GENETIC ASSESSMENT OF THE NON-DESCRIPT PIG BREEDS ACROSS SELECTED REGIONS OF UTTARAKHAND

Thesis submitted to the

Saurashtra University

Rajkot (Gujarat)

For the award of the Degree of

DOCTOR OF PHILOSOPHY

IN

WILDLIFE SCIENCE

By

KHUSBU SAHU

Registration No: 16797

WILDLIFE INSTITUTE OF INDIA

December 2023

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Under the Supervision of

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December 2023

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DECLARATION

I hereby declare that the thesis "**GENETIC ASSESSMENT OF THE NON-DESCRIPT PIG BREEDS ACROSS SELECTED REGIONS OF UTTARAKHAND, INDIA**" is original research conducted by me under the supervision of Dr. S. K. Gupta, Scientist-F, Wildlife Institute of India and Co-supervision of Dr. Gopi G. V., Scientist-F, Wildlife Institute of India. The Thesis has been submitted to the **Saurashtra University, Rajkot (Gujarat)**, for the award of **Doctor of Philosophy in Wildlife Science** and has not formed the basis for the award of any other degree. It embodies my own work and observations, and in that respect, the investigation appears to advance knowledge on the subject.

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CERTIFICATE

This is to certify that the thesis by **Mrs. Khusbu Sahu** entitled **"GENETIC ASSESSMENT OF THE NON-DESCRIPT PIG BREEDS ACROSS SELECTED REGIONS OF UTTARAKHAND, INDIA"**, 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**.

Mrs. Khusbu Sahu has put more than six terms of research work embodied in this thesis under our guidance and supervision. The work presented in this thesis has not been submitted for any degree of any other University or Institution.

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LIST OF PUBLICATIONS

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EXECUTIVE SUMMARY

Modern agriculture relies heavily on a limited number of animal species, for various purposes, such as livestock for meat, dairy, and other products. The domestication of wild animals and changes in human interactions with them have often resulted in the extinction or severe endangerment of their wild ancestors. For example, the wild ass (the ancestor of the donkey) is endangered, while the wild relatives of the dromedary and the cattle are extinct. The domesticated pig (*Sus scrofa domesticus*) is an exceptional livestock species because the presence of its wild ancestor, the wild boar (*Sus scrofa*), still exists worldwide. This can be attributed to various factors, including the extensive geographic distribution of wild boars, their prolific reproductive capabilities, the appeal of hunting them for both sport and sustenance, their adaptability to diverse habitats, cultural significance, invasive populations in certain regions, and ongoing conservation efforts. Wild boars have managed to coexist with their domesticated counterparts in diverse environments across the globe.

Managing and protecting domestic and wild pig populations in ecological areas is a complex endeavour, requiring a multifaceted approach that balances the needs of agriculture and conservation. Research and innovation play a pivotal role in finding solutions that facilitate the coexistence of agriculture and wildlife.

Sus scrofa is traditionally distinguished from other *Sus* species based on morphological characteristics of the lower canine. This distinction results in two groups the scrofic group, also known as non-warty pigs, which includes *Sus scrofa*, and the verrucosic group, encompassing other *Sus* species. Due to the extensive geographic distribution of *Sus scrofa*, numerous subspecies arised. Some of these subspecies are domesticated, while others are wild or feral. The domesticated subspecies include *Sus scrofa domesticus, Sus scrofa ussuricus, Sus scrofa*

cristatus, and *Sus scrofa vittatus*. The wild subspecies include *Sus scrofa scrofa, Sus scrofa leucomystax, Sus scrofa sibiricus,* and *Sus scrofa meridionalis*. The feral subspecies *Sus scrofa affinis, Sus scrofa andamanensis, Sus scrofa celebensis,* and *Sus scrofa papuensis*. These subspecies differ in their morphology, physiology, behaviour, and ecology, reflecting their adaptation to various environmental and human influences.

The global databank currently compiles breed data from 182 countries (15 dependent territories) for 37 species and as reported there has been a marginal increase in the total count of national breed populations within the global databank. Approximately 28% of all mammalian breeds, excluding those that have already gone extinct, are currently designated as being at risk of extinction. Among mammalian species, the largest numbers of breeds facing this risk are observed in sheep, cattle, and horses. The pig category, for instance, comprises 625 documented pig breeds, encompassing local, regional, transboundary, and international transboundary classifications and 70 pig breeds, are documented as extinct. Geographically, the Europe and the Caucasus region prominently records a higher count of extinct pig breeds, followed by Asian regions. The urgent need to act promptly to safeguard genetic diversity within domestic animal breeds cannot be overstated.

India, which has ancient history of pig rearing, is home to several indigenous pig breeds that reflect the adaptation of *Sus scrofa* to different environmental and cultural conditions. However, only 13 of these breeds are currently recognized and documented by the National Bureau of Animal Genetic Resources. The present research was undertaken to identify the spatial distribution, socio-economic status, pig husbandry methods of pig rearers, and the impact of Exotic pig breed on the indigenous breed and ecology of the region. As molecular characterization is a valuable tool that provides insights into the evolutionary history and genetic diversity of the current pig breed raise in a region. Hence by incorporating molecular techniques in understanding of the domestic pig breed and their interactions with ecosystems, promote more precise and science-based management that contributes to biodiversity conservation and sustainable husbandry practices. The present study will also aim to evaluate the maternal lineage, genetic diversity and population structure of the domestic pig breeds at the regional scale, using microsatellite markers and mitochondrial DNA sequences.

The survey and sampling was done in the planar (Dehradun, Haridwar and Nainital) districts of Uttarakhand, a mountainous state of India that lies in the foothills of Himalayas, where rapid urbanisation and continuous migration make us reconsider the necessity of an organise promotion of the piggery sector due to its multiple ecological implications.

28 households were reached by adopting rapid appraisals approach and interviewed by using a semi-structured questionnaire. Household survey analysis showed that 60.7%, 10.7% and 21.4% of the household raised pig near seasonal streams (slum settlements), the residential areas within the house premises and near the forest, respectively. Moreover, 28.6% of pig rearer households' maintained breeding boar of feral origin and 96.94% followed a semi-intensive pig rearing system; hence human settlement is staying in close proximity with pigs (domestic and wild) and other domestic animals. The results reveals that the limited contribution of the piggery sector in Uttarakhand is influenced by geographical, cultural, economic, and infrastructure-related factors. The hilly terrain and temperate climate pose challenges for pig farming, with concentration in plain districts. Traditional dietary preferences, capital-intensive nature, and inadequate government support hinder sector growth. Crossbreeding of local sows with wild boars is widespread. Production and management practices indicate that 85.7% follow a farrow-to-finish approach, with 96.94% adopting a semi-intensive pig rearing system. Natural and controlled breeding techniques are predominant, and 100% of respondents practice castration of old boars. The decline in indigenous pig breeds, despite adaptability, signals genetic diversity loss. The low work participation rate within this community presents an opportunity for targeted support to enhance pig rearing. The semi-intensive system, prevalent

among small-scale producers, offers economic stability. Emphasis is all laid on the need for monitoring pig herds, understanding wildlife-domestic pig and human interactions, and implementing measures to safeguard public health in urban ecosystems. Lastly, the introduction of exotic pig breeds poses potential threat on ecological, genetic, and socio-economic implications, emphasizing the importance of context-specific management strategies. Comparison of the performance of different pig breeds reveals that the hybrid of local cross with wild pigs emerged as the top performers, exhibiting advantages in various criteria such as reproduction, feed efficiency, and disease resistance. It is also analysed that the market value of hybrid of local cross with wild pigs was higher, attributed to enhanced genetic traits, superior carcass characteristics, specific demand-supply dynamics and highly adaptable breed for the local conditions of Uttarakhand. This information can be useful for managing and conserving both domestic and wild pig populations, as well as for understanding their role in ecosystems and human societies.

The maternal lineage of the domestic swine population was traced using mitochondrial DNA control region markers. Analysis of 68 samples reveals 20 haplotypes, the signature of the Pacific Clade (D6), MTSEA (South East Asia), European (D1), and the ubiquitously distributed Chinese (D2) haplotypes are present in the domestic pig of Uttarakhand. The D3 haplotypes, which were reported in wild pigs from North India, were also identified in 47 domestic samples. A unique genepool, UKD (Uttarakhand Domestic), as another lineage specific to this region has been proposed. The incorporation of an additional 15 samples originating from North Indian states, and their subsequent integration with previously published 1,518 mitochondrial D-loop sequences of *Sus scrofa*, provide a comprehensive global perspective on the geographical distribution and phylogenetic positioning of the examined specimens. The resulting global dataset revealed 18 unique haplotypes, and a complex clustering patterns of porcine haplotypes with distinct phylogeographic signals. The study unveiled diverse maternal lineages among the domestic pig populations in northern Indian states situated in the foothills of the majestic Himalayas. Additionally, the study also confirmed the finding of the presence of two distinct subspecies of wild boar in India (W1 and W2), demonstrating their migration routes and offering a strong phylogeographic signal. Evidence for a separate domestication center in the Himalayan foothills and Indian sub-continent was found within Mixed Clade 5 (MC5). A novel clade (MC4) with a localized distribution in the Chotanagpur plateau was detected, suggesting a unique population of wild boar in this region. Diverse maternal lineages among domestic pigs of Uttarakhand were observed, which may reflect human-mediated dispersal of pigs within and into the region; hence, a hypothesis linking ancient human migration with the presence of these maternal lineage clades has been proposed. Overall, the porcine haplotypes discovered in the Indian subcontinent through this study highlight its unique and significant contribution to genetic resources.

67 non-invasive biological samples were collected from the herd of the household survey for molecular analysis using 13 microsatellite loci, recommended by Food and Agriculture Organisation. The study found that the domestic pigs in Uttarakhand have a high level of genetic diversity, as indicated by the observed (Ho) and expected (He) heterozygosities of 0.83±0.02 and 0.84±0.01 respectively. The average polymorphic information content (PIC) values of 0.83±0.01 also indicate the high informativeness of the markers. Further, the domestic pigs in Uttarakhand have a low level of inbreeding, as indicated by the overall mean FIS value of 0.04 ($P<0.01$), which suggests that the animals are randomly mating within the population. However, some loci deviated from Hardy-Weinberg equilibrium (HWE) at a significant level ($p < 0.05$), which may indicate the presence of selection, genetic drift, or non-random mating at these loci. The domestic pigs in Uttarakhand have a low level of genetic differentiation, as indicated by the two clusters identified by the STRUCTURE analysis, which showed

overlapping populations. The low differentiation and significant FST value ($\mathrm{Fst} = 0.09$, $P < 0.001$) support these findings. These results suggest that the traditional management practices in Uttarakhand have allowed for genetic mixing and the sharing of genetic material among pig populations. This can contribute to increased genetic diversity but may also result in the loss of distinct genetic characteristics or breed purity of the local breeds, if not carefully managed.

Therefore, the study recommends that the genetic resources of the local pig breeds in Uttarakhand should be conserved and utilized in a sustainable manner, taking into account their adaptation, productivity, and socio-cultural importance.

CHAPTER 1

INTRODUCTION

1.1BACKGROUND

Modern agriculture is heavily dependent on a limited number of animal species for various purposes, including livestock for meat, dairy, and other products (FAO, 1995). The domestication of wild animals and changes in human interactions with them have often led to the extinction or severe endangerment of their wild ancestors. Some of these ancestral species, like the wild ass (from which the donkey descended), are endangered, while others, like the dromedary's wild relative and the cattle’s wild relative have been extinct (Ajmone-Marsan et al., 2010).

The domestic pig (*Sus scrofa domesticus*) stands out as an exceptional livestock species, owing to the global persistence of its wild progenitor, the wild boar (*Sus scrofa*). This unique situation can be attributed to several factors, including the wide geographic distribution of wild boars, their high reproductive capacity, adaptability to various habitats, cultural significance, invasive populations in some areas, and conservation efforts aimed at preserving the species (Bosse et al., 2019). While local populations may be vulnerable in specific regions, wild boars have managed to coexist with their domesticated counterparts in diverse environments across the globe (Layos et al., 2022).

In the realm of domestic animal species, a diverse spectrum of breeds exhibits distinct characteristics. The Global Databank (Domestic Animal Diversity Information System) currently compiles breed data from 182 countries for 37 species and as reported there has been a marginal increase in the total count of breed populations, ascending from 15,115 in 2021 to 15,313 in 2022 (FAO, 2022). Around 28% of existing mammalian breeds, excluding those already extinct, are presently categorized as facing the risk of extinction.

Among mammalian species, the largest numbers of domestic breeds facing this risk are observed in sheep, cattle, and horses. The pig category, for instance, comprises 625 documented pig breeds, encompassing local, regional, transboundary, and international transboundary classifications. Additionally, a significant number of breeds, including 107 sheep breeds, 101 horse breeds, and 70 pig breeds, are documented as extinct. Geographically, the Europe and the Caucasus region prominently records a higher count of extinct pig breeds, specifically 53, while Asian regions closely follow with 15 (FAO, 2022). The urgent need to act promptly to safeguard genetic diversity within domestic animal breeds cannot be overstated. Failing to undertake this vital mission may culminate in the extinction of numerous breeds, a substantial portion of which remains inadequately understood. Thus, there exists an imperative need to meticulously document breed attributes, including population sizes, geographic distribution, unique traits, historical lineage, and cultural significance (Ruan, 2000).

The FAO introduces a worldwide initiative centred on breed preservation, primarily utilizing their distinct genetic markers (Ajmone et al., 2023). This strategy is designed to protect breeds with exceptional genetic characteristics, ensuring the longevity of agricultural and cultural diversity. Across thousands of years, the transition from pig domestication to their extensive natural selection for agroecological adaptation has culminated in the emergence of numerous indigenous pig breeds in India. Currently, India officially recognizes and characterizes thirteen of these indigenous pig breeds, predominantly originating from the eastern states of the country (www.nbagr.res.in). These Indian indigenous pigs occupy a distinct niche within swine germplasm, distinguished by their remarkable phenotypic diversity, setting them apart from the majority of other domesticated species as their genetic composition also incorporates elements of wild ancestry.

Managing and protecting domestic and wild pig populations in ecological areas is a complex endeavour, requiring a multifaceted approach that balances the needs of agriculture and conservation. This collaborative strategy must consider ecological, economic, and social factors (Wang et al., 2023). To effectively safeguard these populations, a combination of strategies is essential. For domestic pigs, regulation of farming practices, enforcement of biosecurity measures, farmer education, are key components. Wild pig populations require controlled hunting and culling, habitat management, and strategies to control feral pig populations (FAO 2010). Achieving a balance between conservation and agriculture involves integrated pest management, cross-sector collaboration, public awareness, and incentives for sustainable practices. Research and innovation play a pivotal role in finding solutions that facilitate the coexistence of agriculture and wildlife (FAO, 2017).

Molecular characterization is a valuable tool in this management process. It provides insights into genetics, disease transmission, and population dynamics, enabling more informed and targeted management strategies. Incorporating molecular techniques enhances our understanding of these animals and their interactions with ecosystems, promoting more precise and science-based management that contributes to biodiversity conservation and sustainable agriculture (Hohenlohe et al., 2021).

