microsatellite loci: Varsa10; Varsa07 (Fu et al., 2011), VA74 (Fitch et al., 2005), K15; K11; K22 (Ciofi

et al., 2011; Table S6). The forward primer of each locus was connected with one of the 5′ universal sequence tail: T7 (5′-TAATACG ACTCACTATAGGG), M13 (5′-TGTAAAACGACGGCCAGT) and M13R (5′-CAGGAAACAGCTATGACC; Ge et al., 2014). The universal primers M13, M13R and T7 were labelled with fluorescent dyes FAM, HEX and TET, respectively. Multiplexing was carried out in 10-μL polymerase chain reaction (PCR) reaction volumes according to basepair size and dye pattern, containing 5 μL of Multiplex PCR Buffer Mix (QIAGEN), 0.5 μL Q-solution, 0.2 μM labelled forward primer (Applied Biosystems), 0.2 μM unlabelled reverse primer, and 80–100 ng of the template DNA. The PCR cycle was performed under the touchdown conditions, initial denaturation at 95 °C for 15 min, followed by eight cycles of denaturation at 95 °C for 45 s, annealing at 58 °C for 60 s and extension at 72 °C for 75 s, again followed by 15 cycles of denaturation at 95 °C for 45 s, annealing at 58 to 50 °C for 60 s and extension at 72 °C for 75 s, followed by 12 cycles of denaturation at 95 °C for 45 s, annealing at 52 °C for 60 s and extension at 72 °C for 75 s, with final extension of 60 °C for 30 min. Positive and negative controls were included in each reaction to monitor the reliability of the reaction. The alleles were determined using the Liz-500 Size Standard (Applied Biosystems) in an ABI 3500XL Genetic Analyser (Applied Biosystems) and further analysed using GeneMarker 2.7.4 (Applied Biosystems).

Data analysis

mtDNA analysis. PCR products were sequenced for parts of CO*I*, ND5 and Cyt *b* genes for samples of *V. bengalensis* (n = 63), *V. flavescens* (n = 4) and *V. griseus* (n = 2; OP117155–OP117223 and OP141809–OP141954). We also included published sequences to increase the robustness of the analyses (Table S2). To estimate divergence time, we incorporated fragments of ND4 region of *V. bengalensis* (n = 4), *V. flavescens* (n = 2) and *V. griseus* (n = 2; OP141878–OP141885) and analysed these with NCBI sequences (Table S2). Sequences obtained from the forward and reverse direction of samples for each gene were edited manually by confirming electropherograms and assembled using SEQUENCHER® 4.9 (Gene Codes Corporation, Ann Arbor, MI, USA). A BLAST (Altschul et al., 1997) search was done with the generated sequences for ND5, Cyt *b* and CO*I* genes for species identification. *Heloderma suspectum* was used as an outgroup to reconstruct the phylogenetic relationships among different monitor lizard species. Sequences were aligned using the ClustalW program (Thompson et al., 1994) in BioEdit 7.1.3 (Hall, 1999) with default settings. The maximum parsimony analysis was performed in PAUP 4.0a169 (Swofford, 2003). The optimal parsimonious tree was constructed with an heuristic search with 1000 bootstrap replicates of stepwise addition, tree–bisection–reconnection branch swapping.

A Markov chain Monte Carlo (MCMC)-based Bayesian consensus tree was constructed using BEAST 1.7 (Drummond et al., 2012). Bayesian inference analyses were performed using the best-fit model HKY + G + I for CO*I* and ND5, while HKY + I for Cyt *b* genes, using MCMC chains executed for 10 million generations logging every 1000 generations with 10% per run being discarded as burn-in. The runs were evaluated in Tracer 1.6 and resulting phylogenetic trees were visualized in FigTree 1.4.0 (http://tree.bio.ed.ac.uk/software/figtree/). The mean distance between groups for all three genes was calculated using the TN92 + G model with the lowest BIC score value in MEGA X (Kumar et al., 2018).

Species delimitation analysis. Species delimitation uses heuristic methods to interpret groups of individuals either as different populations of a single species or as different species altogether. Uncertainty in morphologies resulting from a few distinctive traits can be addressed by a semi-automated process of delimitation obtained from genomic data and computer algorithms. The samples of *V. bengalensis* used in this study were inspected through species delimitation tests based on phylogenetic trees of CO*I*, ND5 and Cyt *b* genes. Three different analyses were performed: (i) multi-rate Poisson tree processes (mPTP); (ii) Bayesian Poisson tree processes (bPTP); and (iii) generalized mixed Yule-coalescent (GMYC).

The mPTP analysis was performed using a web server (https://mptp.h-its.org). This model uses a fast approach to estimate the maximum-likelihood (ML) delimitation from an input tree. The bPTP analysis was performed using a web server (https://species.h-its.org) keeping parameters for MCMC, thinning, burn-in and seed value as default. This model adds Bayesian support (BS) values to delimit species on the input tree. The multiple threshold GMYC model was performed using a web server (https://species.h-its.org). This model uses the likelihood method to delimit species and reconstructed input tree by fitting within and between species branching model.

Divergence estimation. A strict molecular clock method was implemented in BEAST 1.7 (Drummond et al., 2012) to infer lineage divergence times of the genus *Varanus*. Divergence times of phylogenetic clades were calibrated at two points with normal distribution: one prior point was at 28 Ma (SD = 2), supported by African monitor lizard fossils (Holmes et al., 2010), and the other prior point was at 20 Ma (SD = 1), which is supported by monitor lizard fossils from Australia (Molnar, 2004). We used the Yule speciation model and HKY substitution rate model for tree construction. The analysis included two independent runs, using MCMC lengths of 60 million generations and logging every 6000 generations. Tracer 1.6 was used to evaluate all the runs. As burn-in, the first 10% per run was discarded. We used TreeAnnotator (implemented in the BEAST 1.7 package) to acquire maximum credibility trees. The final phylogenetic tree was visualized in FigTree 1.4.0 (http://tree.bio.ed.ac.uk/software/figtree/).

Microsatellite analyses. For microsatellite analysis, a total of six polymorphic loci were selected and genotyped for *V. bengalensis* (n = 63), *V. flavescens* (n = 4) and *V. griseus* (n = 2). Alleles were identified and scored in GeneMarker 2.7.4 (Applied Biosystems). The genotyping error caused by null alleles was checked in MICROCHECKER 2.2.3 (Oosterhout et al., 2003). All of the loci were examined for Hardy–Weinberg equilibrium in GenAlEx 6.5 (Peakall and Smouse, 2006).

In order to examine genetic partitioning within *V. bengalensis*, a Bayesian clustering analysis was performed using STRUCTURE 2.3.4 (Pritchard et al., 2000). We used a correlated allele frequency model with admixture to analyse the data with a burn-in of 60 000 followed by 600 000 MCMC (Markov chain Monte Carlo) replications. Ten runs were carried out for each cluster set (K) from 1 to 10 and visualized in the web server ClumpaK (http://clumpak.tau.ac.il; Kopelman et al., 2015). The optimal number of K was estimated by examining ΔK with the web server-based program STRUCTURE HARVESTER (Earl and VonHoldt, 2012). Additionally, to identify the number of genetic clusters, a multivariate and model-free approach, the discriminant analysis of principal components (DAPC) was implemented using the ADEGENET package in R to strengthen the data (Jombart et al., 2010). We used CONVERT 1.31 software to obtain the required input file format (Glaubitz, 2004). The pairwise F_{ST} values among the different monitor lizard populations were calculated using GenAlEx 6.5 (Peakall and Smouse, 2006).

Results

Phylogenetic analysis

The MP consensus tree constructed for monitor lizard species with individual genes is represented in

Figures 2, 3 and 4. In addition, we also performed phylogenetic analyses implemented in Bayesian inference and ML, resulting in similar topologies to MP and shown in the Supporting information (Figs S1–S6).

Phylogenetic trees derived from CO*I*, ND5 and Cyt *b* genes indicated node support with bootstrap (BS) >52 and posterior probability value (PP) >0.51 The resulting tree of ND5 and Cyt *b* genes showed the presence of two lineages within *V. bengalensis*: Lineage-I comprising samples from the Himalayan foothills, and Lineage-II samples from the area south of the Himalayan foothills. Conversely, the resultant tree of the CO*I* gene showed the presence of three well-supported clades within *V. bengalensis*: Lineage-I and Lineage-II show congruence with the ND5 and Cyt *b* phylotrees, whereas Lineage-III consists of the sequences submitted from South India in NCBI. Owing to the unavailability of ND5 and Cyt *b* sequences from South India, we could not further assess their evolutionary relationships. Our phylogenetic analyses indicate the presence of two distinct clades within *V. bengalensis*. Lineage-I is spread across the Uttarakhand region and adjoining areas in Uttar Pradesh, Assam and Arunachal Pradesh. Lineage-II covers the species' distribution in the Indian states of Haryana, Uttar Pradesh, Bihar, Madhya Pradesh and Maharashtra (considered as the remainder of the mainland populations). Lineage-I is restricted to foothills of the Himalaya and presents a distinct signature. The placement of the *V. bengalensis* sequences from South India at a basal position in the CO*I* gene tree led us to speculate that there may be other distinct lineages of *V. bengalensis,* which warrants further extensive pan-Indian research.

The other species, *V. flavescens* and *V. griseus*, showed well-supported clades ranging from BS 53-99 in all three phylotrees. Furthermore, we calculated pairwise genetic differentiation based on three mitochondrial genes. The result suggested clear genetic differentiation between Lineage-I and Lineage-II of *V. bengalensis* with 2.5%, 5.6% and 3.5% in CO*I*, ND5 and Cyt *b* gene, respectively. Additionally, the genetic differentiation between Lineage-I and Lineage-II with Lineage-III of *V. bengalensis* through the CO*I* gene is 2.4% and 2.7%, respectively (Tables S3 and S4).

The resultant pairwise genetic differentiation validates two genetic lineages of *V. bengalensis*, one recorded from the Himalayan foothills and the second from south of these foothills, comprising the remaining mainland populations other than those in South India (Fig. 7).

Divergence dating

Our divergence results show congruence with the recent timeline provided by Brennan et al. (2021) for modern monitor lizard species. Resultant diversification through partial fragments of the ND4 gene also suggests a crown age in the early to mid-Oligocene. When we focus on the extant monitor species in the Indian subcontinent, the split between Desert monitor (*V. griseus*) of the subgenus *Psammosaurus* and the subgenus *Polydaedalus* (including the Nile monitor, *V. niloticus*), occurred in the late Oligocene–Miocene, around 24.7 Ma ($CI_{95\%}$: 21.57–27.82). The split between the subgenus *Empagusia* (including Yellow monitor, *V. flavescens*) and Bengal monitor (*V. bengalensis*) occurred in the mid-Miocene, around 12.45 Ma ($CI_{95\%}$: 9.97–15.11). Our analysis indicated that the split between *V. bengalensis* from the Himalayan foothills (Lineage-I) and remainder of the mainland (Lineage-II) took place in the mid-Pliocene around 3.06 Ma ($CI_{95\%}$: 1.98–4.33; Fig. 5).