1.2 SYSTEMATICS OF THE EXTANT SUOIDEA

The extant Suoidea are categorized into two living families: Suidae (encompassing suids, hogs, or pigs) and Tayassuidae (comprising tayassuids, javelinas, or peccaries). Suoidea, in turn, is part of Artiodactyla also known as “Cetartiodactyla”, one of the major clades within the mammalian evolutionary tree (Asher & Helgen, 2010). While the exact relationships among ruminants are challenging to decipher but within the Artiodactyla order, Tayassuidae, known as peccaries (New World pigs), are commonly regarded as the closest relatives of Suidae. These families, occasionally grouped together as "Suina," are considered more primitive branches of Artiodactyla, characterized by less advanced digestive systems. The key differentiation between these two families is found in the structure of their upper canines. Suids are characterized by upward-turned canines, whereas Tayassuids have canines that point downward (Asher & Helgen, 2010). The molecular level of taxonomy using the Cyt *b* gene has supported the position of Suidae (Figure 1.1) (Agnarsson et al., 2008).

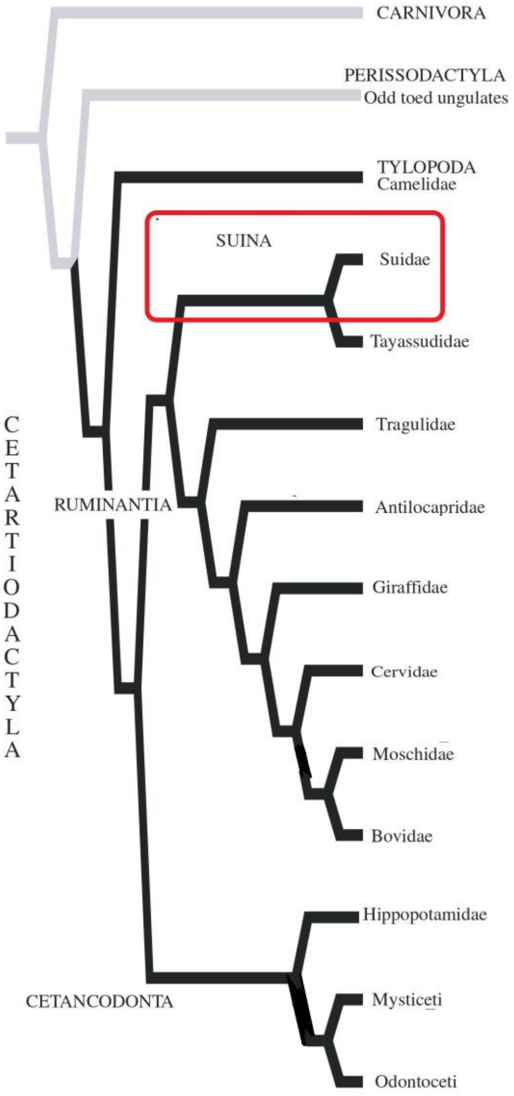

Figure 1.1 Summary cladogram of the full analyses using cytochrome *b* showing position of suidae within Cetartiodactyla (adapted from Agnarsson & May-Collado, 2008).

Suids (Suidae) are found in diverse environments, from tropical rainforests to arid open woodlands, grasslands, highlands, deserts, and islands across Africa, Europe, and Asia (Meijaard et al. 2011). *Sus scrofa* is classified within the genus *Sus*, which falls under Suidae, a family of even-toed ungulates includes 14–30 species, organized into six genera: *Sus* (comprising domestic and wild pigs) from Eurasia; *Babyrousa* (consisting of babirusas) from Southeast Asia; and *Potamochoerus* (bush pigs), *Phacochoerus* (warthogs), and *Hylochoerus* (forest hogs) all from sub-Saharan Africa . *Porcula* (housing the pygmy hog) from the Indian subcontinent; *Porcula salvanius,* has been considered a new genus, distinct from *Sus*. While it shares a close relationship with *Sus*, the precise placement of *Porcula* concerning other genera remains uncertain (Gongora et al., 2011).

Pigs are systematically categorized as: Kingdom-Animalia, Phylum- Chordata, Class-Mammalia, Order-Artiodactyla, Family-Suidae, Genus-*Sus*, and Species-*scrofa L*. This scientific nomenclature delineates the hierarchical taxonomic structure reflecting their evolutionary relationships within the animal kingdom.

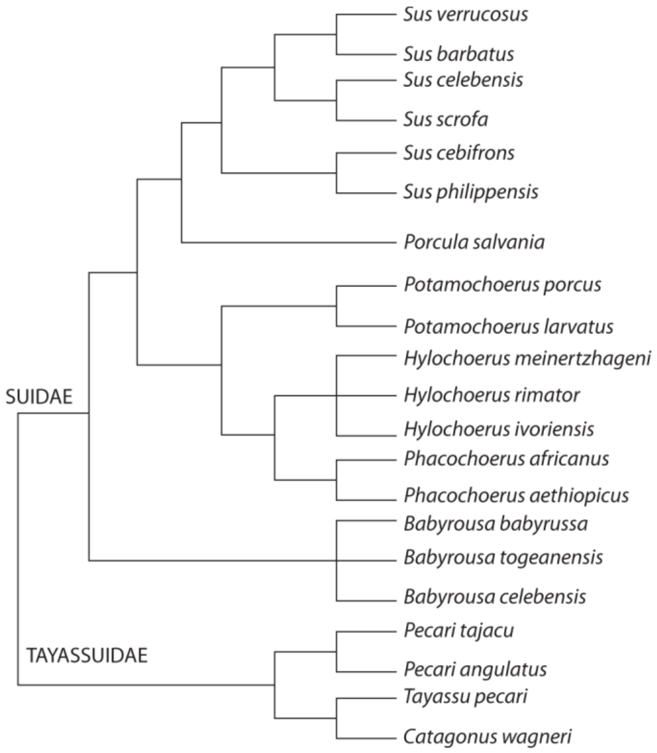

Figure 1.2: Cladogram illustrating morphological affinities among species of the genus *Sus*. Polytomies within the diagram signify either relationships that are not conclusively resolved or instances where data is unavailable (Gongora 2017).

1.3 ORIGIN AND EVOLUTION OF *Sus scrofa*

Hyotherium, the earliest documented suid fossil, flourished during the Early Oligocene epoch within the Indian subcontinent (Orliac et al., 2010). The Suidae family experienced a diversification, giving rise to around 20 genera during the Neogene period. This diversification included the development of Suinae, believed to have originated towards the conclusion of the Middle Miocene, approximately 11-10 million years ago (Orliac et al., 2010). By the onset of the Pliocene epoch, multiple genera within the Suinae subfamily had already emerged.

Genetic evidence indicates that the divergence of *Porcula* and *Sus*, two suid subgroups, occurred during the Miocene epoch, approximately 14.9-13.6 million years ago. However, the paleontological record lacks definitive evidence to substantiate this claim. Within the *Sus* genus, genetic analyses suggest that the three principal lineages of extant species diverged from a common ancestor around 7.3-5.5 million years ago (Gongora et al., 2017). These estimations align, to some extent, with existing fossil evidence, indicating that the initial diversification of *Sus* species likely occurred between the Late Miocene and the Pliocene/Pleistocene epochs. Several extinct *Sus* species may serve as representative examples of this early diversification.

Within the genus *Sus*, *Sus scrofa* is usually distinguished from other *Sus* species based on morphological characteristics of the lower canine. Based on this difference two groups were recognised: the scrofic group (non-warty pigs), which includes *Sus scrofa*, and the verrucosic group, encompassing other *Sus* species (Meijaard, 2006).

The widespread geographic distribution of *Sus scrofa* has led to the emergence of numerous subspecies. In India, *S. s. davidi* is identified in Pakistan, south-eastern Iran, and northwest India, particularly in the Sub-Himalayan region, with suggestions of its potential classification as a distinct species. Another subspecies, *S. s. cristatus*, spans from northern India to Western Thailand, reaching down to the Isthmus of Kra, and is also under consideration as a candidate for a separate species. *S. s. affinis* is found in southern India and Sri Lanka. This subdivision of *Sus scrofa* into subspecies reflects its diverse and widespread range in India as well (Figure 1.3) (Oliver et al., 1993).

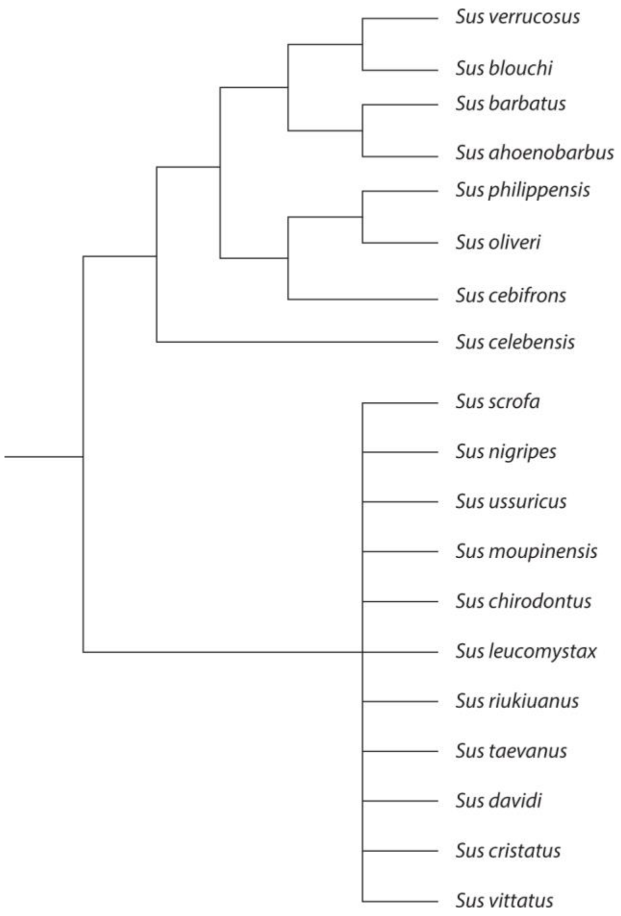

Figure 1.3: A cladogram depicting morphological affinities among species within the genus *Sus* is presented below. Polytopies within the diagram indicate either unresolved relationships or unavailable data (Gongora et al. in 2017). The taxonomic arrangement follows the classification outlined by Groves & Grubb in 2011.

1.4 ORIGIN OF DOMESTIC PIG

The domestication of *Sus scrofa* is regarded as an earliest instances of animal domestication, dating back to the beginning of the Holocene period. This period coincided with the rise of Neolithic cultures worldwide, leading to the domestication of key farm animals such as sheep, goats, and cattle (Vigne et al., 2011).

Pigs hold a unique place among domestic animals for several reasons. Firstly, their wild ancestor, the wild boar (*Sus scrofa*), had an extensive natural range, spanning from Island Southeast Asia, where genetic evidence indicates its origin, to western Europe (Evin et al., 2015). This is in contrast to the wild ancestors of sheep and goats, which had more restricted geographic distributions. Secondly, wild boars persist in their natural habitats, unlike the wild precursor of cattle, the aurochs, which became extinct in 1627 (Upadhyay et al., 2017). Currently, the history of domesticating pigs stands out as one of the most thoroughly researched topics among all domesticated animals.

Within the genus *Sus*, only *S. scrofa* and *S. celebensis* have undergone domestication from wild pig (Groves, 1981). The domestication of *S. celebensis* took place on South Pacific islands, leading to the development of breeds that have since remained isolated geographically (Larson et al., 2007). While the precise date of the earliest domestication remains uncertain, both archaeological and molecular evidence suggest that humans initiated this process around 9000 years ago (Giuffra et al., 2000, Larson et al., 2007, Vigne et al., 2009).

Evidence from archaeozoological records indicates the presence of at least two independent *Sus scrofa* domestication centers. The first center is located in western Asia, near the eastern edge of Anatolia, dating to around 8500 BC (Conolly et al., 2011), the second center is situated in China, along the Yellow River valley, around 6500 BC (Jing et al., 2002). Conclusive evidence indicates that domestication occurred independently of wild boar subspecies in both Europe and Asia (Giuffra et al., 2000).

More recent genetic studies of modern wild and domestic pig populations has led to the advancement of the understanding of the origins, migration, domestication, and dispersal of *Sus scrofa* and suggested that several wild boar populations contributed to the genetic makeup of modern domestic pig stocks that are distributed globally (Larson et al., 2005; 2007; 2010).

Numerous indigenous pig breeds have been identified across the native range of *S. scrofa*. Frantz et al., (2015) laid a robust foundation for understanding the domestication and diversification of various pig breeds. Their analysis, employing genome sequences from Asian and European wild boar as well as domestic pig breeds, challenges the traditional model of domestication that assumes human-driven domestication process and reproductive isolation between wild and domestic forms, proves inconsistent with genetic evidence. To reconcile genetic findings with zooarchaeological evidence indicating a constrained domestication process, the researchers propose a model involving continuous gene flow between wild and domestic populations. According to the study domestication, in this perspective, is not a one-time isolation event but a dynamic process allowing genetic exchange over time. Factors such as escapees from domestication or interbreeding between domestic pigs and nearby wild boars influence this gene flow. Continuous gene flow elucidates the observed genetic diversity in domestic pig breeds and their retention of genetic influences from diverse wild boar populations. It emphasizes the necessity of recognizing the dynamic and interconnected nature of domestication processes, challenging the previous assumption of strict isolation between domestic and wild populations. The study suggests that recurrent selection for domestic traits counteracted the homogenizing effect of gene flow from wild boars, leading to the formation of distinct "islands of domestication" within the pig genome where desirable domestic traits were consistently favoured. All subspecies of *S. scrofa* are capable of interbreeding, and domestic pigs from various geographic regions have also undergone hybridization. An illustrative case is the crossbreeding of Asian and European breeds initiated in the late 1700s, resulting in the amalgamation of swine lineages previously separated by millennia of divergence (Jones, 1998).

1.5 GEOGRAPHICAL DISTRIBUTION

Wild pig (*Sus scrofa*) is a highly adaptable and prolific animal, as it has high reproductive potential, wide dietary and habitat preferences, and strong behavioural and physiological adaptations to different environments (Ballari et al., 2014).

1.5.1 Distribution of the wild and feral pigs (*Sus scrofa*)

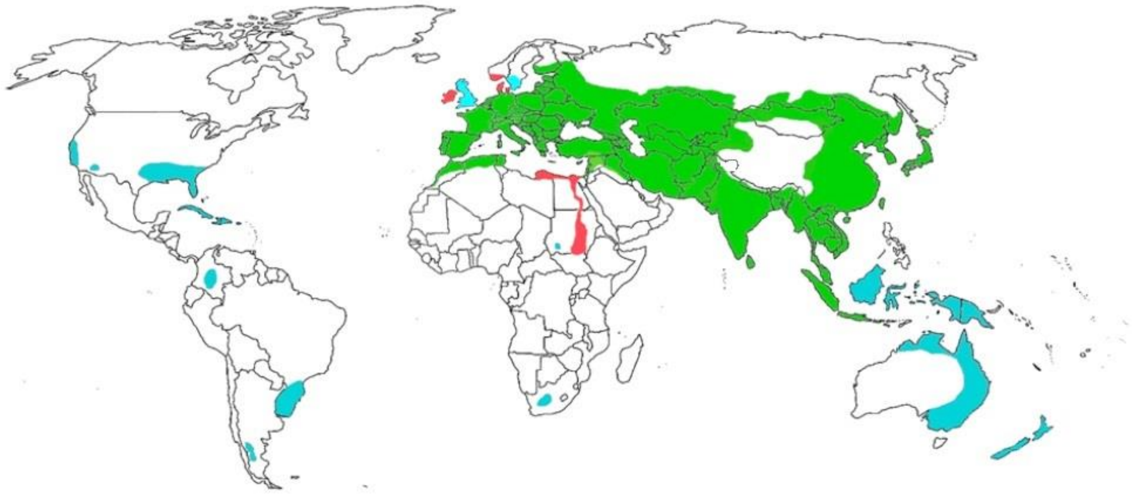

Figure 1.4: A global map depicting the distribution of the suiform group and populations of feral pigs. (Source: IUCN Red List of Threatened Species, Designed by Daniele Baisero of the Global Mammals Assessment, University 'La Sapienza', Rome.

The wild boar (*Sus scrofa*) is a widespread and adaptable suid that originally inhabits (green) a large part of Eurasia, from the westernmost edge of Europe to Japan, and from Manchuria in north-eastern China and south-eastern Russia to Java in Indonesia (Figure 1.4). The distribution is constrained by geographical features such as the Sahara and Arabian deserts in the Middle East, the colder climates prevalent in higher latitudes and altitudes, and the bodies of water that separate the Sunda Islands (comprising the Malayan Peninsula, Sumatra, and Java) from Borneo and the remaining eastern Indonesian islands

(Meijaard et al., 2011; Oliver & Leus, 2008). Historically, wild boars inhabited regions that are now recognized as Egypt, Libya, the British Isles, and Scandinavia (red). However, over time, the wild boar population faced various challenges leading to their extinction in these specific regions. In the British Isles and Scandinavia, the historical absence of wild boars was addressed through intentional reintroduction efforts orchestrated by human intervention (light blue) (Meijaard et al., 2011; Oliver & Leus, 2008).

Human activities have also led to the introduction of wild boars beyond their original range (dark blue). These introductions have occurred in various regions worldwide, including the Americas (North, South, and the Caribbean), the sub-Saharan region, and the Australasian region, which comprises eastern Indonesia, Australia, New Zealand, and Polynesian islands (Meijaard et al., 2011; Oliver & Leus, 2008). It has become an invasive species in these introduced region causing ecological, economic and social impacts and a threat to native biodiversity and agriculture (IUCN, Species Survival Commission (SSC) on invasive species).

1.5.2 Distribution of Domestic pigs

Following the initial domestication events of wild pigs in multiple regions, the subsequent generations witnessed the emergence of various pig breeds. This diversification was propelled by a combination of crossbreeding, selective breeding, and, in some instances, breeding with wild populations. These breeding practices were guided by human preferences and specific needs, resulting in the development of distinct breeds tailored for various purposes. The intricate interplay of selective traits and interbreeding has given rise to a wide spectrum of domestic pig breeds, each uniquely adapted to fulfill specific roles in agriculture, production, and cultural practices.

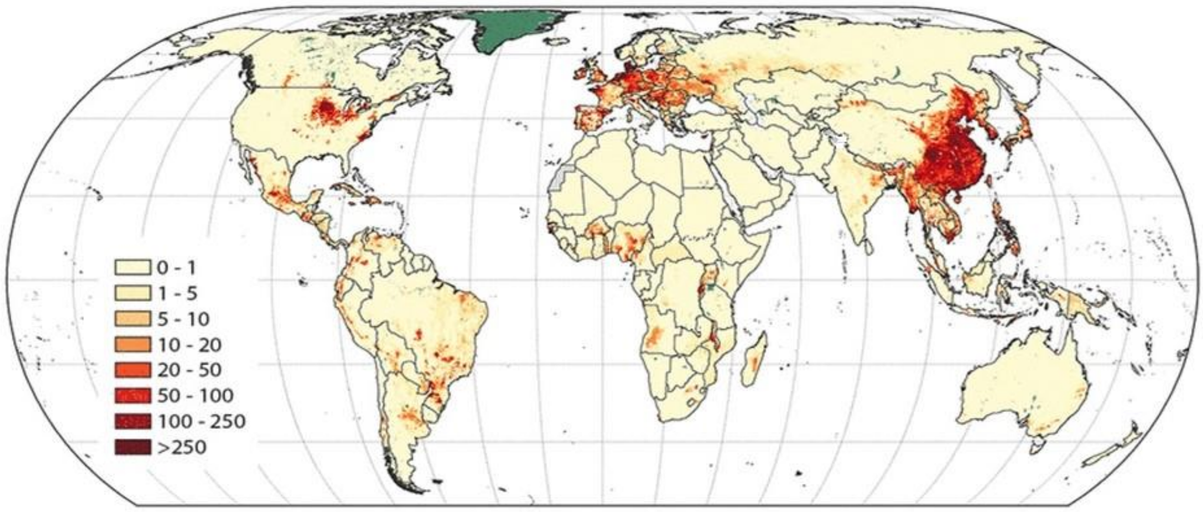

Figure 1.5: Number of pigs per square kilometer in 2015 (Dark grey are areas considered unsuitable and dark green areas correspond to IUCN protected areas) (Gilbert et. al., 2018). (The dataset is developed and maintained by FAO's Livestock Information, Sector Analysis and Policy Branch (NSAL) in collaboration with the Spatial Epidemiology Lab (SpELL), Université Libre de Bruxelles).

The Gridded Livestock of the World (GLW 4) of pig distribution is a spatial dataset is an updated version of GLW 3 that shows the global distribution and abundance of pigs for the reference year 2015 (Figure 1.5). It is part of the GLW 4 dataset that covers eight livestock species: buffaloes, cattle, chicken, ducks, goats, horses, pigs and sheep. The pig distribution dataset is based on census data and spatial modelling techniques, and it provides two versions of the pig distribution: one that disaggregates pig numbers within census polygons according to weights established by statistical models using high resolution spatial covariates (dasymetric weighting). This method uses data about various spatial factors to provide a more accurate representation of where livestock populations are likely to be concentrated. It refines the distribution based on available environmental and socioeconomic data and distributes pig numbers homogeneously with equal densities within their census polygons (areal weighting).

The domestic pig (*Sus scrofa domesticus*) primarily serves as a meat source, although its leather and hair are also utilized. The distribution of pigs is significantly shaped by religious and cultural factors, with fewer occurrences in predominantly Islamic countries. Asia hosts large domestic pig populations, and China alone is home to almost half of the world's domestic pigs. Pigs exhibit remarkable versatility, being raised across a spectrum of systems.

1.6 THREATS

From the 1800s onward, driven by consumer demand and government requirements, there has been a prolific development of numerous new pig breeds through systematic crossing and artificial selection (Jones, 1998). Presently, the diversity is exemplified by over 566 local domestic pig breeds across the world, including up to 232 local breeds from Asia alone (FAO, 2022). This diversity provides a robust foundation for comprehending the domestication and diversification of various pig breeds.

Intensive artificial as well as natural selection and environmental factors have created marked phenotypic modifications in domestic pigs. These adaptations encapsulate behavioural traits, body composition, disease resistance, reproductive characteristics, and coat coloration, contributing to the development of diverse local pig populations characterized by distinct genetic compositions and varied production performances. Pigs (*Sus scrofa domestica*) globally serve as a significant meat source and hold cultural importance in various communities. However, market demands for leaner fatteners and shifts in European agriculture have posed challenges to the preservation of this diversity. A trend towards genotype standardization and mass rearing of high-yielding breeds has led to the dominance of only few breeds in the global pork production landscape.

Consequently, the number of local and more adaptable pig breeds has markedly decreased, with some facing the threat of extinction.

India, home to several indigenous pig breeds, currently recognizes and documents only 13 of them. Similar challenges related to market preferences and agricultural shifts have affected the preservation of breed diversity in the Indian context. The global trend of preferring a handful of high-yielding breeds has implications for the survival of traditional breeds, some of which are now at risk of extinction.

Recognizing the cultural and economic significance of indigenous pig breeds, efforts are being made by institutions like the National Bureau of Animal Genetic Resources (NBAGR) in India to conserve and enhance these breeds. Preserving the genetic diversity of pig breeds is crucial not only for maintaining cultural heritage but also for potential contributions to the country's economy. Cataloguing and tracing the phylogeny and genetic diversity of native pig breeds offer multifaceted benefits. These include advancements in agriculture, conservation efforts, and valuable insights for scientific research. Preservation and sustainable management of pig genetic diversity stand as essential components in navigating the challenges posed by shifts in market preferences and agricultural practices.

This investigation holds significant potential to inform and guide breeding initiatives for pigs in Uttarakhand, where accelerated urbanization and population demands in urban districts are concurrently accommodating the livestock population. It is also imperative to consider the recurrent interactions between indigenous and non-descript pig breeds with exotic and wild pig varieties, thereby contributing to the gradual erosion of the longstanding genetic distinctiveness within the domestic as well as wild pig population and posing a threat to their overall existence.

Selective breeding and human manipulation often lead to the concentration of specific genetic traits deemed desirable for domestic purposes. Over time, this risk of genetic depletion in domesticated animals can result in a limited gene pool, leading to the reduction in genetic diversity within domestic populations making the population more vulnerable to diseases, environmental changes, or other stressors (Liu et al., 2019)

Modern genomic studies highlight that the domestication process involved extensive interbreeding over thousands of years between domestic stock and their wild counterparts. This interbreeding had various motives, including reducing the need for herders to manage males, addressing challenges in husbandry practices, and perpetuating desirable traits found in wild species (Smith et al., 2022). The implications of these dynamics underscore the complex interplay between domestication, conservation, and the genetic integrity of both domestic and wild populations.

However another threat of extinction in wild population of domestic animals is through genetic swamping which involves the interbreeding between domesticated and wild populations. This may result in the dispersion or erosion of distinctive genetic traits within wild species. This process, known as genetic introgression or swamping, occurs when genes from domesticated individuals flow into the wild population through interbreeding (Rutherford et al., 2019). The species, such as wild yaks, Bactrian camels, wild pigs and jungle fowl, face this risk. When domestic breeds interbreed with their wild relatives, the resulting hybrids may carry genes that are better adapted to the conditions of domestication but might not be well-suited for survival in the wild. Over time, if this hybridization continues, it can lead to the loss of distinct traits and behaviours that are crucial for the survival of the wild population (Redford et al., 2018)

The consequences of genetic swamping can include reduced fitness, adaptability, and overall viability of wild populations. Conservationists and researchers are concerned about this phenomenon because it can compromise the integrity of wild species and their ability to thrive in their natural habitats. Managing and mitigating the risks associated with genetic swamping requires a balance between the conservation of wild populations and the sustainable practices of domestication.

1.7 STUDY SPECIES

The domestic pig (*Sus domesticus*) is usually given the scientific name *Sus scrofa domesticus*, although some taxonomists, including the American Society of Mammalogists, call it *S. domesticus*, reserving *S. scrofa* for the wild boar.

In India, the pig is classified as a livestock species, contributing approximately 1.69% to the overall livestock population (20th Livestock census 2019). The majority of the pig population in the country consists of indigenous breeds, constituting a substantial 76% of the total population. These breeds/varieties exhibit distribution across diverse regions, manifesting distinctive morphometric traits and production parameters. A considerable number of these breeds' remains inadequately characterized using precise scientific methodologies. Characterized by smaller size, limited conservation efforts and selective breeding initiatives have been directed toward enhancing economic traits, including litter size, birth weight, weaning weight, average daily gain, feed conversion efficiency, and carcass traits. These pig breeds exhibit notable adaptation to hot and humid climates, demonstrating heightened disease tolerance (Boro et al., 2016; Banik et al., 2016; Chusi et al., 2016; Danapat et al., 2010). Remarkably, they display prolificacy and adaptability to low management inputs, emphasizing their resilience. In specific regions, the local pig breeds are preferred over exotic and crossbred counterparts due to the perceived superior quality and taste of pork, coupled with their demonstrated disease tolerance. A distinctive attribute is the early maturation observed in some native pig breeds (Kumaresan et al., 2011).

While these pigs may exhibit moderate prolificacy, they display commendable mothering ability, directly influencing the survival rates of their piglets during the early stages of life. The indigenous pig population in India is recognized as a distinctive group, evolving gradually through the domestication process of wild pigs within their respective environments. These indigenous pigs exhibit variations in characteristics and attributes across different regions of the country, influenced by the diverse topographical and climatic conditions prevalent in each area.

A total of eighteen varieties of indigenous pigs have been delineated based on discernible physical attributes and geographical distribution (ICAR- National Bureau of Animal Genetic Resources, www.nbagr.res.in). Of this subset, thirteen varieties have been formally registered that include (Agonda Goan, Doom, Niang Megha, Andaman Local, Ghungroo, Nicobari, Andaman Wild, Golla, Pondi, Ankamali, Lepchamoun, Purnia Desi, Burudi, Mali, Tenyi Vo, Dome, Manipur Local, Zovawk) (Figure: 1.6) and acknowledged as distinct pig breeds through comprehensive classification processes. Nevertheless, there remain numerous non-descript pig varieties that are yet to be systematically classified and identified within the prevailing taxonomy. Through this research and concerted efforts, the genetic characterization of currently unclassified pig populations raised in the specified districts of Uttarakhand will enhance our understanding of the biodiversity within the indigenous pig species in India.

GHUNGROO (WEST BENGAL)

NIANG MEGHA (MEGHALAYA)

AGONDA GOA (GOA)

MALI (TRIPURA)

TENYI VO (NAGALAND)

NICOBARI (ANDAMAN AND NICOBAR)

DOOM (ASSAM)

ZOVAWK (MIZORAM)

GHURRAH (UTTAR PRADESH)

PURNEA (BIHAR AND JHARKHAND)

BANDA (JHARKHAND)

MANIPURI BLACK (MANIPUR)

WAK CHAMBIL (MEGHALAYA)

Figure 1.6: Registered pig breed (ICAR- National Bureau of Animal Genetic Resources).

1.8 LITERATURE REVIEW

Pigs (*Sus scrofa*) display exceptional adaptability, blending their inherent traits and human interactions for survival across diverse environments. Their capacity to thrive in different habitats, diets, and reproductive strategies, paired with their economic and cultural significance, underscores their global prevalence and utilization. Wild boars, belonging to the species *Sus scrofa*, have a long and ancient evolutionary history that predates the domestication of pigs by humans (Larson et al., 2007). Both archaeological and genetic methods have been used in recent publications to study the domestication of pig (Price et al., 2020; Groenen, 2016).

Fossil evidence indicates that wild boars were present during the Early Middle Pleistocene period, around 800-900 thousand years ago. Before the initiation of pig domestication by human wild boar populations had already differentiated and established themselves in mainland East Asia and Europe (Larson et al., 2007; 2010). The process of wild boar dispersal and differentiation is thought to have occurred through natural mechanisms such as migration and adaptation to different environments over a long period of time. These processes shaped the genetic diversity and distribution of wild boar populations across Eurasia.