Species delimitation

The mPTP, bPTP and GYMC analyses suggested four independent clades in our dataset, namely *V. bengalensis* Lineage-I (Himalayan foothills), *V. bengalensis* Lineage-II (remainder of mainland), *V. flavescens* and *V. griseus* (Figs 2, 3 and 4). All three analyses delimited the same taxonomic units as inferred from BEAST phylogenetic analysis. The *V. bengalensis* sequences KF766940 and MN148451 in Cyt *b* and CO*I* genes showed exceptions and delimited as different units. This subdivision is probably a consequence of the shorter length of these sequences. The results support previously recognized taxonomic divisions and provide new insights corroborated by our analyses.

Population genetic structure and genetic differentiation

The Bayesian clustering analysis using the admixture model identified the highest ΔK when K was set at 3 (Mean LnP(K) = 1914.1400; ΔK = 74.575293). Evanno et al. (2005) recommended a cautionary interpretation of K; hence, to identify the possible hidden structure and population subdivision, we further analysed the data accordingly. Thus, we adopted the value of adjacent value of $K = 4$ (Mean LnP(K) = −2007.0300; ΔK = 1.123932), where individuals from our dataset fell into four different clusters, namely *V. flavescens*, *V. griseus*, and *V. bengalensis* differentiated in two distinct groups (Figs S7 and S8). The samples of *V. bengalensis* from the Himalayan foothills showed genetically distinct clusters compared to the remainder of the mainland *V. bengalensis*. The multivariate DAPC corroborated the Bayesian clustering analysis that differentiated the populations into four genetic clusters (Fig. 6). A parallel pattern between genetic cluster assignment and phylogenetic clades was

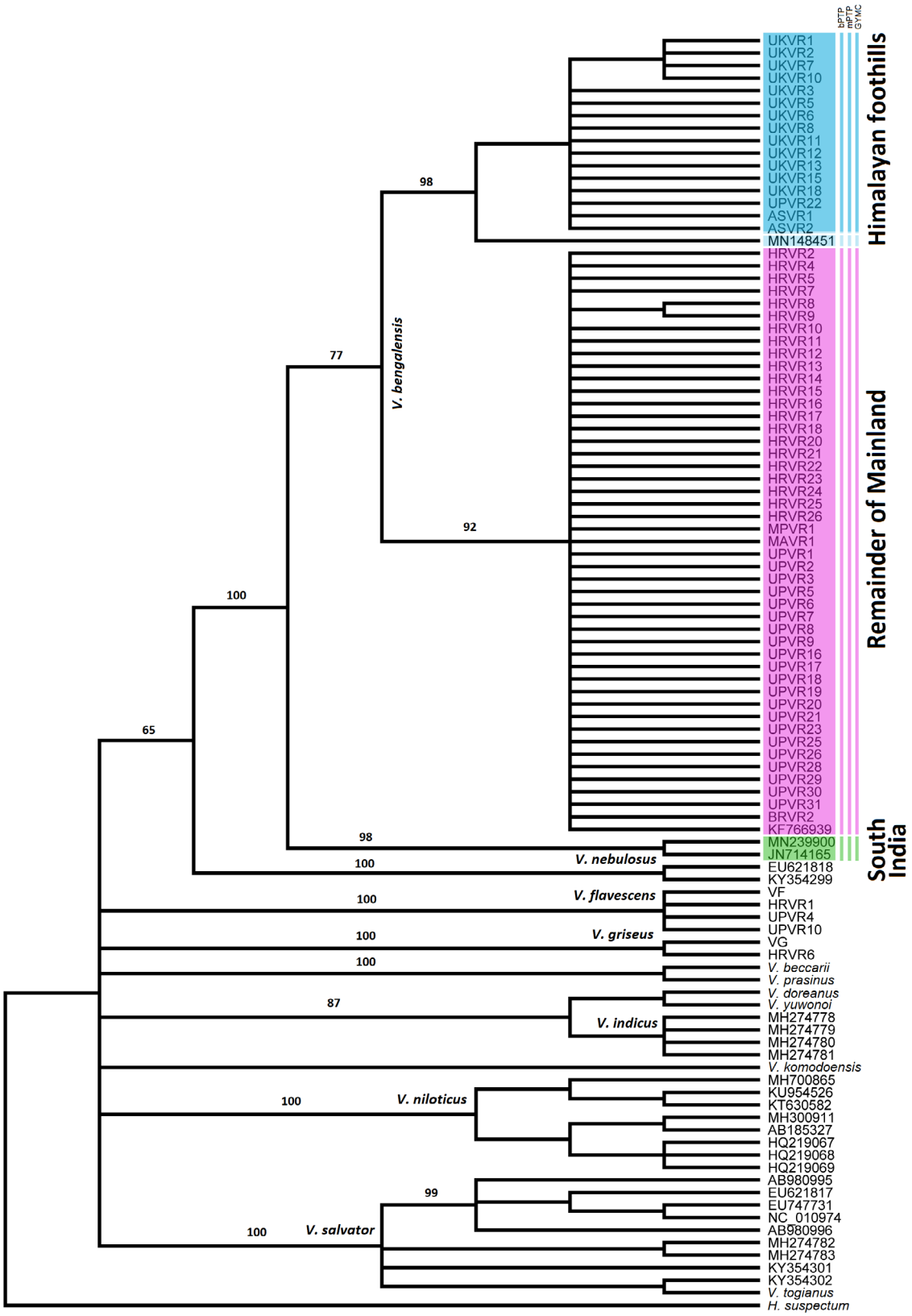

Fig. 2. Cladogram through Maximum parsimony (MP) of *V. bengalensis* and other monitor lizard species based on CO*I* gene. Numbers on clades indicate bootstrap (BS) for the node. The tree also represents the result of three different molecular species delimitation methods.

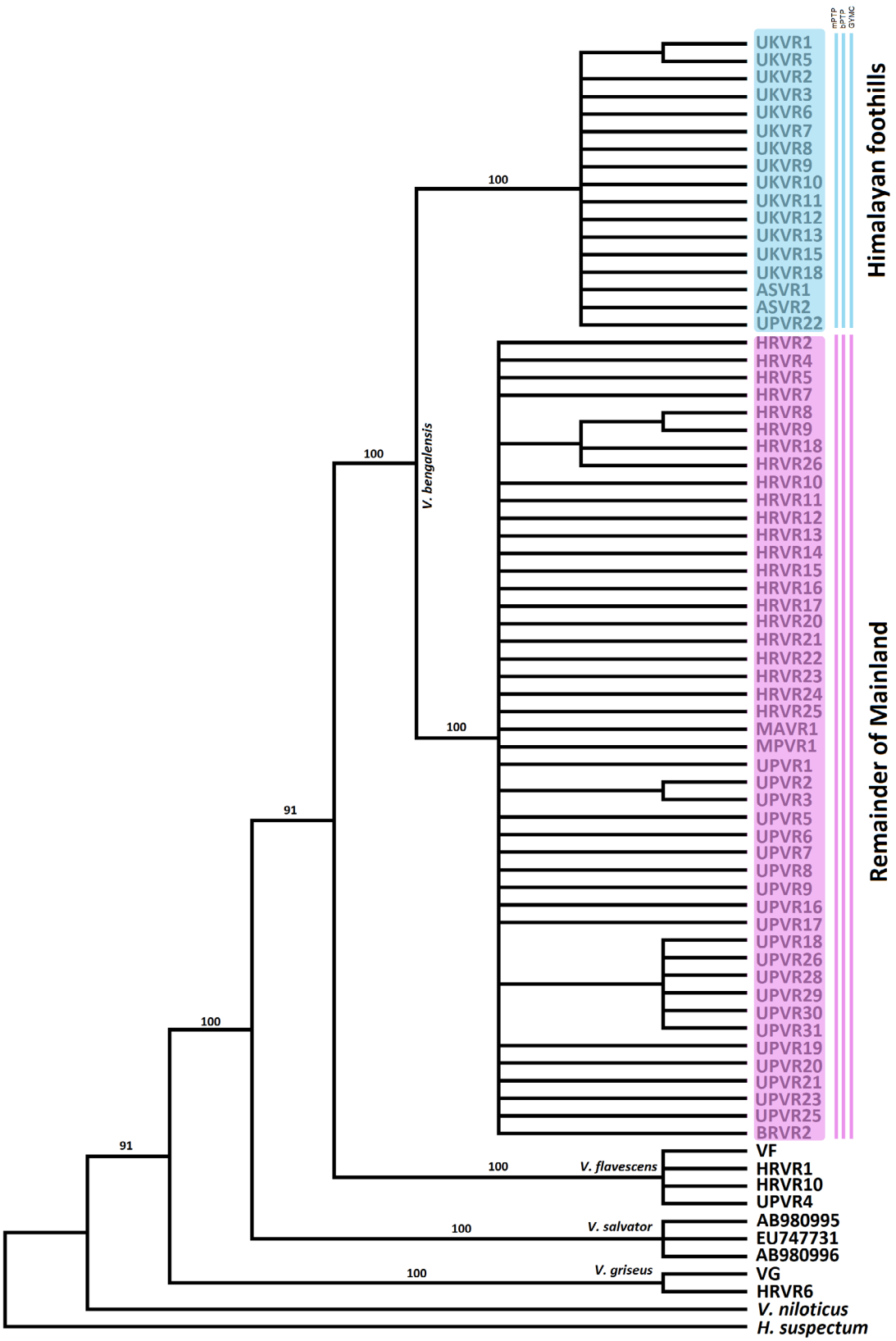

Fig. 3. Cladogram through Maximum parsimony (MP) of *V. bengalensis* and other monitor lizard species based on ND5 gene. Numbers on clades indicate bootstrap (BS) for the node. The tree also represents the result of three different molecular species delimitation methods.