The domestication of pigs by humans happened independently in multiple regions, with the earliest evidence of pig domestication dating back to around 9,000-10,000 years ago in the Near East and China. Archaeozoological evidence shows that pigs were domesticated independently in western Asia (around 8500 BC) and China (around 6500 BC) (Evin et al., 2017). The domestication of pigs involved the selection and breeding of specific traits for human purposes, leading to the development of distinct domestic pig breeds (Xiang et al., 2017).

The genetic diversity and evolutionary history of wild boars and domestic pigs have intertwined to some extent, as wild boar populations have contributed genetic material to domestic pig populations through hybridization and selective breeding. However, it is important to recognize that wild boars and domestic pigs are separate entities, with wild boars representing the ancestral species from which domestic pigs were derived (Frantz et al., 2012).

Different types of molecular markers can reveal different aspects of the evolutionary and population history of vertebrate species, including domestic animals like pigs. Some of the most commonly used markers are polymorphisms from the mitochondrial and nuclear genomes (Avise, 1994). For example, the control region (D-loop) of the mitochondrial genome is a fast-evolving sequence that can help reconstruct the maternal lineage and biogeographic origin of species (Brown et al., 1979). In the nuclear genome, microsatellite (MS) loci are highly variable and useful for assessing gene flow, population structure, and parentage (Lutkemeier et al., 2009). Single nucleotide polymorphisms (SNP) are another type of nuclear marker that can provide high-resolution and genome-wide information on the genetic diversity and phenotypic variation of species (Rhorer et al. 2007, Gray et al. 2009). While numerous neutral markers contribute to evolutionary and population genetics studies, mitochondrial DNA (mtDNA), microsatellites, and SNPs emerge as the most practical choices for molecular research on domestic animals, especially pigs. This preference is attributed to the availability of primers and assays for laboratory work and the widespread use of these markers in global studies of both wild and domestic pigs (Scandura et al., 2011). The individual contributions of these markers to molecular investigations of pigs and other vertebrate species should be carefully considered.

The use of mtDNA has been very informative for studying the phylogeography, haplotypic variation and evolution of many large vertebrate species such as horses and donkeys (*Equus spp.*), bison (*Bison bison*), tigers (*Panthera tigris*), and deer (*Cervus spp.*) (Cracraft et al. 1998, Randi et al. 2001) and domestic species such as sheep (Rocha et al., 2011), goats (Peng et al., 2022), cows (Edwards et al., 2007). In pigs, mtDNA has helped to identify the centres of domestication in Asia and Europe, the patterns of human-mediated dispersal across continents, and the hybridization events between domestic pigs and wild boar (Giuffra et al., 2000, Gongora et al., 2004, Fang and Andersson 2006, Larson et al., 2005; 2007; 2010; Scandura et al., 2008; Luetkemeier et al., 2010, Fang et al., 2006) hence there is the large availability of a global dataset of published mtDNA sequences.

However, mtDNA also has some limitations that should be considered. MtDNA is a single haploid marker that is only inherited from the mother, which means that it cannot track the movement of male pigs beyond the first generation. Also, it is unclear whether mtDNA reflects the evolutionary history of the whole organism or just the history of an organelle (Ballard and Whitlock 2004). Therefore, the use of mtDNA alone for phylogeographic studies has been criticized, and the use of multi-locus nuclear DNA markers has been recommended (Edwards & Bensch, 2009).

The work conducted by Larson et al. (2005) has laid a substantial groundwork for advancing the study of origins, migration, domestication, and the dispersal of *Sus scrofa* through analysis of mtDNA control region (D-loop) sequences obtained from global wild boar, domestic pig, and ancient samples.

Microsatellites, characterized by short tandem repeats are inherited from both parents, have been widely employed since the mid-1990s for molecular studies in humans and other vertebrate species. In pigs, numerous polymorphic MS markers have been identified, proving useful for various genetic research applications in both domestic and wild forms. These markers have facilitated analyses of population structure, evolutionary relationships, hybridization events, and gene flow between different domestic breeds of pigs at global, continental, and regional scales (Martinez et al., 2000; Fang et al., 2005; San Cristobal et al., 2002; Scandura et al., 2008; Nidup 2011). Therefore, understanding the genetic diversity and phylogeny of indigenous pigs can help in developing conservation strategies, designing effective breeding programs, and formulating management policies (Notter, 1999).

The admiration and importance of the wild boar have persisted throughout ancient times in India (Krishna, 2010; Dornadula, 2007; Math, 2018). For centuries, pigs have been domesticated in diverse regions of India, serving as a vital source of sustenance and livelihood for numerous communities throughout the nation. While the practice of pig rearing has evolved over time, it is important to recognise that the influence of the caste system, which is the oldest form of surviving social stratification, has had a significant impact on how pig rearing is approached and integrated into the cultural and social fabric of India. In India, the practice of pig rearing is primarily linked to the Scheduled Castes and Tribes, allowing them to maintain their social identity and preserve their traditional customs (Stevenson, 1954; Banik et al., 2019). The local swine meat is an essential ingredient in many traditional dishes in India (Govindasamy, 2018) and is very popular within select communities. This highlights the important role that pigs play in the food culture and traditions of certain communities and also offering income and livelihood to the communities that raise them.

While policies are in place to improve the performance of pig farming in India, its overall contribution to the farmed species at the national level is still relatively low at 1.69% (20th Livestock census 2019). This could be due to a variety of factors, including cultural and social factors that limit the practice of pig farming to certain communities as well as limited access to resources and markets. Over the past several years, Indian pig breeds diversity has been studied in detail (Kaul et al., 2001; Behl et al., 2005; Zaman et al., 2013a, b; 2014 a, b,c,d; Sahoo et al., 2015, 2016, 2017; Boro et al. 2019). Hence, a combination of ecological and genetic assessment are necessary to evaluate the pig husbandry practices, phylogeny and genetic diversity of non-descript pig population status in Uttarakhand.

1.9 SIGNIFICANCE OF THE STUDY

Pigs and humans have a long history of interaction in India, dating back to the Upper Paleolithic period when they were depicted in cave paintings at Bhimbetaka (Wakankar, 1973; Dubey, 2014). Lipid profile studies on the diet of the ancient Harappan civilization, one of the oldest in the world, show that pigs made up about 2-3% of animal remains at Indus sites, suggesting they may have been part of the Indus people diet (Suryanarayan et al., 2021). The wild boar in northern India today are descendants of *Sus scrofa* that migrated from ISEA to Asia (Larson et al., 2005). Additionally, the genetic research provides support for separate and independent domestication pathways at the foothills of Himalayas and suggests that there was at least one population of wild boar in India that underwent domestication, from different wild populations in the region (Larson et al. 2005). Recent studies discovered the existence of two novel wild boar clades, in South Asia (Nidup 2011) and also confirm the migration route of the newly discovered wild boar clade in India from ISEA (Choi et al., 2020). However, there is no evidence to suggest that wild boars belonging to these clades have made any maternal genetic contribution to modern domestic pigs. Nevertheless, the distribution and variation of mtDNA lineages in Indian subcontinent have not thoroughly been explored, and the origin and domestication of these pigs remain poorly understood. Hence this study will provide a valuable insight about the presence of a dominant haplotype at the foot hills of Himalayas.

This study stands to significantly benefit the pig sector and the pig-rearing community in Uttarakhand by comprehensively investigating the geographical distribution of pig rearers and profiling their socio-economic status and husbandry practices. The exploration of these aspects serves multiple purposes. It unveils the genetic diversity of the non-descript pig breeds using microsatellite marker and also the dynamics involved in pig production systems, emphasizing their pivotal role in sustaining the livelihoods of pig-rearing communities. The generated profiles facilitate a holistic assessment of the environmental impacts associated with pig rearing, encompassing considerations such as waste generation, disposal practices, and land-use changes. The collected data provides a foundational basis for the formulation of effective policies and interventions geared towards optimizing the social and economic returns from pig husbandry while concurrently mitigating negative externalities. The study plays a crucial role in the ongoing monitoring and containment of pig diseases and zoonotic threats, contributing to the enhancement of animal health and welfare standards of the community that reared pig within the region (Robinson 2014).

1.10 OBJECTIVES AND RESEARCH QUESTIONS

The overall aim of the study is to identify putative global origins and types of pigs (e.g., domestic breeds) raised by the pig rearer in selected regions of Uttarakhand and to develop hypotheses for pathways of dispersal at national and global scale. Another aim will be to evaluate genetic diversity and population structure of domestic breed at the regional scale. The study also examines the pig breeding system and its stakeholders in Uttarakhand, providing insights into the local context. The will serve as an entry point to planned broader-scale characterization and development of pig breeding systems in Uttarakhand.

The objective and research question of the study are as follows:

a. To characterize the genetic feature of non-descript pig breed’s genetic resources using microsatellite markers.

- Does the pig breed raised exhibit significant genetic diversity and heterozygosity?

- Does the breed has any population structure?

b. To examine the exotic pig breeds and problems associated with its use in commercial farming in the selected areas.

- What is the cause for hesitant in adopting the intensive farming of the pig breeds by local livestock keeper?
- What is the overall ecological implication of pig farming in the selected region of Uttarakhand?
- Does the exotic breed has any implication in defining the population structure?
- Does the introduction of exotic pig breeds is leading to potential economic constraints due to high management requirements?

c. To evaluate the phylogenetic relationship of non-descript pig breeds with Indian wild pig (*Sus scrofa cristatus*) and other wild pigs.

- What is the precise phylogenetic position of non-descript pig breeds with respect to other wild and domestic pig breeds available in India and worldwide?

d. To examine the comparative economics associated with locally adapted and improved breeds.

- Does locally adapted pig breeds demonstrate economic advantages over improved breeds?

1.11 HYPOTHESIS

Hypothetical statements for each of the specified objectives in the study of pigs:

a) The non-descript pig breeds of Uttarakhand will exhibit higher genetic diversity, as compared to other indigenous breed and European pig breed.

b) The introduction of exotic pig breeds into commercial farming areas may lead to challenges such as lower adaptability to local environments, increased susceptibility to certain diseases, and potential economic constraints due to high management requirements.

c) Non-descript pig breeds in Uttarakhand share a common phylogenetic ancestry with the Indian wild pig (*Sus scrofa cristatus*) and exhibit distinct genetic relationships compared to other wild pig species lineages.
d) Locally adapted pig breeds demonstrate economic advantages over improved breeds in terms of feed efficiency, reproductive performance, and disease resistance, reflecting the benefits of adaptation to specific local conditions.

These hypothetical statements provide a foundation for testing and exploring the specified objectives in the study, guiding the research toward meaningful findings and insights.

1.12 STUDY AREA

Uttarakhand, the 27th state of India, was established on November 9, 2000, following its separation from northern Uttar Pradesh. Nestled on the southern slope of the formidable Himalayan mountain range, it spans from latitude 28º43'N to 31º27'N and longitude 77º34'E to 81º02'E. The state shares international borders with China (Tibet) to the north and Nepal to the east. Himachal Pradesh lies to the northwest, while Uttar Pradesh borders it to the south.

Geographically, Uttarakhand is divided into two distinct regions: Garhwal, encompassing seven districts (Uttarkashi, Chamoli, Tehri, Pauri, Dehradun, Haridwar, and Rudraprayag) in the northwest, and Kumaon, comprising six districts (Almora, Nainital, Pithoragarh, Champawat, Bageshwar, and Udham Singh Nagar) in the southeast. In total, the state comprises 13 districts. The National Bureau of Soil Survey & Land Use Planning (NBSS & LUP) has categorized Uttarakhand into Agro-ecological regions, broadly classifying them into Plain and Hill Zones. The Plain Zone encompasses the Tarai and Bhavar regions, including Udham Singh Nagar, Haridwar, and parts of Dehradun Districts. In contrast, the Hills Zone includes Uttarkashi, Tehri, Pithoragarh, Pauri, Chamoli, Rudraprayag, Nainital, Almora, Bageshwar, and Champawat.

The entire geographic expanse of Uttarakhand has been divided into four physiographic zones based on mean altitude from sea level (Table 1.1). Domestic animals are distributed across these zones, spanning from the plains to the hilly regions.

Table 1.1: Various physiographic Zone in Uttarakhand (source SAP 2012).

Sl. No.	Physiographic zone	District	Livestock raised
1	Zone A Valley (upto 1000m)	U.S Nagar, Haridwar, parts of Dehradun District	Buffalo, Cattle, goat
2	Zone B Low Hills (1000-1500m)	Parts of Dehradun, Nainital, Pauri Garhwal, Tehri Garwal	Cattle sheep goat
3	Zone C High Hills (1500-2500m)	Uttarkashi, Nainital, Rudraprayag, Pauri-Garwal Bageshwar, Champawat, Almora, Parts of Chamoli	Cattle, Sheep, Goat
4	Alpine Zone (above 2500m)	Parts of Uttarkashi, Pithoragarh, Chamoli	Sheep, goat

No information was found regarding the districts in Uttarakhand where pig farming is prevalent. However, according to the 2012 and 2019 Census of India, the number of pigs raised in various districts of Uttarakhand is presented in the Table 1.2 below.

Table 1.2: District wise pig population in Uttarakhand during census 2012 and 2019.

District Name	**Breed type**											
	Exotic (male+female)						**Indigenous(Desi)**					
	under 6 month		**6 month and above**		**total**		**under 6 month**		**6 month and above**		**Total**	
	2012	2019	2012	2019	2012	2019	2012	2019	2012	2019	2012	2019
Uttarakashi	32	60	41	58	73	118	71	66	91	85	162	151
Chamoli	19	0	15	0	34	0	126	110	188	177	314	287
Rudraprayag	16	0	37	0	53	0	0	18	3	26	3	44
Tehri	107	82	90	121	197	203	170	178	242	242	412	420
Dehradun	588	411	1020	358	1608	769	868	256	1976	667	2844	923
Pauri	49	4	76	3	125	7	261	494	300	591	561	1085
Haridwar	1663	1168	2088	1105	3751	2273	2289	1602	3165	2254	5454	3856
Pithoragarh	12	9	45	54	57	63	80	37	93	61	173	98
Bageshwar	0	0	0	0	0	0	38	65	31	49	69	114
Almora	25	4	54	4	79	8	590	138	366	213	956	351
Champawat	4	0	76	1	80	1	97	26	122	53	219	79
Nainital	354	168	269	228	623	396	130	158	209	225	339	383
UdhamSingh Nagar	4209	2406	333	1871	4542	4277	1006	490	689	747	1179	1753

The table distinctly illustrates that Dehradun, Haridwar, and Udham Singh Nagar, situated in Zone A (Valley up to 1000m) of the physiographic classification, exhibit higher numbers of pig farming. Additionally, the Nainital region is identified as suitable for the development of sheep, goat, and piggery (SAP, 2012).