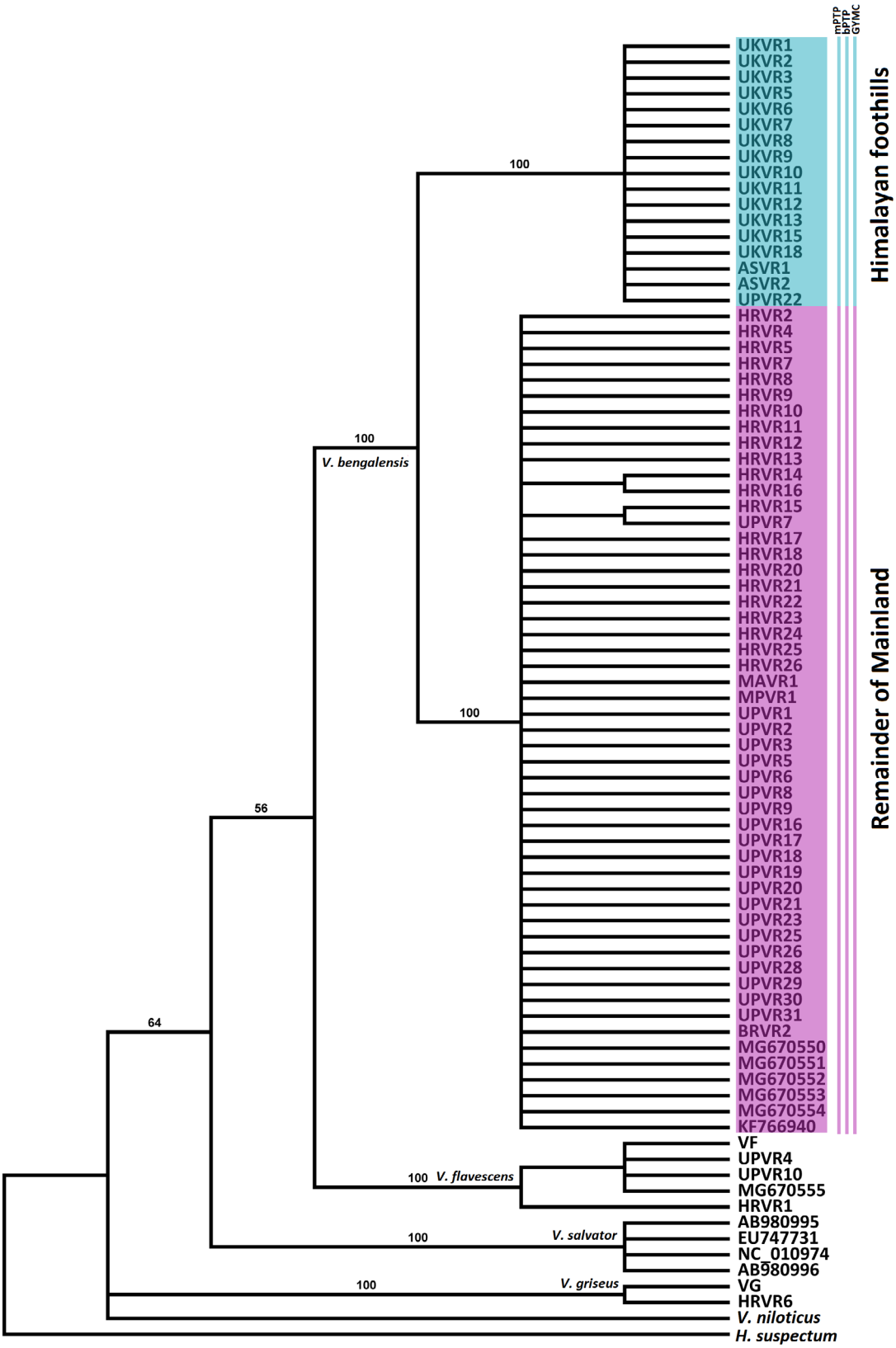

Fig. 4. Cladogram through Maximum parsimony (MP) of *V. bengalensis* and other monitor lizard species based on Cyt *b* gene. Numbers on clades indicate bootstrap (BS) for the node. The tree also represents the result of three different molecular species delimitation methods.

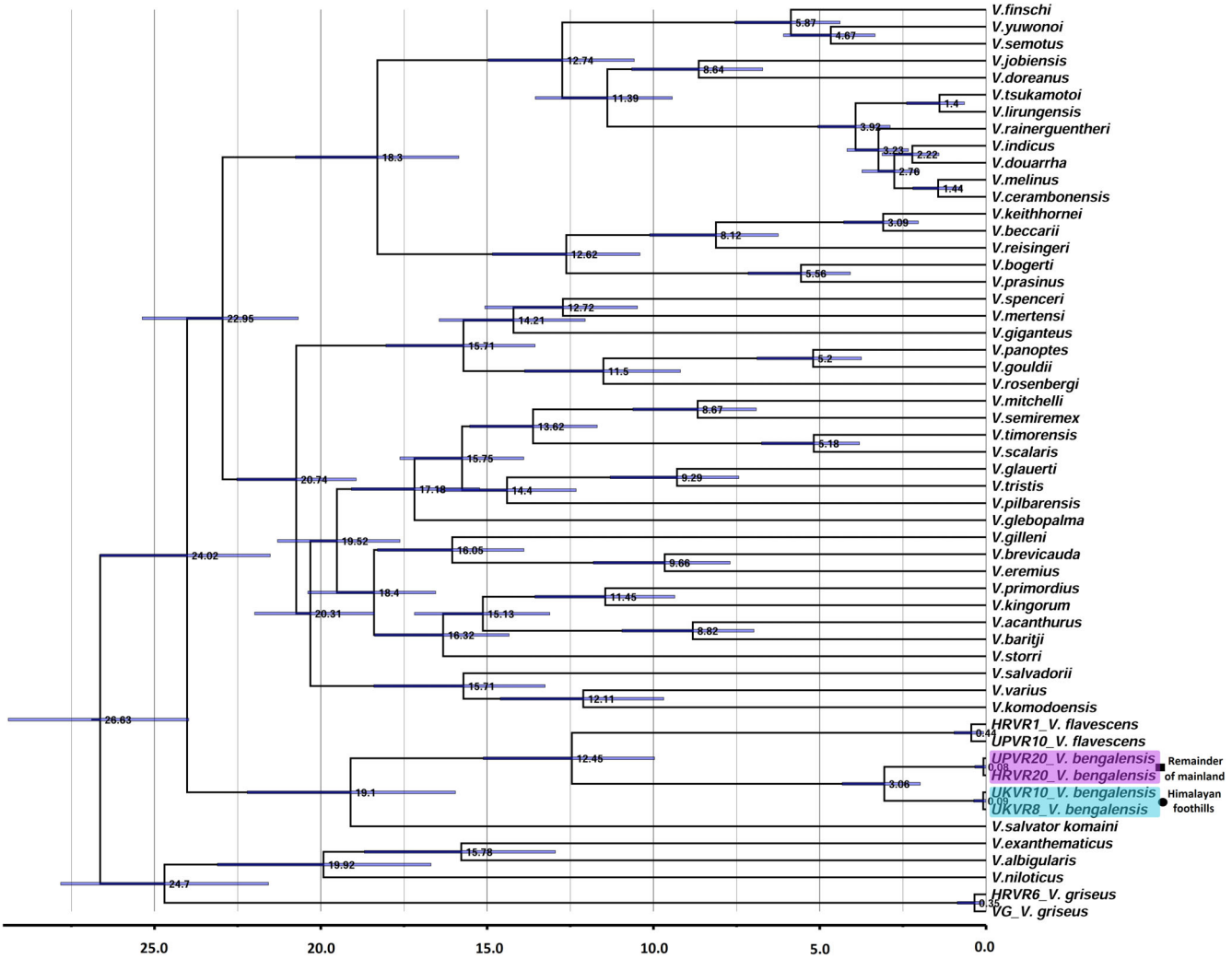

Fig. 5. Divergence time estimation based on maximum credibility tree using ND4 gene generated from BEAST analysis. X-axis denotes million year ago (mya). The square denotes *V. bengalensis* from remainder of mainland, while the circle denotes *V. bengalensis* from Himalayan foothills.

observed. We found evidence of genetic differentiation of 6.3% between Lineage-I Himalayan foothills and Lineage-II remainder of the mainland populations of *V. bengalensis*. We further observed well-supported distinctiveness between other monitor lizard species; *V. flavescens* is 25.3% and 16.4% distinct from Lineage-I and Lineage-II of *V. bengalensis,* respectively. Likewise, *V. griseus* is 27.2% and 26.4% genetically different from *V. bengalensis* Lineage-I and Lineage-II, respectively. Moreover, the genetic differentiation between *V. griseus* and *V. flavescens* is 48.4% (Fig. 7; Table S5).

Discussion

The Himalayan region encompasses noteworthy species diversity and endemism. Temporal records of biological processes reveal high-resolution information about the Himalayan history and environmental changes. This study aimed to understand the genetic structure and evolutionary history of *V. bengalensis* in India, focusing on the Himalayan foothills. It provides a novel molecular database of monitor lizard species from India. It compiles extensive analyses of mitochondrial and microsatellite loci to understand the geological events that may have influenced the demographic pattern and population genetic structure of *V. bengalensis*. The phylogeny found in this study is aligned with the recent phylogenetic assessment of monitor lizards suggested by Brennan et al. (2021), supporting the monophyly of the genus *Varanus,* which is differentiated into African, Indo-Asian and Indo-Australian clades, and placed *V. bengalensis*, *V. flavescens* in the Indo-Asian group, and *V. griseus* in the African group. The widely distributed

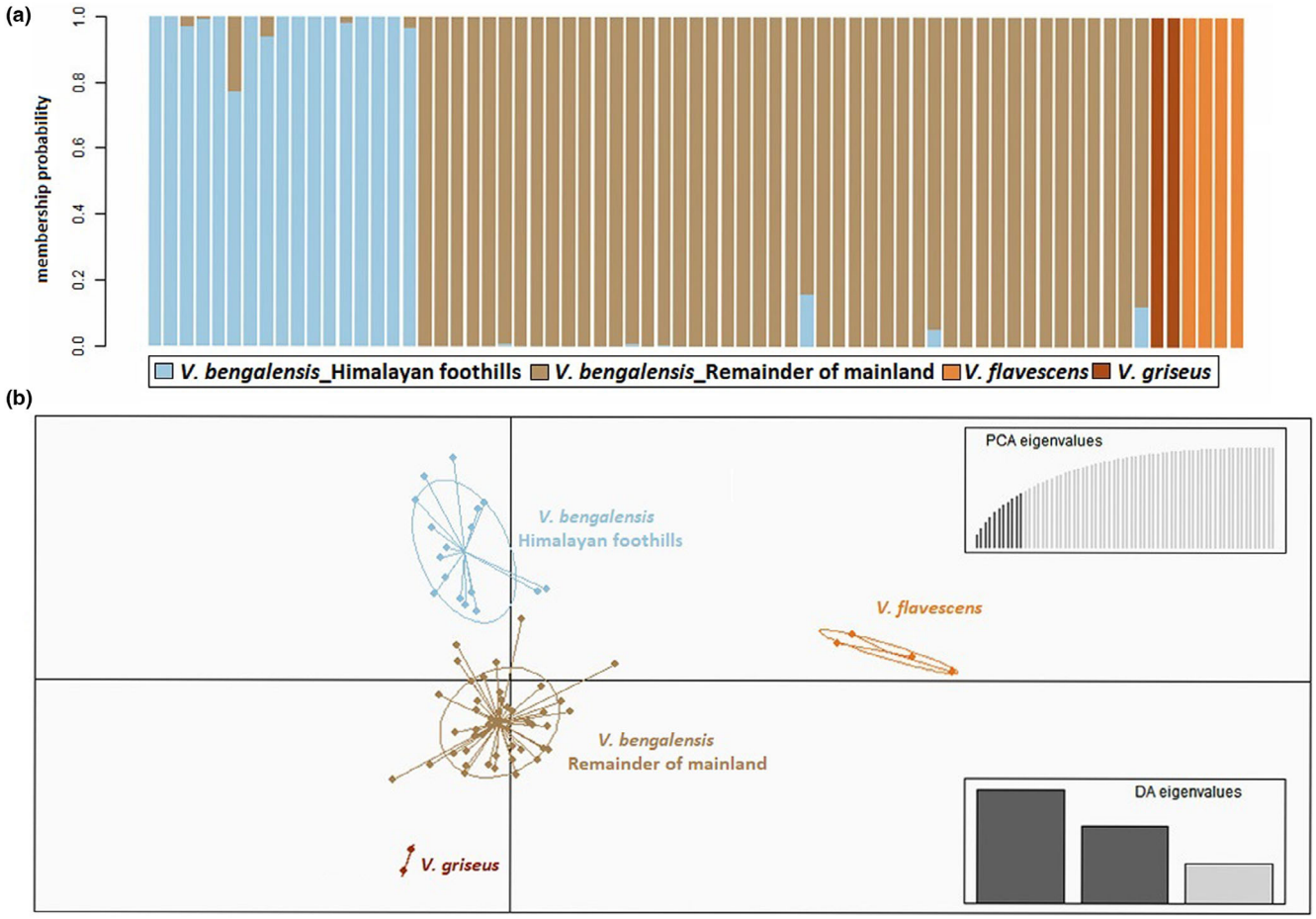

Fig. 6. The bar plot results show genetic clustering implemented in Discriminant Analysis of Principal Components (DAPC; a). Each column along the X-axis represents one *Varanus* individual. The Y-axis represents the assignment of the membership probability of each individual. Scatter plots of DAPC using a hierarchical islands model and shown by different colours and inertia ellipses (b). The DA and PCA eigenvalues of the analyses are displayed in the inset.