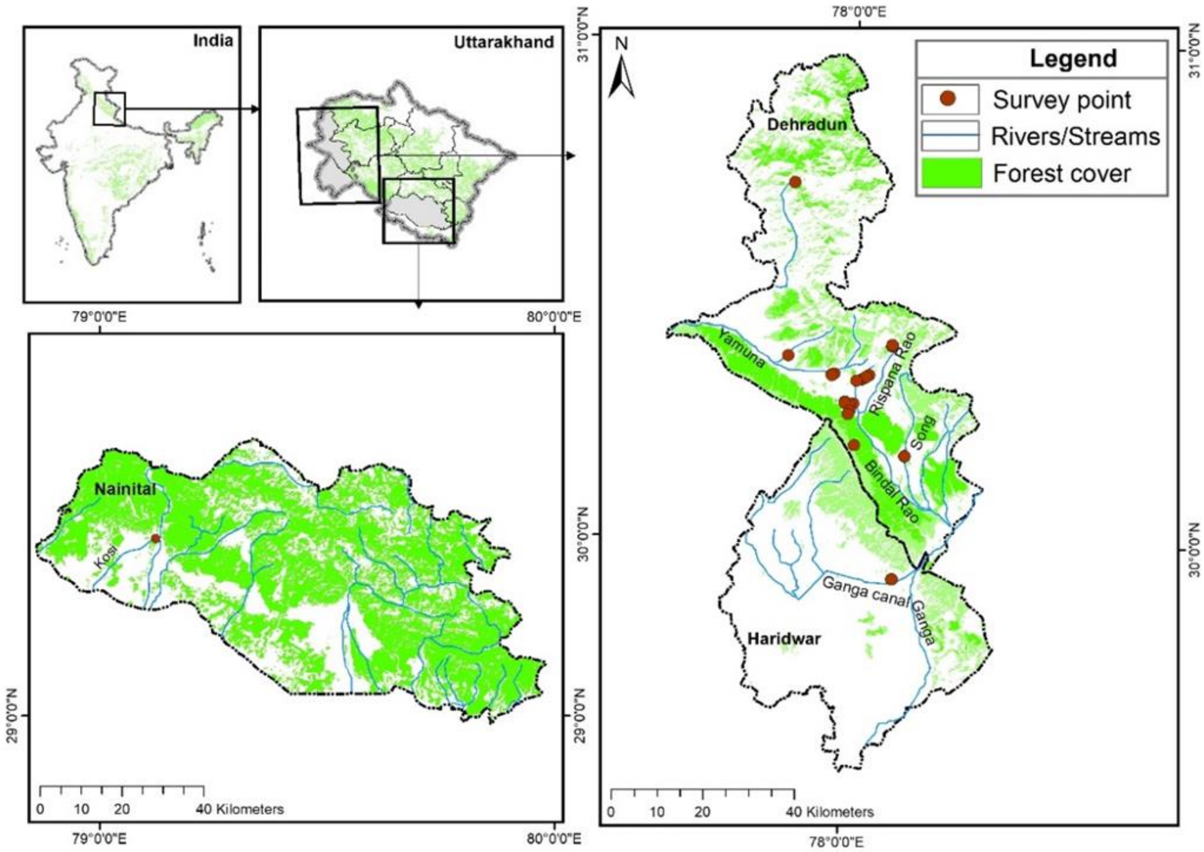

Figure 1.7: Map showing the study districts and the GPS of the surveyed household and the sample collection site.

1.13 ORGANISATION OF THE THESIS

The thesis is organized into five chapters Chapter 1 provides a concise introduction to the systematics of extant Suoidea. This includes an exploration of the origin and evolution of *Sus scrofa*, delving into the emergence of the domestic pig (*Sus scrofa domesticus*). The chapter further investigates the geographical distribution of the scrofic group and feral pigs, as well as the distribution patterns of domestic pigs. Special attention is given to the various threats faced by domestic pigs, drawing on existing literature for a comprehensive review of the study species and role of various molecular markers in its genetic assessment. It also elucidates the significance of the study and outlines its objectives, providing insights into the areas under investigation. Chapter 2 delves into the geographical distribution of pig breeders in a selected region of Uttarakhand, scrutinizing the pig breeding system and providing insights into the local context. This section also evaluates the adaptability of existing pig breeds to diverse environmental conditions in Uttarakhand. Further, it investigates the influence of imported exotic breeds on the diversity of local pig breeds, addressing potential consequences and ecological implications of pig farming in the region. Chapter 3 demonstrates the economic advantages of local non-descript pigs over improved breeds. In Chapter 4, the phylogenetic positioning of pigs raised in Uttarakhand is elucidated, offering a comprehensive understanding within both regional and global contexts. Chapter 5 conducts a genetic assessment of domestic non-descript pigs raised by pig rearers, utilizing nuclear markers to explore their genetic characteristics. Finally, Chapter 6 consolidates and summarizes the research findings.

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CHAPTER 2

CHALLENGES AND OPPORTUNITIES OF EXOTIC PIG BREEDS IN COMMERCIAL FARMING IN THE SELECTED REGION

2.1 SUMMARY

Pig farming has a profound caste-based and cultural association in India. In Uttarakhand, pigs are raised predominantly by the specific community contributing to their livelihood. Unlike other livestock species in the State, the piggery sector is unorganised and the pig farming is not sufficient to meet the increasing demand for pork. This chapter aims to identify the spatial distribution, socio-economic status, and pig husbandry methods of pig rearers, in the planar (Dehradun, Haridwar and Nainital) districts where rapid urbanisation and continuous migration make us reconsider the necessity of an organise promotion of the piggery sector due to its multiple ecological implications. It also examines the types of pig breeds raised in different agro-ecological systems and its impact on the indigenous breed. 28 households were reached by adopting rapid appraisals approach and interviewed by using a semi-structured questionnaire. Analysis showed that 60.7%, 10.7% and 21.4% of the household raised pig near seasonal streams (slum settlements), the residential areas within the house premises and near the forest, respectively. Moreover, 28.6% of pig rearer households' maintained breeding boar of feral origin and 96.94% followed a semi-intensive pig rearing system; hence human settlement is staying in close proximity with pigs (domestic and wild) and other domestic animals. The discussion reveals that the limited contribution of the piggery sector in Uttarakhand is influenced by geographical, cultural, economic, and infrastructure-related factors. The hilly terrain and temperate climate pose challenges for pig farming, with concentration in plain districts. Traditional dietary preferences, capital-intensive nature, and inadequate government support hinder sector growth. 65 individual pigs was identified with 32 classified as exotic/crossbred and 33 as indigenous based on coat colour and rearers recall. Crossbreeding of local sows with wild boars is widespread. Production and management practices indicate that 85.7% follow a farrow-to-finish approach, with 96.94% adopting a semi-intensive pig rearing system. Natural and controlled breeding techniques are predominant, and 100% of respondents practice castration of old boars. The decline in indigenous pig breeds, despite adaptability, signals genetic diversity loss. The low work participation rate within pig rearer community presents an opportunity for targeted support to enhance pig rearing. The semi-intensive system, prevalent among small-scale producers, offers economic stability. However, the rise in slaughtering activities and challenges in market access complicate the sector's dynamics. The study also emphasizes the need for monitoring pig herds, understanding wildlife interactions, and implementing measures to safeguard public health in urban ecosystems. Lastly, the introduction of exotic pig breeds poses potential ecological, genetic, and socio-economic implications, emphasizing the importance of context-specific management strategies. The basic understanding of socio-economic landscapes, geographical distributions, and pig husbandry practices contributes valuable insights for informed decision-making and sustainable pig farming development in Uttarakhand. Before planning for a holistic piggery development program in Uttarakhand, the government may consider this study as a pilot study that can be replicated in larger pig population.

2.2 INTRODUCTION

The modern world is the repercussion of a historical act of the human race, which is the paradigm shift from a hunter-gatherer to food producer based on the domestication of a few wild plants and animal species (Frantz et al., 2020). The domestication events and the sedentary settlement resulted in the formation of societies that embodied different cultures, linguistics, and food habits (Diamond et al., 2003). Unlike the other domesticated animal species, the abundance of domestic pigs (*Sus scrofa domesticus*) in a particular region is influenced by the religion, culture, and tradition for pork consumption. Except for Jews and Muslims, almost all other religions have included pork in their food regime after Pig domestication (Pappas, 2013).

India is a secular country with the coexistence of diverse cultures and ethnic groups and 79.8% of the total population, i.e. 966.3 million people, follows Hinduism (Census, 2011). A significant portion of people's cultural beliefs and customary practices find their roots in Hinduism. Within Hindu scriptures, animals were held in high regard and often elevated to the status of deities based on their qualities and utility. In line with this, the wild boar came to symbolize both food production and fertility, owing to its remarkable ability to till the earth using its tusks (Krishna, 2010). Hindu mythology emphasizes the recurring incarnation of Lord Vishnu in different eras, each time to vanquish evil forces. His third incarnation manifested as Varaha, the wild boar, with the mission of rescuing the earth from the clutches of demons. To this day, numerous temples across India continue to offer prayers and devotion to Vishnu's Varah avatar, underscoring its enduring significance (Krishna, 2010; Dornadula, 2007). Various Indian empires throughout history honoured animals by featuring them on their coins and flags. The Chalukya, Kakatiya, and Vijayanagara dynasties all chose to prominently depict the boar on their currency and banners, as a tribute to this revered creature (Krishna, 2010; Math, 2018). Therefore, the admiration and importance of the wild boar have persisted throughout ancient times.

Pigs have been domesticated for centuries in various regions of India, while the practice of pig rearing has evolved over time, it is important to recognise that the influence of the caste system, which is the oldest form of surviving social stratification, has had a significant impact on the approach of pig rearing practices. In modern India, the longstanding social stratification represented by the caste system has been officially organized by the government into specific groups. These categories include the Scheduled Castes (SC), which consist of historically disadvantaged castes facing social and economic challenges. Certain communities has also been designated as Scheduled Tribes (ST), while other disadvantaged castes are classified as Other Backward Castes (OBC) and the remainder of the population falls under the General Caste category (Sankaran et al., 2017). This official classification sheds light on the intricate social landscape of contemporary India. As a result, in India, the practice of pig rearing is primarily linked to the Scheduled Castes and Tribes, allowing them to maintain their social identity and preserve their traditional customs (Stevenson, 1954; Banik et al., 2019). Despite the upper caste's avoidance of consuming domestic pigs, the consumption of wild boar were favoured by them due to its perception as a clean-feeding animal (Stevenson, 1954). However, it's important to note that Wild boars are listed as wild animals under Schedule III of the Indian Wildlife (Protection) Act, 1972, which means they are protected from hunting and killing, except in certain circumstances. Anyone who consumes or sells the meat of wild boars may face legal consequences, such as fines or imprisonment, according to the provisions of the Act (Wildlife (Protection) Act, 1972). Domestic pigs are recognized as primary contributors to meat production globally. Their natural habitat stretches from the western part of Europe to Eastern Siberia, covering a vast geographical range. The domestication of pigs has its roots in populations of European and Asian wild boars, with independent domestication events occurring in diverse regions of East Anatolia, Europe and China, (Larson etal.; 2005; 2007; 2010; Giuffra et al.; 2000).The domestication timeline reveals that pigs were first domesticated nearby 8500 BC in the Middle East (Asia) and approximately 6600 years ago in Central Europe. The dynamic nature of human activities and changing agricultural practices have brought about substantial alterations in the phenotypic traits of domestic pigs. As a result, domestic pigs exhibit a diverse array of characters, including behaviour, colour, body structure, disease resistance, and reproduction. In India, the pig is classified as a livestock species, contributing approximately 1.69% to the overall livestock population (20th Livestock census 2019). The majority of the pig population in the country consists of indigenous breeds, constituting a substantial 76% of the total population. The major contribution of the pig population in the country comes from the eastern and north-eastern regions, which is around 63%. The highest pig population was in the State of Assam (2.10 million), followed by Jharkhand (1.28 million), Meghalaya (0.71million) and West Bengal (0.54 million) (20th Livestock census 2019).

Despite the existence of numerous domestic breeds the global market's demand for lean fatteners and changes in European agricultural practices, has resulted in a trend toward standardizing genotypes and mass-producing high-yielding breeds. In the current landscape of global pork production, dominance is concentrated in only five domestic pig breeds, Large White Yorkshire, Middle White Yorkshire, Hampshire, Berkshire, and Landrace. These breeds have superior traits such as high growth rate, prolificacy, feed efficiency, carcass quality, and adaptability to various climatic conditions. They are also used for crossbreeding with the indigenous pigs to enhance their genetic potential and performance. This intensification of animal production practices, coupled with the focus on a limited number of breeds, has resulted in environmental challenges. The negative impacts on the environment, coupled with potential sustainability concerns, highlight the need for a careful balance between meeting market demands and preserving the genetic and ecological diversity inherent in indigenous pig populations. The 20th livestock census 2019 in Uttarakhand reported a pig population of 17,659, with indigenous pigs constituting 54.05%, down from 63.72% in the 19th Livestock census 2012. Spread across all physiographic zones of Uttarakhand, the decline raises concerns about the impact on smallholders' food security and economies Genetic analysis in Chapter 2 revealed that Uttarakhand indigenous breeds were genetically distant from European breeds, exhibiting a higher number of alleles per gene locus and greater genetic heterogeneity. The consequential decline in the number of indigenous breeds, attributed to the replacement or crossbreeding with high-yielding exotic breeds, raises concerns about the potential loss of biodiversity in the state. The introduction of exotic breeds, recommended by the National Commission on Agriculture in 1976, aims to enhance overall production performance of Indigenous pig breed. It poses a crucial question for policymakers and pig rearer alike on how to sustainably integrate high-performance breeds without compromising the biodiversity and resilience embedded in the indigenous pig farming traditions of Uttarakhand.

The porcine sector within a given location stands to gain substantial advantages through a systematic analysis of the spatial dispersion of pig rearers, coupled with a comprehensive profiling of their socio-economic status and pig husbandry methodologies. Firstly, such an examination can reveal the diversity and dynamics of pig production systems, highlighting their crucial role in supporting the livelihoods of the pig rearer communities. Secondly, such a profile can enable a holistic evaluation of the environmental consequences of pig rearing, such as waste production and disposal, land-use changes. Thirdly, such data can serve as a basis for designing effective policies and interventions that aim to maximize the social and economic returns from pig husbandry while minimizing negative externalities. Lastly, it plays a pivotal role in the monitoring and containment of pig diseases and zoonotic threats, concurrently enhancing animal health and welfare standards (Robinson, 2014)

Given the limited available data on the pig farming sector in Uttarakhand and its potential impact on growth of pig farming in the context of urbanization, this chapter seeks to achieve the following objectives:

1. It aims to investigate the spatial distribution of individuals engaged in pig rearing within urban districts of Uttarakhand, with a specific focus on delineating the socio-economic

status of these practitioners and understanding the various pig husbandry practices they employ.

2. It further investigates the type of pig breeds and crossbreed prevalent in diverse agro-ecological systems across Uttarakhand and study the challenges and opportunities of exotic pig breeds in commercial farming.
3. Assess the ecological implications on the urban landscape and human settlements that emanate from the pig husbandry practise within Uttarakhand.

2.3 METHODOLOGY

A comprehensive analysis of census data from the 17th to the 20th Livestock Census (GOI) were utilized to examine the changing trend of pig population dynamics in Uttarakhand. The districts for the survey were selected based on the pig population density in each district, as reported in the 2012 livestock census data (Figure 2.1). The districts with the highest pig populations were Dehradun, Haridwar, Udham Singh Nagar, and Nainital, These districts also coincided with the major urban centers in the state, which witnessed rapid urbanization and significant population growth (Sati, 2013).