V. bengalensis was divided into *V. b. bengalensis*, *V. b. irrawadicus* and *V. b. nebulosus* as its subspecies (Ziegler and Böhme, 1997; Böhme, 2003; de Lisle, 2009). This study analysed the genetic structure of *V. bengalensis* and revealed two genetically distinct lineages, one from the Himalayan foothills and the other from south of the foothills (i.e. the remainder of the mainland). As the name suggests, the Himalayan foothills lineage of *V. bengalensis* inhabits the foothills of Indian Himalayan states of Uttarakhand, Uttar Pradesh, and Assam to Arunachal Pradesh. The two genetic clusters were also confirmed by DAPC and structure analyses. These distinctions findings within *V. bengalensis* were supported by species delimitation methods, corroborating the divergence between *V. bengalensis* from the Himalayan foothills and *V. bengalensis* from the remainder of the mainland as separate evolutionary significant units (ESUs). The level of genetic distinctiveness between the two lineages of *V. bengalensis* found in this study compared with the levels among existing recognized monitor lizard species supports the presence of the distinct Himalayan and remainder of mainland lineages. Interestingly, the genetic signature found in this study within *V. bengalensis* is not affected by major river systems such as the Ganga and Brahmanputra, as was also in small-bodied lizards such as *Sitana* and *Cyrtodactylus* (Agarwal et al., 2014; Deepak and Karanth, 2018). The Himalayan lineage of *V. bengalensis* shows genetic differentiation ranging from 2.5% to 5.6% and 6.3% in the mtDNA and microsatellite loci, respectively. We compared these new findings with previous morphological data from Auffenberg's monograph on *V. bengalensis* where 495 specimens were examined for scale counts and did not reveal any explicit pattern of geographical variation. Two populations of *V. bengalensis* were recognized on the basis of scalation, one west of Myanmar (i.e. *V. b. bengalensis*) and the other east of Myanmar (i.e. *V. b. nebulosus*), with an intermediate zone from lower West Bengal through

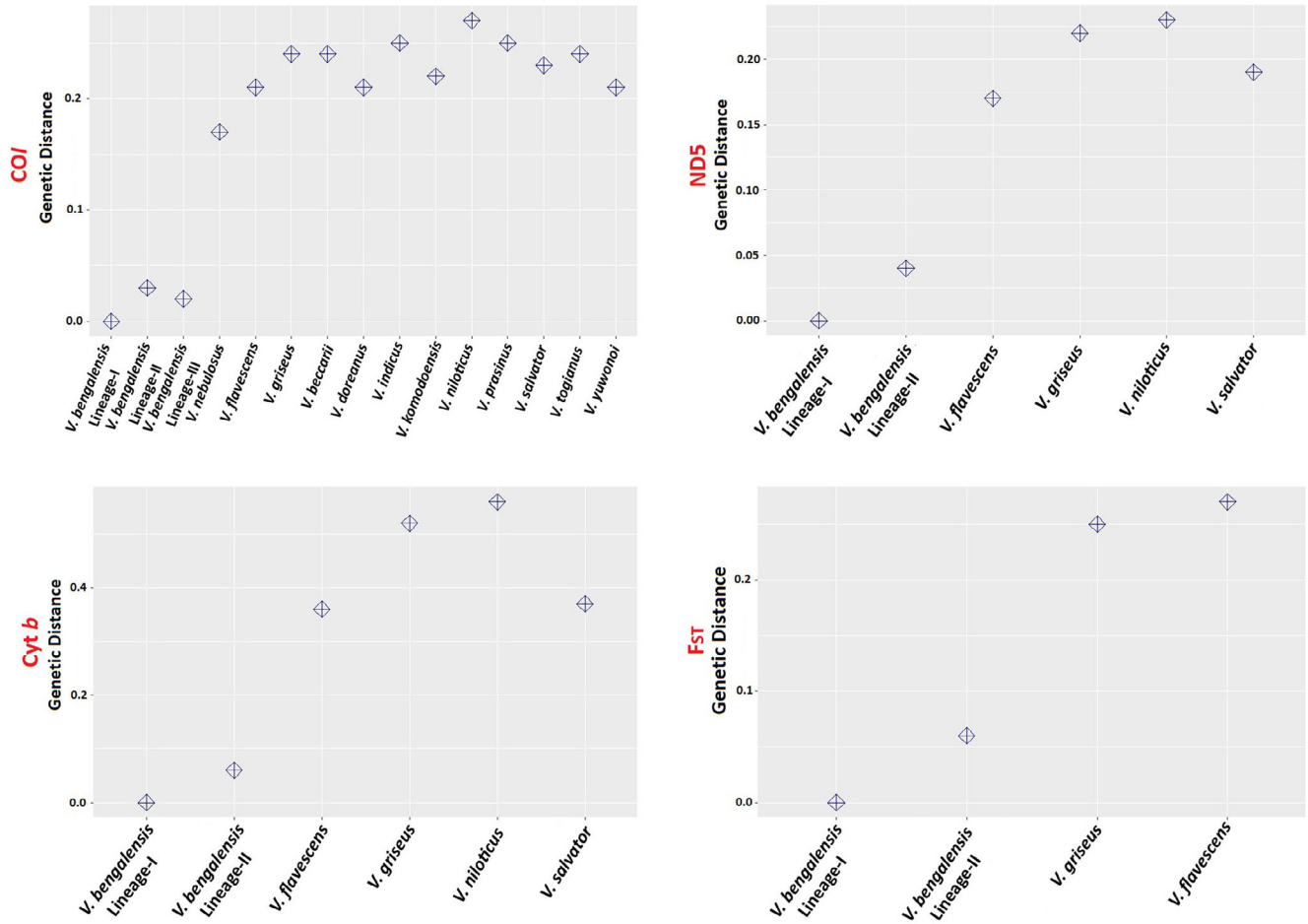

Fig. 7. Genetic differentiation among *V. bengalensis* and other monitor lizard species from mitochondrial genes and microsatellite loci.

Bangladesh to the eastern highland border area of Myanmar and Thailand (Auffenberg, 1994). Within these two major populations of *V. bengalensis* recognized by Auffenberg (1994), several subsets also were observed. However, the CO*I* gene indicated >15% differentiation between *V. b. nebulosus* and the three *V. b. bengalensis* lineages studied here, surpassing the subspecies level. Hence, previously reported morphology data were ambiguous and the present study focuses more on the genetics of *V. bengalensis*. In the future, with robust sampling along with morphological data, conclusive evidence could be provided for this intraspecific differentiation within *V. bengalensis* in the Indian subcontinent.

The intraspecific differentiation observed in this study was also seen in other sister monitor lizard species. Smissen et al. (2013) found three geographically separated clades derived from the ND4 gene and microsatellite data in their study of the biogeography of *V. varius*. Range-wide multilocus assessment of the *V. niloticus* group exhibited genetic differentiation above that typically found between sister monitor lizard species (Dowell et al., 2016). However, new monitor lizard species have also been recorded highlighting both local and regional endemism with 2.4–13.5% and 2.3–0.6% pairwise distances in the ND4 and 16S rRNA genes, respectively (Weijola et al., 2017). Welton et al. (2014) reported a distinct species within the *V. salvator* complex by with 1.00–3.5% divergence based on ND1 and ND2 genes. Among Indo-Australian varanids, Fitch et al. (2006) reported genetic differentiation ranging from 3.4% to 27.7% using the ND4 region. The divergence found here between *V. bengalensis* in the Himalayan foothills and remainder of the mainland suggests that some barriers or factors might have played a role in intraspecific differentiation. The diversification within *Cyrtodactylus* allowed exploration of the India–Asia collision and provided insights into Himalayan biogeography (Agarwal et al., 2014). Another example of intraspecific differentiation is in the wide-ranging king cobra, with four independently evolving genetic lineages yet a low

level of morphological divergence (Shankar et al., 2021). Several phylogenetic studies on birds (Johansson et al., 2007; Martens et al., 2011), insects (Schmidt et al., 2012), amphibians (Hofmann et al., 2017) and reptiles (Xu et al., 2021) provide information and understanding regarding divergence in the Himalayan biota.

India separated from Madagascar around 85–90 Ma and advanced northwards until about 20–30 Ma (Acton, 1999). The collision of India with the Neotethyan intraoceanic arc is considered as a 57 Ma event and the subsequent sudden drop in northward speed was a result of the joining of India and Asia around 55–50 Ma (Huang et al., 2000; Valdiya, 2002). The formation of the Himalaya as a result of the northward movement of the Indian plate in a phased manner started around 45 Ma and major uplift occurred 8–6 Ma at the eastern edge (mostly the Tibetan plateau; Favre et al., 2015; Spicer, 2017). The divergence split between the subgenus *Soterosaurus* including the *V. salvator* group and *V. flavescens* of the subgenus *Empagusia* was recorded in the early Miocene ~19.1 Ma ($CI_{95\%}$: 15.95–22.21). The split of *V. bengalensis* from *V. flavescens* was observed around the mid-Miocene ~12.45 Ma ($CI_{95\%}$: 9.97–15.11). A study by Brennan et al. (2021) using a nuclear exon dataset suggested the divergence of *V. bengalensis* and *V. flavescens* occurred around 9.5 Ma, which was also supported our study where the divergence age estimated by mtDNA falls within $CI_{95\%}$. The slight variation in the divergence date is likely to be the result of the different evolutionary rates observed in mtDNA and nuclear genes. The study shows concurrence with a Laurasian origin, and corresponds with Hugall and Lee (2004), Sweet and Pianka (2007), Amer and Kumazawa (2008) and Vidal et al. (2012), thus countering the Gondwanan hypotheses proposed by Schulte et al. (2003). The divergence within *V. bengalensis* correlates with the split between Himalayan foothills and remainder of mainland lineages in the mid-Pliocene ~3.06 Ma ($CI_{95\%}$: 1.98–4.33), suggesting that the newly identified lineage was formed during Shiwalik broadening. The Shiwalik Basin spans from the Sindh region in the west to Tripura, Cachar and Arunachal Pradesh regions in the east (Valdiya, 2002). The uplift of the Himalayan foothills caused Shiwalik succession at 18.3, 11, 5.3 and 0.22 Ma, respectively, as a result of accelerated erosion following the deposition of detritus (Valdiya, 2002). The Himalayan uplift increased already variable environmental conditions. The rise in elevation of the Himalaya resulted in the intensification of the South Asian monsoon, which may have facilitated a climatic gradient along the range and disconnected the landmasses of India, Sino-Japan and South-east Asia (Pandit, 2017). The uplifted Himalayan range with a strengthened monsoon may have provided diverse climatic niches, and new habitats, and dispersal barriers may have influenced evolutionary opportunities to accelerate speciation rate (Xu et al., 2021). Repeated climatic changes in mountainous regions and stochastic interannual weather fluctuations may have led to a high rate of speciation facilitated by repeated isolation, *in situ* diversification and remixing of gene pools during favourable conditions. Recent range extension of *V. bengalensis* at 3000 m above sea-level indicates species movement to higher elevation either under global climate change pressure or lack of robust field studies (Singh et al., 2020).

The eastern Himalayas are older than the western Himalayas and, hence, most Himalayan reptiles are largely Indo-Chinese and Malayan derivatives, such as *Japalura tricarinata*, *Japalura kumaonensis*, *Paraxenodermus* and *Tropidophorus assamensis* (Wang et al., 2019; Deepak et al., 2021; Lalremsanga et al., 2022). The Himalayan uplift has helped in the evolution and diversification of Asiatic ancestral stock into endemic high-altitude elements (Mani, 1974). An increase in the number of species by rapid speciation and isolation on highlands was manifested via evolutionary changes during Pliocene (Valdiya, 2002). Furthermore, Pleistocene glaciations and associated climatic factors may have played a role in the evolution of Pliocene endemic forms and given rise to numerous subspecies and species (Mani, 1974). The late Pliocene Shiwalik fauna included a diverse mammalian megafauna (Dennell et al., 2006). During the Plio-Pleistocene, warm and moist climatic conditions became the precursor to survival and proliferation of species.

Interestingly, fossils of the genus *Varanus* have been reported from India, such as the Pleistocene deposits of *V. bengalensis* from Billa Surga and Kurnool caves areas that are still inhabited by the species (Lydekker, 1886, 1888; de Fejervary, 1918, 1935; Prasad and Yadagiri, 1986). A rare large-bodied fossil known from only two vertebrae and a part of the humerus from Pliocene rocks of the Shiwalik Hills was recorded and named as *V. sivalensis* (Falconer, 1868). The two vertebrae align with the *V. salvator* fossil, whereas the humerus is morphologically different from *V. komodoensis* even though it is of similar size (Hocknull et al., 2009; Conrad et al., 2012). Hocknull et al. (2009) suggested the rarity or extinction of *V. sivalensis* is a result of the absence of records from younger deposits in the same region. Considering the fossil record of *V. sivalensis* from the Pliocene with respect to the divergence time of the Himalayan foothills lineage of *V. bengalensis*, we can only speculate an affinity between these two because no studies have compared *V. sivalensis* with *V. bengalensis* in a reliable investigation of the theoretical affinity. Hence, future

confirmation warrants further resolution in the Bengal monitor lizard at the population, clade and species levels because robust recognition is the foundation for efficient conservation.

Conclusions

The present study aimed to understand the geological factors and associated climatic changes affecting niche dynamics of *V. bengalensis*. Our results suggested that biogeographical changes during the mid-Pliocene epoch may have influenced lineage delimitation in the Himalayan foothills. The results reveal genetic structuring within *V. bengalensis,* identifying the Himalayan foothills and the remainder of mainland population as two distinct lineages. Hence, we recommend a comprehensive taxonomic investigation to address the status of the newly revealed lineage in the Himalayan foothills as a distinct ESU. This study highlights the need for taxonomic revision within *V. bengalensis* and speculates about the affinity between the Himalayan foothills lineage and the *V. sivalensis* fossil. Furthermore, an extensive study based on robust genetic markers as used in this study is required for *V. bengalensis* populations throughout its distribution range with comprehensive sampling to identify distinct lineages and their geographical limits.

Acknowledgements

We acknowledge the support provided by the Director and Dean, Wildlife Institute India. This study was carried out in the Wildlife Forensic and Conservation Genetic Cell, WII and thankful for the support from the members. We acknowledge the support from Subhashree Sahoo, Bhim Singh, Vishal K. Prasad and Himangshu Borah. We are grateful to MoEF&CC and State Forest Departments of Uttarakhand, Uttar Pradesh, Bihar, Haryana, Assam, Madhya Pradesh and Maharashtra for granting permission for biological sample collection and field support. We thank Niazul H. Khan and Dhruv Jain for map preparation.

Funding

This research work is part of the PhD of Kumudani Bala Gautam with self-funding in fieldwork. Other expenditures were met from the resources of Wildlife Forensic and Conservation Genetic Cell, Wildlife Institute of India through Grant-in-aid.

Conflict of interest

The authors declare that they have no known competing interests.

Data availability statement

All the data supporting this study's findings are available in the supplementary materials. Newly generated DNA sequences have been uploaded to GenBank with accession numbers OP117155-OP117223, OP1418 09-OP141954 and OP141878-OP141885.

References

Acton, G.D., 1999. Apparent polar wander of India since the Cretaceous with implications for regional tectonics and true polar wander. Mem. Geol. Soc. India 44, 129–175.

Agarwal, I. and Ramakrishnan, U., 2017. A phylogeny of open-habitat lizards (Squamata: Lacertidae: *Ophisops*) supports the antiquity of Indian grassy biomes. J. Biogeogr. 44, 2021–2032.

Agarwal, I., Bauer, A.M., Jackman, T.R. and Karanth, K.P., 2014. Insights into Himalayan biogeography from geckos: a molecular phylogeny of *Cyrtodactylus* (Squamata: Gekkonidae). Mol. Phylogenetics Evol. 80, 145–155.

Allendorf, F.W., Luikart, G. and Aitken, S.N., 2007. Conservation and genetics of populations. Mammalia 2007, 189–197.

Altschul, S.F., Madden, T.L., Schäffer, A.A., Zhang, J., Zhang, Z., Miller, W. and Lipman, D.J., 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. 25, 3389–3402.

Amer, S.A.M. and Kumazawa, Y., 2008. Timing of a mtDNA gene rearrangement and intercontinental dispersal of varanid lizards. Genes Genet. Syst. 83, 275–280.

Auffenberg, W., 1994. The Bengal Monitor. Univ Press of Florida, Gainesville.

Auliya, M. and Koch, A., 2020. Visual Identification Guide to the Monitor Lizard Species of the World (Genus *Varanus*). Bundesamt für Naturschutz, Bonn, Germany.

Avise, J.C., 2000. Phylogeography: The History and Formation of Species. Harvard University Press, London, England.

Bhattacharya, S. and Koch, A., 2018. Effects of traditional beliefs leading to conservation of water monitor lizards (*Varanus Salvator*) and threatened marshlands in West Bengal, India. Herpetol. Conserv. Biol. 13, 408–414.

Böhme, W., 2003. Checklist of the living monitor lizards of the world (family Varanidae). Zool. Verh. 341, 4–43.

Brennan, I.G., Lemmon, A.R., Lemmon, E.M., Portik, D.M., Weijola, V., Welton, L., Donnellan, S.C. and Keogh, J.S., 2021. Phylogenomics of monitor lizards and the role of competition in dictating body size disparity. Syst. Biol. 70, 120–132.

Brown, R.M., Siler, C.D., Grismer, L.L., Das, I. and McGuire, J.A., 2012. Phylogeny and cryptic diversification in southeast Asian flying geckos. Mol. Phylogenet. Evol. 65, 351–361.

Ciofi, C., Tzika, A.C., Natali, C., Watts, P.C., Sulandari, S., Zein, M.S. and Milinkovitch, M.C., 2011. Development of a multiplex PCR assay for fine-scale population genetic analysis of the Komodo monitor *Varanus komodoensis* based on 18 polymorphic microsatellite loci. Mol. Ecol. Resour. 11, 550–556.

Conrad, J.L., Balcarcel, A.M. and Mehling, C.M., 2012. Earliest example of a giant monitor lizard (*Varanus*, Varanidae, Squamata). PloS One 7, e41767.

Das, I., 1989. Indian monitor lizards: A review of human utilisation patterns. Hamadryad 14, 16–19.