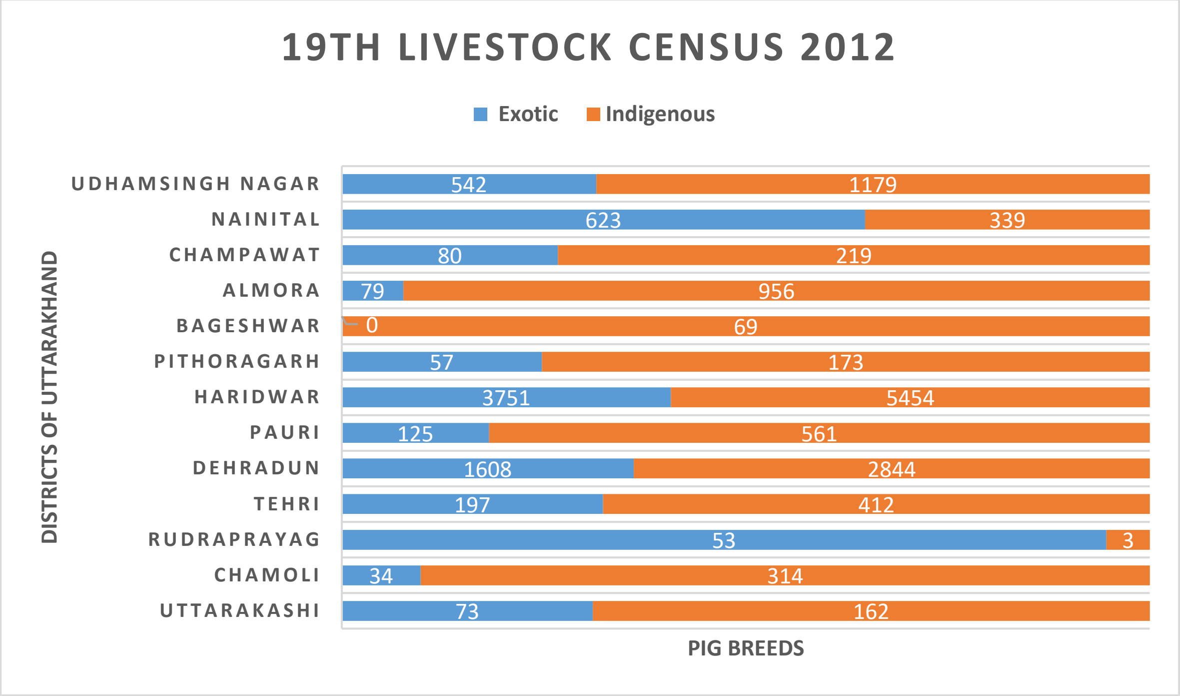


Figure 2.1: District wise population of pig in Uttarakhand (Redrawn from 19th Livestock census 2012).

At the initiation of the investigation, inclusive data concerning pig rearers and their respective locations was absent. Local pig rearers were contacted with the aid of a field assistant. The Rapid Appraisal Approach, recognized as a "survey and monitoring tool" (FAO 2011), was applied to identify and incorporate additional pig-rearing sites in Uttarakhand that had not been previously documented.

Prior to data collection, a semi-structured questionnaire (ILRI, 2011) was prepared in alignment with the study's objectives (Appendix 1). To ensure its effectiveness, a preliminary version of the questionnaire underwent a pre-test involving 5 households in Dehradun. Based on the responses, requirements, and feedback from the participants, the questionnaire was improved. The final questionnaire, administered to the heads of households responsible for pig farming, required approximately 30 minutes for completion. Interviews were conducted in the local language (Hindi). The role of 'key informants' was pivotal in the survey, as they facilitated rapport-building with pig-rearing households. The geographical coordinates of each participating Household were recorded using Android-based Google Maps software.

The sampling methods used in the study involved both random selection to ensure diversity and purposeful sampling to target specific groups of interest, such as pig-owning households near forest areas.

28 households (HH) were surveyed for this study, with the distribution of households being 25 in Dehradun, 2 in Haridwar, and 1 in Nainital. The survey was conducted over the period from March to December 2019, with the exception of July and August 2019 due to the monsoon season. Data pertaining to the socio-economic status of pig rearers and the management practices associated with pig farming were collected, compiled, and subjected to analysis using Microsoft Excel 2007.

2.4 RESULTS

2.4.1 POPULATION TREND OF PIGS IN UTTARAKHAND

Livestock species are distributed across all four physiographic zones of Uttarakhand (SAP 2017). The species-wise contribution of the livestock population in Uttarakhand and its population trend during various livestock censuses of India is shown in (Figure 2.2). The population trend reveals that the piggery sector makes a relatively modest contribution compared to other livestock species in Uttarakhand, and its population has shown a consistent decline in each successive census year.

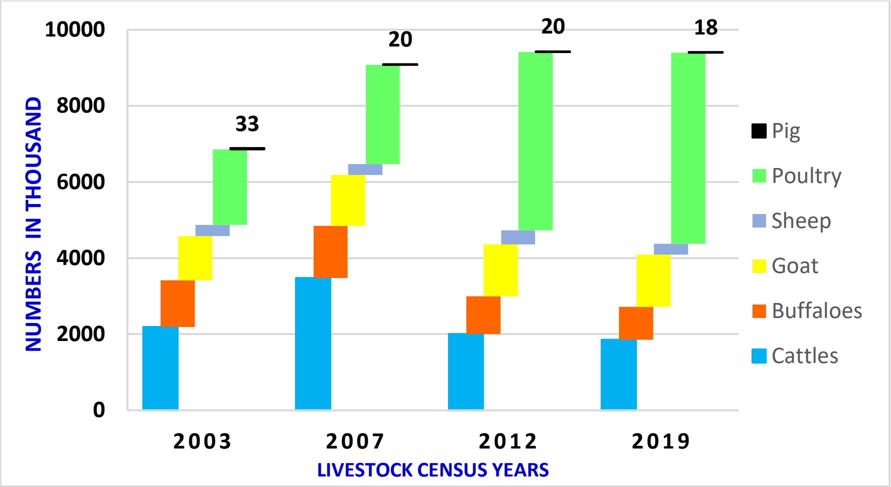


Figure 2.2: Comparative population trend of various livestock species in Uttarakhand from 17th to 20th Livestock census (GOI).

The present pig population in Uttarakhand comprises two main categories: indigenous/non-descript types and exotic/crossbred pigs (Figure 2.3). Census data analysis reveals a notable decline in the population of indigenous breeds since the 17th livestock census, compared with a corresponding increase in the population of exotic breeds. In the high-altitude districts of Rudraprayag, Pauri-Garhwal, Bageshwar, Champawat, Almora, and Chamoli, situated in elevations ranging from 1500 to 2500 meters (High Hills) and above 2500 meters (Alpine Zone) in Uttarakhand, there is a noticeable scarcity or absence of exotic pig breeds (Table 2.1, Figure 2.4)

Exotic pig breeds were introduced in India prior to independence and subsequently integrated into government breed improvement programs with the aim of enhancing economic traits like rapid growth rate, efficient feed conversion, prolific litter size, and improved carcass quality (Deka, 2015). The preference among pig breeders in Uttarakhand is shifting towards exotic breeds, including Landrace, Yorkshire, and their crossbreeds (Breed Survey, 2013).

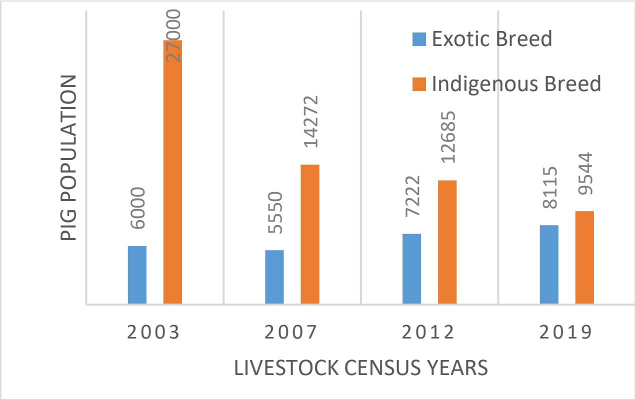


Figure 2.3: Population trend of Indigenous and Exotic breed in Uttarakhand (Redrawn from 17th-20th Livestock census of India).

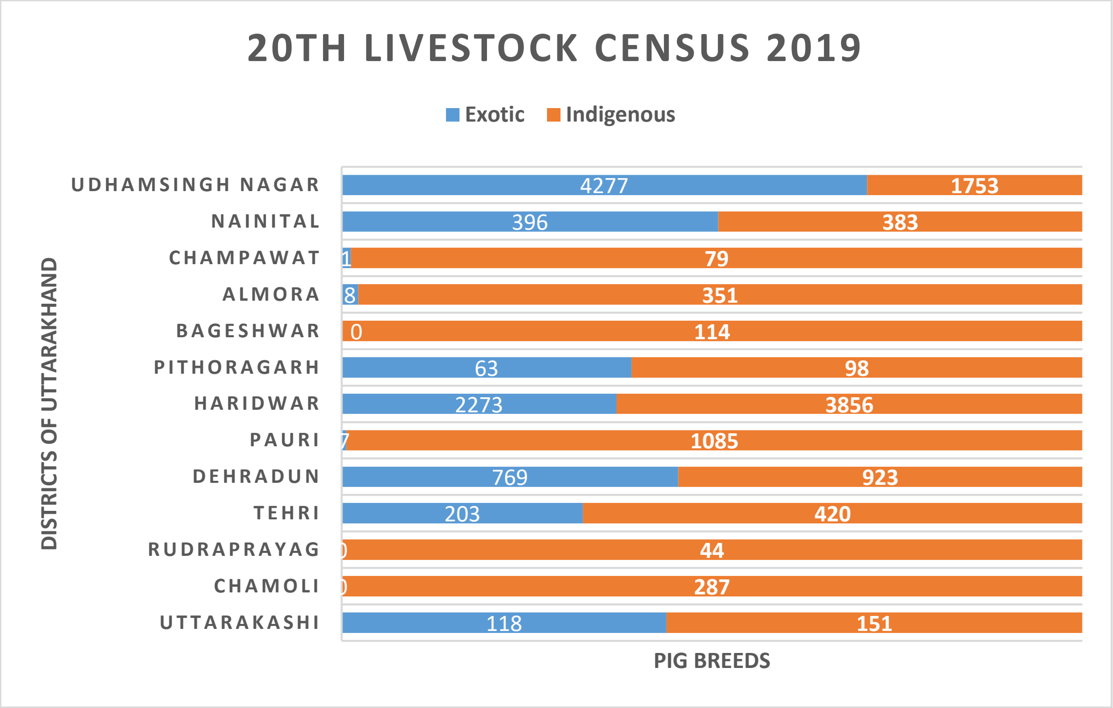


Figure 2.4: District wise population of pig in Uttarakhand (Redrawn from 20th Livestock census 2019).

Table 2.1: District wise population of exotic pig in Uttarakhand (Redrawn from 20th Livestock census 2019)

Districts	Exotic	Indigenous	Total	% of Exotic pig among total pig
Uttarakashi	118	151	269	43.87
Chamoli	0	287	287	0
Rudraprayag	0	44	44	0
Tehri	203	420	623	32.58
Dehradun	769	923	1692	45.45
Pauri	7	1085	1092	0.64
Haridwar	2273	3856	6129	37.09
Pithoragarh	63	98	161	39.13
Bageshwar	0	114	114	0
Almora	8	351	359	2.23
Champawat	1	79	80	1.25
Nainital	396	383	779	50.83
UdhamSingh Nagar	4277	1753	6030	70.93

The pig population in Uttarakhand saw a substantial decrease from 31,000 in the 17th Indian Livestock Census (2003) to 17,659 in the 20th Livestock Census (Figure 2.5). This decline represents a two-fold reduction since the state's formation. In contrast to the declining pig population, it's important to highlight that the annual pig slaughter rate in Uttarakhand consistently exceeds the number of pigs residing in the state (Figure 2.6). This intriguing trend support in-depth investigation and analysis to understand the factors contributing to this apparent disparity between pig population figures and slaughter rates in the region.

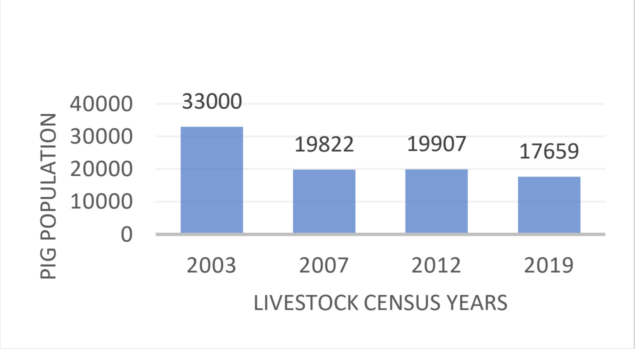


Figure 2.5: Population trend of pig in Uttarakhand after the formation of State (Redrawn from 17th- 20th Livestock census of India).

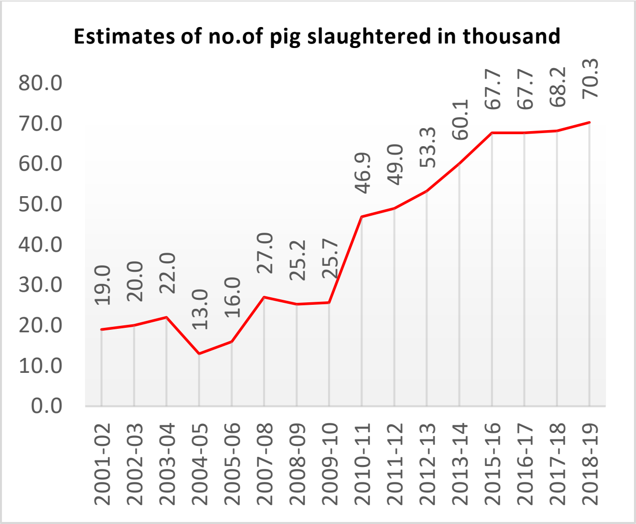


Figure 2.6: Year-wise estimates of the number of pig slaughtered in Uttarakhand (Redrawn from Basic Animal Husbandry and Fisheries Statistics).

2.4.2 MAP

Global Positioning System (GPS) coordinates were initially collected and subsequently mapped using Google Maps. Further cartographic processing and map preparation were conducted using ArcGIS software (Figure 2.7). Our survey results indicate that the distribution of pig-rearing households exhibited a random spatial pattern across Dehradun, Haridwar, and Nainital.