Deepak, V. and Karanth, P., 2018. Aridification driven diversification of fan-throated lizards from the Indian subcontinent. Mol. Phylogenetics Evol. 120, 53–62.
Deepak, V., Lalronunga, S., Lalhmingliani, E., Das, A., Narayanan, S., Das, I. and Gower, D.J., 2021. Phylogenetic relationships of xenodermid snakes (Squamata: Serpentes: Xenodermidae), with the description of a new genus. Vertebr. Zool. 71, 747–762.
Dennell, R., Coard, R. and Turner, A., 2006. The biostratigraphy and magnetic polarity zonation of the Pabbi Hills, northern Pakistan: an upper Siwalik (Pinjor stage) upper Pliocene–lower Pleistocene fluvial sequence. Palaeogeogr. Palaeoclimatol. Palaeoecol. 234, 168–185.
Dowell, S.A., Portik, D.M., de Buffrénil, V., Ineich, I., Greenbaum, E., Kolokotronis, S.O. and Hekkala, E.R., 2016. Molecular data from contemporary and historical collections reveal a complex story of cryptic diversification in the *Varanus* (Polydaedalus) *niloticus* species group. Mol. Phylogenetics Evol. 94, 591–604.
Drummond, A.J., Suchard, M.A., Xie, D. and Rambaut, A., 2012. Bayesian phylogenetics with BEAUti and the BEAST 1.7. Mol. Biol. Evol. 29, 1969–1973.
Earl, D.A. and VonHoldt, B.M., 2012. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conserv. Genet. Resour. 4, 359–361.
Esri, 2018. ArcGIS Desktop: Release 10.6.1. Environmental Systems Research Institute, Redlands, CA.
Evanno, G., Regnaut, S. and Goudet, J., 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol. Ecol. 14, 2611–2620.
Falconer, H.P., 1868. Paleontological Memoirs and Notes of the Late Hugh Falconer. Robert Hardwicke Publishers, Picadilly, p. 465.
Farr, T.G., Rosen, P.A., Caro, E., Crippen, R., Duren, R., Hensley, S., Kobrick, M., Paller, M., Rodriguez, E., Roth, L., Seal, D., Shaffer, S., Shimada, J., Umland, J., Werner, M., Oskin, M., Burbank, D. and Alsdorf, D., 2007. The shuttle radar topography mission. Rev. Geophys. 45, RG2004. https://doi.org/10.1029/2005RG000183.
Favre, A., Päckert, M., Pauls, S.U., Jähnig, S.C., Uhl, D., Michalak, I. and Muellner-Riehl, A.N., 2015. The role of the uplift of the Qinghai-Tibetan plateau for the evolution of Tibetan biotas. Biol. Rev. 90, 236–253.
de Fejervary, G.J., 1918. Contributions to a monography on fossil Varanidae and on Megalanidae. Ann. Mus. Nat. Hungarici Pars Zool. 16, 341–467.
de Fejervary, G.J., 1935. Further contributions to a monograph of Megalanidae and fossil Varanidae-with notes on recent varanians. Ann. Mus. Nat. Hungarici Pars Zool. 29, 1–130.
Fitch, A.J., Goodman, A.E. and Donnellan, S.C., 2005. Isolation and characterisation of microsatellite markers for the Australian monitor lizard, *Varanus acanthurus* (Squamata: Varanidae) and their utility in other selected varanid species. Mol. Ecol. Notes 5, 521–523.
Fitch, A.J., Goodman, A.E. and Donnellan, S.C., 2006. A molecular phylogeny of the Australian monitor lizards (Squamata: Varanidae) inferred from mitochondrial DNA sequences. Aust. J. Zool. 54, 253–269.
Fu, M., Yu, D., Peng, J., Wang, Y., Gao, S., Wang, L., Liu, X., Hu, S. and Gao, L., 2011. Isolation and characterization of novel microsatellite markers in water monitor (*Varanus salvator*). Conserv. Genet. Resour. 3, 777–779.
Gasteiger, E., Gattiker, A., Hoogland, C., Ivanyi, I., Appel, R.D. and Bairoch, A., 2003. ExPASy: The proteomics server for in-depth protein knowledge and analysis. Nucleic Acids Res. 31, 3784–3788.
Ge, C., Cui, Y.N., Jing, P.Y. and Hong, X.Y., 2014. An alternative suite of universal primers for genotyping in multiplex PCR. PLoS One 9, 92826.
Gibbons, J.W., Scott, D.E., Ryan, T.J., Buhlmann, K.A., Tuberville, T.D., Metts, B.S., Greene, J.L., Mills, T., Leiden, Y., Poppy, S. and Winne, C.T., 2000. The global decline of reptiles, Déjà vu amphibians: reptile species are declining on a global scale. Six significant threats to reptile populations are habitat loss and degradation, introduced invasive species, environmental pollution, disease, unsustainable use, and global climate change. Bioscience 50, 653–666.
Glaubitz, J.C., 2004. Convert: a user-friendly program to reformat diploid genotypic data for commonly used population genetic software packages. Mol. Ecol. Notes 4, 309–310.
Hall, T.A., 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for windows 95/98/NT. Nucleic Acids Sym. Ser. 41, 95–98.
Hocknull, S.A., Piper, P.J., van den Bergh, G.D., Due, R.A., Morwood, M.J. and Kurniawan, I., 2009. Dragon's paradise lost: palaeobiogeography, evolution and extinction of the largest-ever terrestrial lizards (Varanidae). PloS One 4, e7241.
Hofmann, S., Stöck, M., Zheng, Y., Ficetola, F.G., Li, J.T., Scheidt, U. and Schmidt, J., 2017. Molecular phylogenies indicate a paleo-Tibetan origin of Himalayan lazy toads (*Scutiger*). Sci. Rep. 7, 1–12.
Holmes, R.B., Murray, A.M., Attia, Y.S., Simons, E.L. and Chatrath, P., 2010. Oldest known *Varanus* (Squamata: Varanidae) from the upper Eocene and lower Oligocene of Egypt: support for an African origin of the genus. Palaeontology 53, 1099–1110.
Huang, C.Y., Yuan, P.B., Lin, C.W., Wang, T.K. and Chang, C.P., 2000. Geodynamic processes of Taiwan arc–continent collision and comparison with analogs in Timor, Papua New Guinea, Urals and Corsica. Tectonophysics 325, 1–21.
Hugall, A.F. and Lee, M.S.Y., 2004. Molecular claims of Gondwanan age for Australian agamid lizards are untenable. Mol. Biol. Evol. 21, 2102–2110.
Johansson, U.S., Alström, P., Olsson, U., Ericson, P.G., Sundberg, P. and Price, T.D., 2007. Build-up of the Himalayan avifauna through immigration: a biogeographical analysis of the *Phylloscopus* and *Seicercus* warblers. Evolution 61, 324–333.
Jombart, T., Devillard, S. and Balloux, F., 2010. Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. BMC Genet. 11, 1–15.
Klaus, S., Morley, R.J., Plath, M., Zhang, Y.P. and Li, J.T., 2016. Biotic interchange between the Indian subcontinent and mainland Asia through time. Nat. Commun. 7, 12132.
Koch, A., Ziegler, T., Boehme, W., Arida, E. and Auliya, M., 2013. Pressing problems: Distribution, threats, and conservation status of the monitor lizards (Varanidae: *Varanus* spp.) of Southeast Asia and the indo-Australian archipelago. Herpetol. Conserv. Biol. 8, 1–62.
Kopelman, N.M., Mayzel, J., Jakobsson, M., Rosenberg, N.A. and Mayrose, I., 2015. Clumpak: a program for identifying clustering modes and packaging population structure inferences across K. Mol. Ecol. Resour. 15, 1179–1191.
Kumar, S., Stecher, G., Li, M., Knyaz, C. and Tamura, K., 2018. MEGA X: molecular evolutionary genetics analysis across computing platforms. Mol. Biol. Evol. 35, 1547–1549.
Kumazawa, Y. and Endo, H., 2004. Mitochondrial genome of the Komodo dragon: efficient sequencing method with reptile-oriented primers and novel gene rearrangements. DNA Res. 11, 115–125.
Lajmi, A. and Karanth, P.K., 2020. Eocene–Oligocene cooling and the diversification of *Hemidactylus* geckos in peninsular India. Mol. Phylogenetics Evol. 142, 106637.
Lalremsanga, H.T., Decemson, H., Vabeiryureilai, M., Malsawmdawngliana, F., Lalhlimpuia, V., Muansanga, L. and Biakzuala, L., 2022. Phylogenetic position of *Tropidophorus assamensis* Annandale, 1912 with updated morphological data and distributional records. J. Herpetol. 32, 1–4.
Lehner, B. and Grill, G., 2013. Global river hydrography and network routing: Baseline data and new approaches to study the world's large river systems. Hydrol. Process. 27, 2171–2186.
Lehner, B., Verdin, K. and Jarvis, A., 2008. New global hydrography derived from spaceborne elevation data. Eos Transactions AGU 89, 93–94.