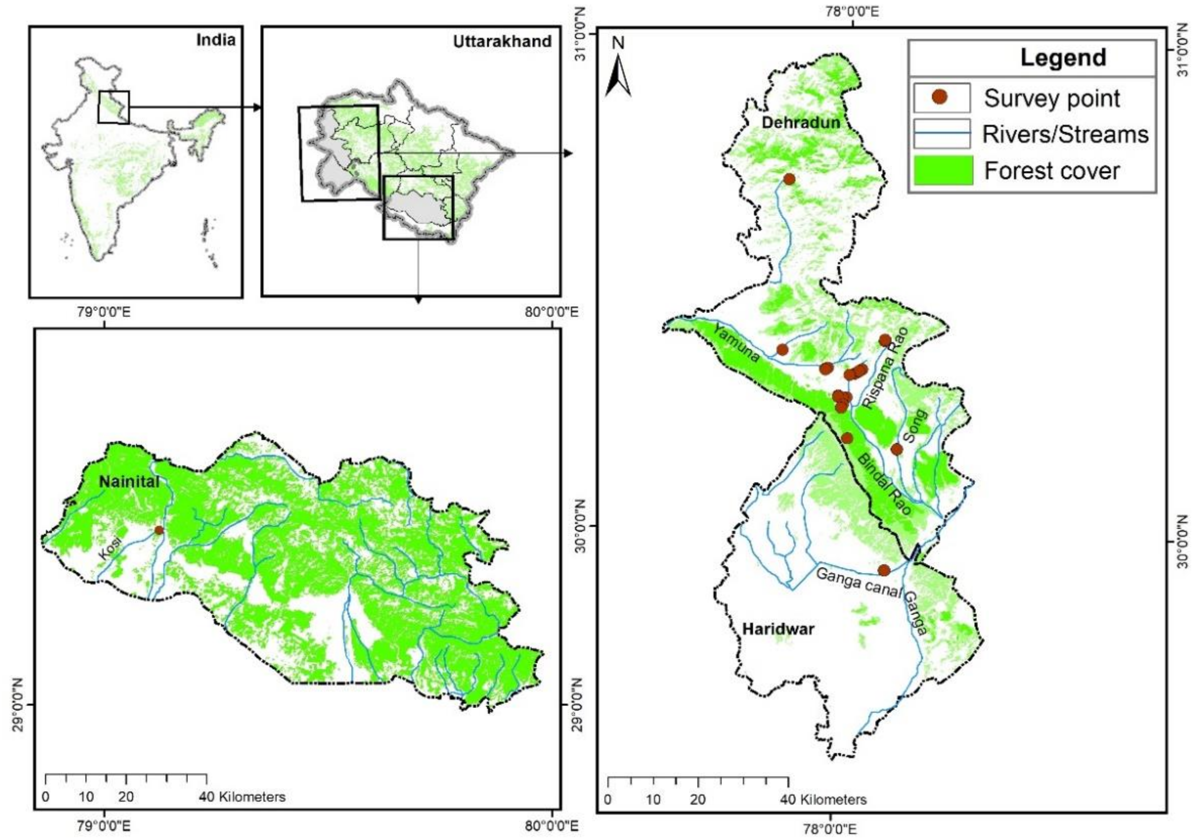


Figure 2.7: Map showing the location of surveyed household in the study area of Uttarakhand.

60.7% of households engaged in pig farming are situated in proximity to seasonal streams or rivers. The household locations are concentrated within areas identified as slums or poverty pockets in these urban districts (PCA slums 2011). In Dehradun, slums are concentrated along the banks of perennial streams such as Bindal and Rispana Rao, which have transitioned from once-freshwater bodies into drainage systems for the city (Dehradun City Development Plan 2007). A parallel trend was noted in the districts of Haridwar and Nainital. In Haridwar, surveyed households were located in densely populated areas of Jwalapur, situated near the Ganga canal (Haridwar City Development Plan 2007). Similarly, in Nainital, slum settlements were observed in areas adjacent to the river Kosi, specifically in the vicinity of Ramnagar (District census handbook Nainital 2011). 21.4% of the surveyed household were engaged in swine husbandry activities within proximity to forest regions. 10.7% of households practiced pig rearing within residential areas characterized by limited open space and establish suitable shelters within the confines of their residences (Figure 2.8)

(a) (b)

(c) (d)

(e) (f)

Figure 2.8: Location of pig rearer (a) (b) proximity to seasonal streams which is now a drainage (c) (d) proximity to forest regions (e) (f) within residential areas.

2.4.3 PIG HUSBANDRY PRACTICES IN UTTARAKHAND

HOUSEHOLD CHARACTERISTICS

The households included in the survey were predominantly under the leadership of male heads, constituting 100% of the sample and exclusively male members assumed the role of the primary respondents. In terms of the age distribution among household heads, 85.7%, fell within the middle age bracket (30-55 years), while a minor proportion, comprising only 4.3%, belonged to the younger age group (20-29 years). 100% of the surveyed household belonged to the Scheduled Caste community, with specific affiliation to a particular caste.

The highest education level attained by the household heads was primary for half of the sample (50%), followed by secondary for 46.4%, while only 3.6% had no formal education. The majority of the households (89.29%) had a nuclear family structure, with 3-6 members per household, while 10.71% had a joint family structure, with more than 6 members per household.

All the surveyed households had solid construction houses made of bricks and cement. They also had access to water supply from hand pumps or other sources and electricity supply. Television was the only source of entertainment for all the households (100%). Pig rearing was the primary activity for only 21.4% of the households, while the rest (78.6%) had other odd jobs and reared pigs in small herds. 64.3% of them worked as sweepers in various institutes and municipal corporations. As evidenced by our survey data, urbanization and improved community literacy have led to occupational diversification, with 10.71% of individuals pursuing careers in the armed forces and 3.6% in miscellaneous professions.

For livelihood sustenance, some households also participated in additional activities such as ceremonial music, poultry farming, and engagement in sporting events involving game animals like cockfighting and pigeon flying. None of these households possessed agricultural land for cultivation in alternative sectors.

LIVESTOCK INVENTORY

The survey revealed that chicken farming was a popular livelihood activity among the households, with 39.29% engaged in it. 25% of the household also kept cattle for milk production. Some households had a penchant for pigeons and wild cocks, which they used for gambling and betting purposes. The households also raised pigs of various breeds and sizes, usually having 2-4 female pigs and 1-3 male pigs per herd. The pigs were either indigenous, crossbred with exotic breeds, or suspected hybrids with wild boars. The hybrid pigs with wild boars were especially valued for their high market demand and easy accessibility (Figure 2.9(b)). Interestingly, 28.6% of the pig rearers secretly maintain breeding boar of feral origin, which could be the result of natural or intentional hybridization events with wild boars. However, to confirm the origin and identity of these piglets, further analysis using molecular genetic techniques is required.

TYPES OF PIG BREED PRESENT IN THE PIG HERD

Due to the absence of comprehensive documentation of the pig breeds present in the herd, the categorization of breeds was done either as indigenous (includes Local X Wild, Local) or crossbred/exotic. A total of 65 animals were identified, with 32 classified as exotic/crossbred and 33 as Indigenous. The Local X Wild and Local emerged as the predominant breeds, followed by the Exotic and their crossbreds were prevalent on smallholder farms.

The indigenous/local pig was predominantly found in suburban areas or regions distant from urban settings. In the mountainous region of India, indigenous breeds were kept throughout the seasons, while exotic piglets were raised during summer, reaching adulthood for eventual slaughtering. All surveyed household maintained exotic/crossbred pigs, and those in proximity to forest areas raised them from weaning to fattening due to a peculiar disease that causes recurrent abortion among female pigs.

Crossbreds were favoured for their taste in local tourism, while local people generally preferred indigenous breeds due to their lower fat content. Several composite breeds were observed, but the breeding history, genetic makeup, or breed standardization of pigs in the study area has not been achieved. Exotic pigs, including Yorkshire and Landrace, were introduced sporadically by pig rearers. Uncontrolled breeding and extensive management led to intermingling among exotic and local pigs, resulting in significant diversity in the pig population as observed in Chapter 5.

In various pig farming systems and regions of Uttarakhand, different breeds serve distinct purposes, each tailored to meet specific agricultural needs. The local breed, renowned for its suitability in both fattening and as reproductive sows, is predominantly raised in semi-intensive environments within planar and mountainous districts. This breed is characterized by a medium litter size, with its meat flavour highly favoured by the local population. The local breed exhibits excellent mothering abilities, contributing to its popularity among rearer (Table 2.2). A hybrid variety resulting from the crossbreeding of the local and wild pig, also finds its place in semi-intensive farming, particularly in planar and mountainous districts. This hybrid demonstrates a large litter size and is sought after for its distinctive meat flavour, aligning with the preferences of the local community. These hybrids exhibit commendable mothering abilities, reinforcing their value in pig farming systems. On the other hand, exotic pig breeds are primarily raised for fattening purposes in semi-intensive settings within planar districts. These breeds possess a moderate litter size and are favoured for their meat flavour, catering specifically to commercial interests. A crossbreed between exotic and local pig varieties also plays a role in semi-intensive farming within planar districts, serving the dual purpose of fattening and reproduction. This hybrid exhibits a medium litter size, aligning with the requirements of commercial enterprises. The meat flavour of this crossbreed caters to the preferences of the broader consumer market, making it a valuable asset in the pig farming industry.

Table 2.2: Pig breeds types and its husbandry practises

Breeds	Purpose of keeping pig	Prevalent systems and zones	Note
Local	Fattening and reproductive sows	semi-intensive/ planar and mountainous districts	Medium litter size, meat flavour preferred by local, good mothering ability.
Local x Wild	Fattening and reproductive sows	semi-intensive/ planar and mountainous districts	Large litter size, meat flavour preferred by local, good mothering ability.
Exotic	Fattening	semi-intensive/ planar districts	medium litter size, meat flavour preferred for commercial purposes
Exotic x Local	Fattening and reproductive sows	semi-intensive/ planar districts	medium litter size, meat flavour preferred for commercial purposes

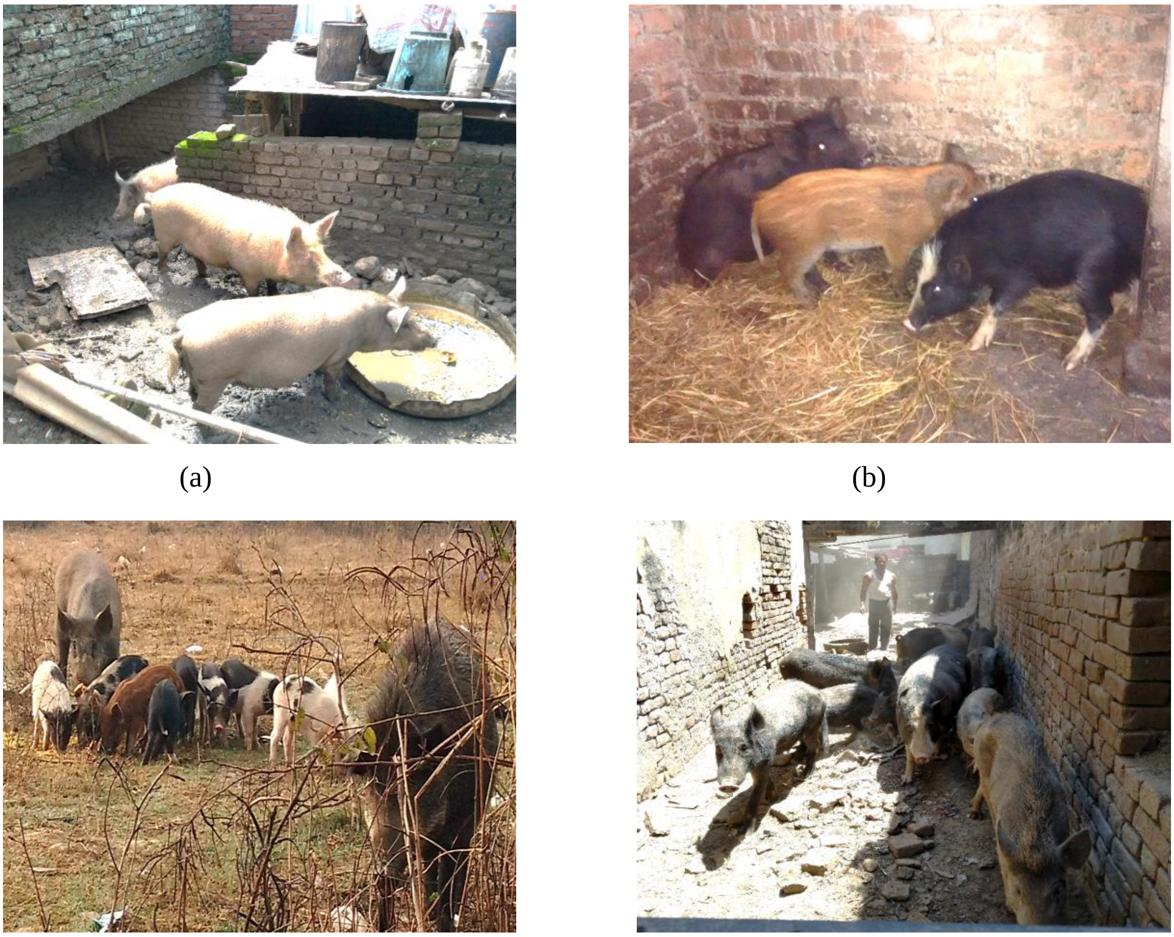
PRODUCTION AND MANAGEMENT OF PIG

A prevalent production system observed among 85.7% of the surveyed households entails a combined approach of farrow-to-finish, where piglets born within the household herd are nurtured until they reach slaughter age, and piglet production, wherein multiple sows are maintained to produce piglets for subsequent sale. A smaller proportion, constituting 14.3% of households, engaged in a fattener production system, acquiring piglets from external sources and raising them for eventual slaughter.

The predominant rearing pattern in the study area was characterized as a semi-intensive system, adopted by 96.94% of the households. The semi-intensive pig rearing system combines aspects of both intensive and extensive methods (Figure 2.9 a and c). Pigs were housed in enclosures during night and have outdoor access for foraging throughout day. They're fed a mix of commercial feed, kitchen wastes, and by-products, and the rearer contributes in managing the health, including treatment for the disease, deworming, and breeding of the pigs.

A few households, around 3.5%, used an extensive system. The extensive pig rearing system permits pigs to roam freely in natural or semi-natural settings rearer and apart from foraging in natural environment their diet were supplemented with limited grains, vegetables, or kitchen waste. Minimal care was provided by the pig rearer. Nearly all pig farmers in the study area supplemented their herds' diets with swill, sourced from restaurants and kitchens, incurring an average monthly cost of INR 500 (approximately 6.57 USD). Feeding and watering were administered twice daily to the household herds adhering to the combination production system. During the winter season, warm water were incorporated into the pigs' food to mitigate cold exposure. The pig rearers also did not employ measurements to quantify the feed quantities provided to their pigs. None of the households used girth tethering for their pigs, which was a method of restraining them with ropes around their chests.

Animal health services were accessible with a consultation fee of INR 15 (equivalent to 0.20 USD); however, no veterinary doctors were available to visit the sick pigs at their farms. All surveyed households unanimously reported the absence of vaccination and treatment services for ailing animals by the government employed animal service provider. Common health issues observed among pigs encompass reduced appetite, classical swine fever, nail disorders, diarrhoea, worm infestations, and foot-and-mouth disease.