de Lisle, H.F., 2009. Catalog of the genus *Varanus* (Reptilia: Squamata: Varanidae) with new designations of a neotype and a lectotype. ZooNova 1, 8–32.
Lydekker, R., 1886. Fauna of the Karnul caves. Palaeontologica Indica 10, 1–58.
Lydekker, R., 1888. Catalogue of the fossil Reptilia and Amphibia in the British Museum (Natural History), Cromwell Road, S.W. Pt.1. The Orders Ornithosauria, Crocodilia, Dinosauria, Squamata, Rhynchocephalia, and Preterosauria. The Trustees, London, p. 245.
Mani, M.S., 1974. Ecology and Biogeography in India. Dr. W. Junk. Publishers, The Hague.
Martens, J., Tietze, D.T. and Päckert, M., 2011. Phylogeny, biodiversity, and species limits of passerine birds in the Sino-Himalayan region—a critical review. Ornithol. Monogr. 70, 64–94.
Maryan, B., Oliver, P.M., Fitch, A.J. and O'Connell, M.O.R.G.A.N., 2014. Molecular and morphological assessment of *Varanus pilbarensis* (Squamata: Varanidae), with a description of a new species from the southern Pilbara, Western Australia. Zootaxa 3768, 139–158.
Merrell, D.J., 1981. Ecological Genetics. University of Minnesota Press, Minneapolis, MN.
Molnar, R.E., 2004. Dragons in the dust: the paleobiology of the giant monitor lizard Megalania. Indiana University Press, Bloomington, Indiana.
Oosterhout, C.V., Hutchinson, W.F., Wills, D.P.M. and Shipley, P.F., 2003. Micro-checker version 2.2.3. Department of Biological Sciences and Department of Computer Science, University of Hull, Hull, UK.
Pandit, M.K., 2017. Life in the Himalaya: an ecosystem at risk. Harvard University Press, London, England.
Peakall, R.O.D. and Smouse, P.E., 2006. GENALEX 6: genetic analysis in excel. Population genetic software for teaching and research. Mol. Ecol. Notes 6, 288–295.
Pianka, E. and King, D., 2004. Varanoid lizards of the world. Indiana University Press, Bloomington, Indiana.
Portik, D.M. and Papenfuss, T.J., 2012. Monitors cross the Red Sea: the biogeographic history of *Varanus yemenensis*. Mol. Phylogenet. Evol. 62, 561–565.
Prasad, K.N. and Yadagiri, P., 1986. Pleistocene cave fauna, Karnool District, Andhra Pradesh. Rec. Geol. Surv. India 115, 71–77.
Pritchard, J.K., Stephens, M. and Donnelly, P., 2000. Inference of population structure using multilocus genotype data. Genetics 155, 945–959.
Rissler, L.J. and Apodaca, J.J., 2007. Adding more ecology into species delimitation: ecological niche models and phylogeography help define cryptic species in the black salamander (*Aneides flavipunctatus*). Syst. Biol. 56, 924–942.
Schmidt, J., Opgenoorth, L., Hoell, S. and Bastrop, R., 2012. Into the Himalayan exile: the phylogeography of the ground beetle Ethira clade supports the Tibetan origin of forest-dwelling Himalayan species groups. PLoS One, 7, e45482.
Schulte, J.A., Melville, J. and Larson, A., 2003. Molecular phylogenetic evidence for ancient divergence of lizard taxa on either side of Wallace's line. Proc. Royal Soc. B 270, 597–603.
Shankar, P.G., Swamy, P., Williams, R.C., Ganesh, S.R., Moss, M., Höglund, J., Das, I., Sahoo, G., Vijayakumar, S.P., Shanker, K. and Wüster, W., 2021. King or royal family? Testing for species boundaries in the King cobra, *Ophiophagus hannah* (cantor, 1836), using morphology and multilocus DNA analyses. Mol. Phylogenetics Evol. 165, 107300.
Sharma, C.P., Kumar, A., Sharma, V., Singh, B., Kumar, G.C. and Gupta, S.K., 2019. Online selling of wildlife part with spurious name: a serious challenge for wildlife crime enforcement. Int. J. Leg. Med. 133, 65–69.
Singh, A., Rana, B.S., Thakur, M., Tripathy, B., Sharma, L.K. and Chandra, K., 2020. Range extension of the Bengal monitor (*Varanus bengalensis*) for the trans-Himalayan region with an altitude record for monitor lizards. Zootaxa. 2, 4732.
Smissen, P.J., Melville, J., Sumner, J. and Jessop, T.S., 2013. Mountain barriers and river conduits: phylogeographical structure in a large, mobile lizard (Varanidae: *Varanus varius*) from eastern Australia. J. Biogeogr. 40, 1729–1740.
Smith, W., Scott, I.A. and Keogh, J.S., 2007. Molecular phylogeography of Rosenberg's goanna (Reptilia: Varanidae: *Varanus rosenbergi*) and its conservation status in New South Wales. System. Biodiver. 5, 361–369.
Spicer, R.A., 2017. Tibet, the Himalaya, Asian monsoons and biodiversity–in what ways are they related? Plant Divers. 39, 233–244.
Sweet, S.S. and Pianka, E.R., 2007. Monitors, mammals and Wallace's line. Mertensiella 16, 79–99.
Swofford, D.L., 2003. PAUP*: phylogenetic analysis using parsimony (*and other methods), version 4.0b10. Sinauer Associates, Sunderland, MA.
Thompson, J.D., Higgins, D.G. and Gibson, T.J., 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. 22, 4673–4680.
TRAFFIC MONITOR LIZARD FACTSHEET, 2021 https://join.wwfindia.org/illegal-wildlife-trafficking/MonitorLizardFactsheet2021.pdf
Valdiya, K.S., 2002. Emergence and evolution of Himalaya: reconstructing history in the light of recent studies. Prog. Phys. Geogr. 26, 360–399.
Verma, S.K. and Singh, L., 2003. Novel universal primers establish identity of an enormous number of animal species for forensic application. Mol. Ecol. Notes 3, 28–31.
Vidal, N., Marin, J., Sassi, J., Battistuzzi, F.U., Donnellan, S., Fitch, A.J., Fry, B.G., Vonk, F.J., Rodriguez de la Vega, R.C., Couloux, A. and Hedges, S.B., 2012. Molecular evidence for an Asian origin of monitor lizards followed by tertiary dispersals to Africa and Australasia. Biol. Lett. 8, 853–855.
Wang, K., Che, J., Lin, S., Deepak, V., Aniruddha, D.R., Jiang, K., Jin, J., Chen, H. and Siler, C.D., 2019. Multilocus phylogeny and revised classification for mountain dragons of the genus *Japalura sl.* (Reptilia: Agamidae: Draconinae) from Asia. Zool. J. Linn. Soc. 185, 246–267.
Weijola, V., Donnellan, S.C. and Lindqvist, C., 2016. A new blue-tailed monitor lizard (Reptilia, Squamata, *Varanus*) of the *Varanus indicus* group from Mussau Island, Papua New Guinea. ZooKeys 568, 129.
Weijola, V., Kraus, F., Vahtera, V., Lindqvist, C. and Donnellan, S.C., 2017. Reinstatement of *Varanus douarrha* Lesson, 1830 as a valid species with comments on the zoogeography of monitor lizards (Squamata: Varanidae) in the Bismarck archipelago, Papua New Guinea. Aust. J. Zool. 64, 434–451.
Weijola, V., Vahtera, V., Lindqvist, C. and Kraus, F., 2019. A molecular phylogeny for the Pacific monitor lizards (*Varanus* subgenus *Euprepiosaurus*) reveals a recent and rapid radiation with high levels of cryptic diversity. Zool. J. Linn. Soc. 186, 1053–1066.
Weijola, V., Vahtera, V., Koch, A., Schmitz, A. and Kraus, F., 2020. Taxonomy of Micronesian monitors (Reptilia: Squamata: *Varanus*): Endemic status of new species argues for caution in pursuing eradication plans. Royal Soc. Open Sci. 7, 200092.
Welton, L.J., Siler, C.D., Bennett, D., Diesmos, A., Duya, M.R., Dugay, R., Rico, E.L.B., Weerd, M.V. and Brown, R.M., 2010a. A spectacular new Philippine monitor lizard reveals a hidden biogeographic boundary and a novel flagship species for conservation. Biol. Lett. 6, 654–658.
Welton, L.J., Siler, C.D., Linkem, C.W., Diesmos, A.C. and Brown, R.M., 2010b. Philippine bent-toed geckos of the *Cyrtodactylus agusanensis* complex: multilocus phylogeny, morphological diversity, and descriptions of three new species. Herpetological Monographs. 24, 55–85.
Welton, L.J., Travers, S.L., Siler, C.D. and Brown, R.M., 2014. Integrative taxonomy and phylogeny-based species delimitation of Philippine water monitor lizards (*Varanus salvator* complex)

with descriptions of two new cryptic species. Zootaxa. 3881, 201–227.
World Bank, 2022. River Data [Data file]. Retrieved from https://data.worldbank.org/indicator/ER.H2O.FWAG.ZS.
Xu, W., Dong, W.J., Fu, T.T., Gao, W., Lu, C.Q., Yan, F., Wu, Y.H., Jiang, K., Jin, J.Q., Chen, H.M. and Zhang, Y.P., 2021. Herpetological phylogeographic analyses support a Miocene focal point of Himalayan uplift and biological diversification. Natl. Sci. Rev. 8, 263.
Ziegler, T. and Böhme, W., 1997. On the synonymy and taxonomy of the Bengal monitor lizard, *Varanus bengalensis* (Daudin, 1802) complex (Sauria: Varanidae). Amphib-Reptil. 18, 207–211.

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Sample origin and accession number used in this study.

Table S2. Sequences information used in this study from National Center for Biotechnology Information (NCBI).

Table S3. Genetic differentiation among *V. bengalensis* and other monitor lizard species from CO*I* gene.

Table S4. Genetic differentiation among *V. bengalensis* and other monitor lizard species from ND5/Cyt *b* gene.

Table S5. Pairwise F_{ST} value based on microsatellite loci.

Table S6. Details of microsatellite loci. * shows monomorphic locus.

Fig. S1. Bayesian inference (BI) of phylogenetic tree of *V. bengalensis* and other monitor lizard species based on the CO*I* gene. Numbers on clades indicate posterior probability (PP) for the node. The tree also represents the result of three different molecular species delimitation methods.

Fig. S2. Bayesian inference (BI) of phylogenetic tree of *V. bengalensis* and other monitor lizard species based on the ND5 gene. Numbers on clades indicate posterior probability (PP) for the node. Tree also represents the result of three different molecular species delimitation methods.

Fig. S3. Bayesian inference (BI) of phylogenetic tree of *V. bengalensis* and other monitor lizard species based on the Cyt *b* gene. Numbers on clades indicate posterior probability (PP) for the node. Tree also represents the result of three different molecular species delimitation methods.

Fig. S4. Maximum-likelihood (ML) of phylogenetic tree of *V. bengalensis* and other monitor lizard species based on the CO*I* gene. Numbers on clades indicate posterior probability (PP) for the node.

Fig. S5. Maximum-likelihood (ML) of phylogenetic tree of *V. bengalensis* and other monitor lizard species based on ND5 gene. Numbers on clades indicate posterior probability (PP) for the node.

Fig. S6. Maximum-likelihood (ML) of phylogenetic tree of *V. bengalensis* and other monitor lizard species based on the Cyt *b* gene. Numbers on clades indicate posterior probability (PP) for the node.

Fig. S7. Results of genetic clusters inferred from Structure 2.3.4. (a) Delta *K* values with respect to *K*. (b) Mean of estimates of ln probability of data with respect to *K*.

Fig. S8. Barplot indicating the genetic structure at $K = 1$ to $K = 10$, showing the population assignments for each individuals of *V. bengalensis* (Himalayan foothills), *V. bengalensis* (remainder of mainland), *V. flavescens* and *V. griseus*. The Y-axis depicts the proportion derived from each cluster.

Data S1. The aligned dataset of CO*I*, Cyt *b*, ND4 and ND5 genes used in this study. Microsatellite locus score sheet used in this study.

उत्तराखण्ड शासन

17th Uttarakhand State Science and Technology Congress
Rural Science Congress
10-12 February 2023

Science and Technology Council
uCost
Uttarakhand

Certificate of Participation

THIS IS TO CERTIFY THAT

Kumudani Bala Gautam

has actively participated in the 17th Uttarakhand State Science & Technology Congress 2023, on 10th to 12th February, 2023 and presented a research paper entitled

Himalayan Upliftment and Shivalik Succession driven diversification of Bengal Monitor Lizard, Varanus bengalensis in India

Under the discipline

Biotechnology, Biochemistry and Microbiology

at Uttarakhand State Council for Science & Technology, Vigyan Dham, Dehradun.

Dr Ashutosh Mishra
Convener-17th USSTC
Uttarakhand State Council for Science and Technology
Dehradun

Prof (Dr) Durgesh Pant
Chairman-17th USSTC
Director General
Uttarakhand State Council for Science and Technology
Dehradun

UNIVERSITY OF CAMBRIDGE

Conservation Science Group
The David Attenborough Building
Pembroke Street, Cambridge
CB2 3QZ, UK

Tel: +44 (0)1223 762979
Email: sccs@sccs-cam.org
Web: www.sccs-cam.org

This is to certify that

Kumudani Bala Gautam

has attended the Student Conference on Conservation Science, 28-30 March 2023,

at the University of Cambridge, Downing Street, Cambridge, UK.

and presented a poster titled:

"Linking the origin of hemipenis': A molecular approach for identifying the trade route of Monitor Lizard"

Date: 31 March 2023

Administrator……..

Building links among young conservation scientists and practitioners

14th Student Conference on Conservation Science Bengaluru 2023

9th – 12th October 2023

CERTIFICATE OF APPRECIATION

This is to certify that Ms. Kumudani Bala Gautam presented a **Poster** titled "Influence of Himalayan upliftment on diversification of Bengal monitor lizard" in the Student Conference on Conservation Science (SCCS)-Bengaluru that was held between October 09-12, 2023, at the J N Tata Auditorium, Indian Institute of Science, Bengaluru.

Srinivasan Kasinathan
Member
Organizing Committee

CERTIFICATE
Of Participation

Presented to

Kumudani Bala Gautam

for a Poster Presentation
on the topic

Genetic Approach To Identify The Origin of Monitor Lizard Hemipenis

at the 50 Years of Project Tiger
& First Indian Conservation Conference,
Mysuru, Karnataka, India on 10th April 2023

Bilal Habib
Coordinator ICCON 2023
Scientist - F,
Wildlife Institute of India

Virendra Tiwari
Director,
Wildlife Institute of India

S.P.Yadav
ADG - Project Tiger & Elephant Ministry of Environment, Forest & Climate Change

भारतीय वन्यजीव संस्थान
Wildlife Institute of India

UNIVERSITY OF LADAKH
DEPARTMENT OF FORENSIC SCIENCE
(LEH CAMPUS, TARU-THANG - LEH LADAKH)
e-mail: forensic.uol2022@gmail.com

No. UoL/FS/COO-27/2024 **Date: 26-07-2024**

Mr. Kumudani Bala Gautam
Research Scholar
Wildlife Forensic and Conservation,

Subject: Request for an invited talk at the National Conference on "Emerging Trends and Techniques in Forensic Investigations," scheduled to be held at UoL, Leh Campus from 29th to 30th July, 2024.

!!! *Greetings from University of Ladakh*!!!

It gives me immense pleasure to inform you that, University of Ladakh is going to organize a national conference on " ***Emerging Trends and Techniques in Forensic Investigations***" from 29-30 July, 2024 at University of Ladakh, Leh campus. This event is proposed to be organized with an aim to provide a platform for academicians, scientist, students, and research scholar to come together and deliberate on many important topics related to the **"*Emerging Trends and Techniques in Forensic Investigations*".** We intend to catalyze insightful and rigorous research discussions in forensic investigation techniques. The event offers unique opportunity to meet eminent scientists, learned academicians, students, and research scholar to share knowledge.

On behalf of the organizing Committee, I request you to kindly grace the event and deliver an Invited Talk on the themes mentioned below:

- Wildlife forensics
- Forensic Genetics
- Investigation approaches in Forensic
- Cutting-Edge techniques and Forensics
- Crime ,Society ,Emerging Forensic needs

Your gracious presence at the event will be highly motivating for the participants.

Please send us topic and outline of your talk along with a brief biodata by email on forensic.uol2022@gmail.com

Accommodation: Accommodation will be arranged for the duration of your stay in Leh on payment basis.

Thanking you .

With kind Regards,

Dr. Qutsia Tabasum
(Convener ETTFI, 2024)

From evidence to insights: Practicing forensic science in monitoring the *Monitor*

Author: Kumudani Bala Gautam

Molecular markers have revolutionized biological research and are proving invaluable in diverse forensic investigations, particularly in crime scenarios involving both human and wildlife subjects. Emerging molecular tools and techniques are instrumental in addressing crimes, especially when traditional crime scene evidence is unavailable. Wildlife poaching activities become more problematic to resolve since finding an intact crime scene is often an unattainable task for investigators. On such major trade in reptile is second highest among all illegally traded wild species. The trade in reptiles and amphibians surpasses mammalian species due to their smaller size, making them easier to conceal and evade detection. Despite the enactment of stringent laws WildLife (Protection) Act, 1972, the trafficking of Monitor Lizard hemipenes, falsely marketed as "Hatha jodi," continues to rise in India. With the help of cutting-edge technology of molecular tools our goal is to identify the species, pinpoint specific geographic origins, and trace potential trade routes. To achieve this, species identification, DNA barcoding, and genetic structuring were performed for each hemipenis using the mitochondrial DNA (mtDNA) genes, along with microsatellite loci. The results will be compared with the geo-referenced database generated by the Wildlife Forensics Cell at the Wildlife Institute of India. Analysis of mtDNA gene sequences using a Bayesian tree revealed two well-supported clades within the Bengal Monitor Lizard population in our geo-referenced database. A corresponding pattern was observed between genetic cluster assignments and phylogenetic clades. This analysis enabled us to determine the species and likely geographic origins of the confiscated hemipenes with confidence. The study identified distinct geographic signatures of the Bengal Monitor Lizard, allowing us to confidently individualize and identify species from the confiscated samples. These findings are crucial as they provide a reliable method for tracking the unknown origins, thereby supporting enforcement agencies in identifying probable trade routes. By establishing a clear genetic and geographic link, the results can aid in curbing the illegal wildlife trade and protecting these highly traded species. The use of advanced molecular techniques, combined with a robust geo-referenced database, proves to be a powerful tool in wildlife forensics, ensuring more effective monitoring and enforcement of wildlife protection laws.

Keyword: Population genetics, Wildlife trade, Phylogenetics, Forensic genetics, Wildlife forensics, Hatha Jodi

Gmail **KUMUDANI GAUTAM <balakumudani@gmail.com>**

WCH10 - Letter of Acceptance (A-0285)

1 message

WCH10 <noreply@2024wch10.com> 3 March 2024 at 10:08
To: KUMUDANI BALA GAUTAM <balakumudani@gmail.com>

LETTER OF ACCEPTANCE

A-0285

Dear KUMUDANI BALA GAUTAM,

We thank you for the submission of the abstract for presentation at an **oral session** of the **10th World Congress of Herpetology**, to be held in Kuching, Sarawak, Malaysia, on 5-9 August 2024.

Title of Paper:
When Biogeography meets Forensics: Case study with Bengal Monitor Lizard (Varanus bengalensis)

Paper Code:

Name of Presenter/Corresponding Author:
KUMUDANI BALA GAUTAM (balakumudani@gmail.com)

We are pleased to accept the same for an appropriate session at the Congress. Details of the schedule for your session will be provided at a later date.

Please direct enquiries on logistics, including registration, travel, board, and lodging, if relevant, to the Congress Secretariat: secretariat@2024wch10.com

We look forward to welcoming you in Kuching in a few months from now.

Sincerely yours,
Prof. Indraneil Das, D.Phil (Oxon)
Congress Director
10th World Congress of Herpetology (WCH10)

For latest information regarding the 10th World Congress of Herpetology 2024,
please visit 2024wch10.com

This is an auto generated email. Do not reply to this email.

Kumudani Bala Gautam
Indira Nagar
Lucknow
Uttar Pradesh
226016

20th September 2022

Dear Kumudani

Offer Letter: 37676-1

We are delighted to let you know that, after careful consideration, the Trustees of The Rufford Foundation have approved a 1st Rufford Small Grant of £5953 to support Leveraging community and technology to combat illegal trade of Bengal Monitor lizard in Terai Arc, India (the **Project**). This Offer Letter sets out the terms and conditions of our proposed Grant Agreement.
The following are also included with this letter:

- a Banking Information Form; Please pass on to the organisation for completion.
- a template for information you need to provide us for our website;
- information about, and a copy of, the Final Project Evaluation Report template; and
- a copy of our logo in JPG format, together with conditions of use under point 4 Intellectual Property.

We must point out that once this Offer Letter is signed it will create a legally binding agreement between you and the Foundation. We urge you to raise any questions or concerns that you may have about it as quickly as possible. The main point is that by signing this Offer Letter, you agree that this Grant will only be used for the delivery of the Project and not for any other purpose.

Please note that this offer is valid for 120 days from the date of this letter. If we have not had a response from you by the end of this period, the offer will be formally withdrawn.

The Trustees of the Foundation will require you to complete a Final Project Evaluation Report (see point 2 below). In the event that the Grant exceeds the total cost of the Project, you will be required to reimburse us for any funding not used.

By accepting the Grant you agree that you will comply with the terms of the Grant as set out in this Offer Letter.

1. **Grant Payments and Use**

As you know, we will only pay the grant via an organisation. Please contact the relevant person at the organisation that will receive the funds to ensure the details provided on the Banking Information Form included with this Offer Letter are correct.

Gmail **KUMUDANI GAUTAM <balakumudani@gmail.com>**

SERB-Notification

1 message

SERB_Administrator@serbonline.in <SERB_Administrator@serbonline.in> 27 June 2024 at 14:00
To: info@serbonline.in

Science and Engineering Research Board

(Statutory Body Established Through an Act of Parliament : SERB Act 2008)
Department of Science and Technology, Government of India

Anusandhan National Research Foundation (ANRF)

(A statutory body created by an Act of Parliament - ANRF Act, 2023)

Science & Engineering Research Board (SERB) **International Travel Support (ITS) Scheme**

ANRF
3rd & 4th Floor, Block II
Technology Bhavan, New Mehrauli Road
New Delhi - 110016

File Number: ITS/2024/002913

Dated: 27-Jun-2024

To
Ms. Kumudani Bala Gautam,
Wildlife Institute Of India , Chandrabani, Dehradun, Dehradun, Uttarakhand-248001

Subject: Financial Assistance to Ms. Kumudani Bala Gautam for participating in **"10 World Congress of Herpetology, Malaysia (05 August, 2024 to 09 August, 2024)"**

Sir/Madam

We are happy to inform you that your application seeking financial grant to attend the above mentioned international scientific event has been recommended for support by the Anusandhan National Research Foundation (ANRF). **We will provide to and fro economic class air-fare by the shortest route, airport tax , visa fees & registration fees**. It is hoped that the support will give you an opportunity to interact with leading international experts in the area. The support, however, is subject to the following conditions.
1. You should not have received financial support during the last three years under this scheme.

2. The air tickets are to be booked in economy class at "**Best Available Fare**" on the date of booking. It may be noted that rescheduling/cancellation charges will not be reimbursed.

3. ANRF is directed to instruct the applicant to purchase the air tickets from either of three Authorised Travel Agents viz. i) M/s Balmer Lawrie & Company Limited (BLCL) and ii) M/s Ashok Travels & Tours (ATT) and iii) Indian Railways Catering and Tourism Corporation (IRCTC) vide order no. 19024/03/2021-E.IV dated 31-12-2021 issued by Department of Expenditure, Ministry of Finance. In case of failure of adherence to this guideline, air fare will not be reimbursed.

4. The candidate availing ITS must have to submit the certificate for "**Best Available Fare**" at the time of reimbursement. In case, tickets are booked through IRCTC, it is mandatory to submit a screenshot of the related IRCTC webpage as an evidence to ensure that the tickets has been booked at the "Best Available Fare"

5. The signed print copy of Claim Form along with the original Boarding passes and other relevant documents must be sent to the ANRF immediately after completing the online Claim Form to the following address.

ITS Section

3rd & 4th Floor, SERB Block II

Technology Bhavan, New Mehrauli Road

New Delhi 110016

6. The account details must be in the format available at the home page of the online portal in the format section and it must be endorsed by the competent authority of the institute/university.

7. ANRF will reimburse the grant after deducting the financial assistance received from any other sources, if any.

8. All other expenses such as per diem, taxi fare etc. will not be reimbursed by ANRF.

9. You will have to make your own arrangements for foreign exchange required for the purpose.

10. You will not be treated as a delegate sponsored by the Government of India.

11. We request you to either accept or decline this offer at the earliest online. On acceptance only, you will be able to submit the Claim Form. Please note that once you decline this offer, it will be assumed that you are not interested in availing this offer and no further communication will be entertained in this matter.

12. You must submit Claim Form and other relevant documents online within 90 days of the Last Date of the Event, failing which ANRF will not reimburse the Travel Grant.

13. If any candidate found to have furnished incorrect / misleading information at any stage, his/her candidature will be cancelled and no reimbursement will be made. The candidate will also be debarred for next three years for availing support under this scheme.

Disclaimer:

1. Financial support will be provided for physical attendance in the event. No support will be provided in case of online/web attendance.
2. No financial support will be provided other than approved items as mentioned above. ANRF will not be responsible to bear any other cost borne by the candidate.

With kind regards,

(Dr. Magesh K K)

Scientist E

Ph: Ph: 01126552114

Email: ms.its@serb.gov.in

********** LEGAL DISCLAIMER **********

Please do not reply to this mail !!

[SERB is now on Social-Media. Kindly follow us on Twitter: @serbonline https://www.twitter.com/serbonline]