(a) (b)

(c) (d)

Figure 2.9: Herd structure and pig management practises (a) Semi-intensive system of pig management with housing facility (all the pen is made of brick with tin as a roof within the house premises) (b) Possible hybridised feral origin or boar piglet for sale or mating purpose (c) Pig herd of mixed parental origin with extensive system of pig management with no housing facility (d) Pig herd with indigenous breed in Jwalapur (Haridwar).

REPRODUCTION, BREEDING AND BREED PREFERENCE

The predominant method of pig reproduction was through natural and controlled breeding techniques. Breeding males were typically sourced from local farms or nearby establishments, occasionally even from wild relatives. Notably, all respondents (100%) reported the practice of castrating old boars, a procedure carried out using traditional indigenous methods. These castrated pigs were in demand for religious sacrifices during festivals and ceremonial events. The indigenous pigs in the study area have two farrowing cycles per year, with an average litter size of 6 to 8 piglets per cycle. The piglet survival rate is about 80%. A notable aspect was that all respondents (100%) prioritize specific traits, such as mothering ability, reproductive performance, and disease tolerance, when selecting females for retention within their herds for future breeding purposes. These qualities take precedence over general appearance and scavenging ability in the evaluation of female pig quality by herd owners.

MARKETING AND ANNUAL INCOME

The majority of pig farmers, constituting 100% of the respondents, report annual family incomes within the range of INR 30,000 to 1.0 lakh (equivalent to 394 USD to 1313 USD). Adult pigs were sold live, with selling prices ranging from INR 3500 to 4000 (approximately 45 USD to 52 USD), while piglets command prices in the range of INR 2500 to 3000 (approximately 32 USD to 39 USD). These animals find buyers among traders, butchers, and at local village animal fairs. The prices depend on the weight, age, breed, and health status of the pigs.

The majority (100%) of pig farmers had a family income ranging from INR 30000 - 1.0 lakh (394 USD-1313 USD) per annum. The selling price of the live adult pig range INR 3500-4000 (45-USD -52 USD) and piglets INR 2500-3000 (32 USD- 39 USD). The animals were sold to the traders, butchers and the village animal fair. The slaughtering of pigs was done by other communities who had expertise in this activity. The meat was inspected by veterinary professionals in registered slaughterhouses before being sold in shops at INR 180 to 200 (about 2 USD) per kilogram. In cases where direct sales of live animal to consumers occur, such inspections are typically omitted.

LIMITATIONS TO PIG-KEEPING

In the state of Uttarakhand, pig husbandry practices exist within an unstructured and unorganised framework. All surveyed households unanimously identified the rapid expansion of infrastructure and the mounting pressures of administrative regulations as prominent challenges affecting their pig-rearing endeavours. Some families expressed concerns regarding conflicts with neighbours who adhere to Islam as their religion, revealing a potential cultural clash in the context of pig keeping.

It is intriguing that none of the households cited diseased conditions among their pigs as a significant constraint. This observation could be attributed to the prevalent practice of maintaining mixed parentage herds, which combine indigenous and wild breeds. This hybridization appears to confer a degree of resilience to climatic conditions and necessitates relatively lower levels of management inputs, potentially contributing to the absence of disease-related concerns among the pig-rearing communities.

2.5 DISCUSSION

The relatively limited contribution of the piggery sector in Uttarakhand could be attributed to a combination of geographical, cultural, economic, and infrastructure-related factors. The hilly terrain and temperate climate of Uttarakhand are less favourable for pig farming, as it requires additional care and resources to ensure the well-being of the animals (Riedel et al., 2012; Pietrosemoli et al., 2020). The environmental and ecological challenges of pig rearing in the hilly and forested regions of Uttarakhand, increases the risk of disease outbreaks and conflicts with wildlife and humans. As a result, a majority of pig farming activity were concentrated in the planer or partially planer districts of Uttarakhand, including districts such as Udham Singh Nagar, Dehradun, Haridwar, and Nainital. Moreover, the traditional dietary preferences of the majority of the population in Uttarakhand is influenced by cultural and religious factors, tend to lean towards non-pork protein sources.

Pig farming is a capital-intensive endeavour, necessitating substantial investments in infrastructure, feed, and healthcare. Currently, there are two government-run pig farms in Uttarakhand (SAP 2017). Unfortunately, these facilities have not made significant contributions to the promotion of pig farming and have remained non-functional for several years. In Uttarakhand dairy farming dominates the livestock sector, pig farming faces stiff competition for resources and investments. The presence of specialized infrastructure and veterinary services customized for pig farming in Uttarakhand are often limited, impeding the sector's capacity for growth. This lack of support systems can act as a bottleneck for its development. Finally, market dynamics play a crucial role. Due to its unorganised sector the challenges related to market access and distribution channels further compound the difficulties faced by the sector in the state. The existing policies governing pig farming in Uttarakhand are insufficient to foster the sector's growth. The socio-economic status and livelihoods of the pig rearers is dismal who mostly belong to the scheduled caste communities and face discrimination and stigma from the society.

Some of the respondents in our survey painted a bleak picture of pig rearing. They expressed a desire to discontinue this practice if they could secure employment yielding a monthly income of 15,000 INR (approximately 197 USD). They voiced their concerns regarding the challenges associated with administrative procedures and the social stigma attached to pig rearing, which they found burdensome. They also conveyed that their offspring prioritized education over traditional pig rearing practices and held the perception that pig rearing was considered unclean within their social circles.

The declining population trend observed in the piggery sector of Uttarakhand can be attributed to various factors. One significant factor is the process of rapid urbanization occurring within the surveyed districts. This urbanization trend has led to the reduction of available open land areas due to extensive construction activities. In Uttarakhand, pig farming has traditionally followed semi-intensive management practices, which allowed pigs to roam freely and forage in refuse heaps. However, in line with governmental efforts to maintain sanitation standards in urban districts, municipal authorities have undertaken the removal of free-roaming pigs from public areas. Consequently, pig rearers have been compelled to confine their pig farming operations to smaller-scale herds. Another potential factor is the expanding availability of alternative employment opportunities, diverting individuals away from pig farming. Furthermore, there is a rising social stigma associated with pig farming, which could further deter its adoption and practice.

Indigenous pig breeds are distributed across all districts thus exhibit remarkable adaptability to the diverse geographical landscapes of Uttarakhand, encompassing both lowland plains and hilly terrains. However, a notable decrease in indigenous breed numbers, contrasted with a rise in exotic breed populations has been noticed. The substantial decline in the indigenous pig population necessitates immediate attention from the government, especially in light of the ongoing scientific investigations conducted by the research community over the past two decades. These investigations have been focused on elucidating the origin and genetic relationships between indigenous pig breeds and wild pig populations in the Hindukush Himalayan region (Larson et al., 2005; Nidup, 2006; Tanaka et al., 2008).

The population trends observed within the piggery sector in Uttarakhand hold significant implications across various domains. Firstly, the decline in indigenous pig breeds raises concerns about the potential loss of genetic diversity within the region's pig population. This decline could have lasting consequences, impacting the adaptability and resilience of these pigs to local environmental conditions and diseases. Moreover, the decreasing prominence of the piggery sector in comparison to other livestock species suggests that though it may not be a major driver of the local economy, but it is potentially affecting the livelihoods of pig farmers and their communities. The increase in exotic pig breeds hints at a shift towards more commercially-oriented pig farming practices, often favoured for their higher growth rates and productivity, thus departing from traditional, indigenous pig farming methods. This situation underscores the importance of conservation efforts to preserve indigenous pig breeds through breeding programs and initiatives. Policymakers may also need to address the challenges facing the piggery sector, offering support to pig farmers and promoting sustainable pig farming practices in the region.

The observed population trends within the piggery sector in Uttarakhand have far-reaching implications encompassing genetic diversity, economic contributions, production practices, food security, conservation efforts, and policy considerations. Addressing these implications necessitates a holistic approach that strikes a balance between economic development, the preservation of local genetic resources, and the promotion of sustainable farming practices. Moreover, it is crucial for the government's piggery development policies in Uttarakhand to place significant emphasis on indigenous pig breeds due to their remarkable adaptability to both plain and mountainous districts. Therefore, a rigorous documentation and phenotypic characterization of indigenous pig breeds and their respective production systems become pivotal in making informed decisions regarding their promotion and conservation. Initiating a comprehensive breed study in Uttarakhand, characterized by the utilization of appropriate scientific tools to assess morphometric traits and production parameters, can provide valuable insights and inform evidence-based policy decisions in the realm of piggery development within the region

The population dynamics of pigs in Uttarakhand unveils another notable paradox: despite the unorganized nature of the sector, there has been a consistent and substantial increase in the slaughtering of live pigs over the past decade, exemplified by a continuous upward trajectory in pig slaughtering activities. The local supply of live pigs falls short of meeting this escalating demand. This intriguing dichotomy stems from a multitude of intertwined factors. One probable explanation for the heightened demand for pork and its products is the growing appetite for pork-based cuisine. Pork dishes have steadily become regular offerings on the menus of numerous hotels, restaurants, and institutions throughout Uttarakhand. Consequently, to satisfy consumer preferences, a greater number of pigs are being processed (Banik et al., 2019). The surge in demand for live pigs and the subsequent increase in the number of pigs being slaughtered can also be linked to a higher rate of pig slaughtering, which, in turn, coincides with a noticeable decline in the pig population.

The results of the socioeconomic status analysis suggest a significant correlation between a specific community categorized within the Scheduled Caste (SC) of Uttarakhand and pig farming practices within the region. According to the study, this particular community exhibits a significant urban concentration, with approximately 70.64% of its population residing in urban areas (PCA SC, 2011). Interestingly, this SC community is predominantly concentrated in four specific districts: Haridwar, Dehradun, Udham Singh Nagar, and Nainital. These four districts collectively account for a substantial 86.7% of the total population of this SC community in the state (PCA SC, 2011). These same four districts are also characterized by a significant pig population (Figure 2.4). This alignment in the geographic distribution of the SC community and the pig population within these districts highlights an intriguing link between the community's residence patterns and the prevalence of pig farming.

The data also suggests that despite the high degree of urbanization within this specific community, their economic status remains distressingly disadvantaged. Our research findings reveal that a significant portion, specifically 60.7% of households, are engaged in pig farming within close proximity to slum settlements characterized by suboptimal sanitation conditions. As a consequence of these challenging economic circumstances, many households within this community opt for nuclear family structures as a strategy to avoid the financial burden associated with accommodating additional members (Saggurti et al., 2005).

In the context of Uttarakhand, it is noteworthy that the pig-rearing community continues to be primarily identified by their historically caste-based occupation and also exhibit caste-based spatial segregation (Sidhwani, 2015). A substantial (64.3%) of household heads surveyed were employed as sweepers in various institutes and municipal corporations (Ganguly, 2019). This highlights the persistence of occupational patterns rooted in caste affiliations, shedding light on the socioeconomic challenges faced by this community despite their urban residence.

In comparison to other Scheduled Caste (SC) groups, this particular community demonstrates the lowest work participation rate, which stands at a mere 33.49% (Census data 2011). This low work participation rate indicates that a significant proportion of the community is not actively engaged in economic activities. In essence, the low work participation rate can serve as a platform for promoting and enhancing pig rearing practices within the community. By providing targeted support, training, and resources, it's possible to harness the potential of those who are not actively participating in other economic activities and channel their efforts towards sustainable and productive pig farming, thereby improving their economic prospects and overall well-being. Moreover, the survey also suggest that the female involvement in piggery enterprises is limited. Creating an inclusive and supportive environment that encourages and enhances female participation in pig farming not only empowers women economically but also contributes to the sustainable growth of the pig farming sector in urbanized communities (FAO, 2001).

The household survey findings about the pig husbandry practices revealed a prevalent adoption of the semi-intensive system among the participants for pig rearing. This particular approach, characterized by its low resource requirements, is especially favoured by small-scale producers. And can serve as a valuable source of income and livelihood for these communities, fostering economic stability and poverty alleviation.

By taking into account the intricate interactions among various factors, including geographic location, the socio-economic circumstances of pig farmers, and the specific pig husbandry methods employed, a comprehensive assessment of the status of pig farming has been effectively conducted. Moreover, to advance the piggery sector in Uttarakhand, it is imperative to gain a thorough understanding of its ecological implications within the urban districts of the region.

Hence to comprehensively evaluate the ecological repercussions of pig rearing practices within urban zones of Uttarakhand, two additional facets warrant consideration. These pivotal aspects includes (1) the ongoing pace of rapid urbanization within Uttarakhand and (2) the extent of contact between wild boars, humans, and domestic animals.

Urbanization in Uttarakhand plain districts has accelerated due to infrastructure expansion, influx of migrants, tourism, and increased commercial activities. Uttarakhand has experienced a rapid pace of urbanization, particularly in its plain districts. This urbanization has been driven by factors like infrastructural growth, an influx of migratory populations, a continuous stream of tourists, and various commercial activities. However, much of this urbanization has been unplanned, leading to challenges in urban development. The existing natural resources and ecology in these districts have been stretched to accommodate these activities, including the increasing number of livestock populations, often surpassing the region's carrying capacity. Unplanned urbanization has significantly contributed to the proliferation of slum settlements along the banks of perennial streams such as Bindal and Rispana Rao in Dehradun. This pattern has been observed in Haridwar and Nainital districts as well. Across these districts, human populations reside in close proximity to pigs and other domestic animals. Moreover, the geographical placement of pig rearers amplifies the likelihood of disease outbreaks, whether stemming directly from the animals themselves or resulting from the contamination of urban water bodies.

Our research findings reveal that a noteworthy 60.7% of households in these slum settlements engage in pig farming, often under conditions characterized by inadequate sanitation facilities. Consequently, these areas are at heightened risk for both zoonotic and non-zoonotic diseases. Additionally, the close proximity of humans to pigs residing in these unhygienic environments presents a dual risk, impacting the health of both animals and humans (Alirol et al., 2011; Ahmed et al., 2019).

Uttarakhand boasts a remarkable geographical profile, featuring extensive forestland covering 45.44% of its total area. The urban districts of Dehradun, Udham Singh Nagar, Nainital, and Haridwar collectively account for a substantial 23.32% of this expansive forest cover (State of Forest Report, 2019). However, the surge in urbanization has ushered in an era of rapid deforestation, primarily driven by construction activities. This rampant deforestation has amplified the likelihood of close encounters between wild boars, human and domestic animal populations. Intriguingly, our research findings revealed a noticeable reluctance among all surveyed households when approaching the topic of wild boars as Wild pig is a protected species under Schedule-III of the Indian Wild Life (Protection) Act, 1972. However, few members of the local community disclosed that the hunting of wild boars and the consumption of their meat constitute prevalent practices within Uttarakhand. these dietary preferences are not uniform across all social strata, while Brahmins tend to abstain from such indulgence, Pahari Rajput and other castes savour the meat due to its reputation as a clean-feeding animal, coupled with the attraction of its low-fat content (Sethi et al., 2010). This interplay between wildlife, culture, and human practices underscores the complicated dynamics at play within the Uttarakhand landscape.

Wild boars serve as reservoirs for a diverse array of viruses, bacteria, and parasitic pathogens that have the potential to transmit to humans, particularly through the consumption of their meat or close contact with domestic animals (Meng et al., 2009). Our survey data indicate that 21.4% of the households were engage in pig farming in proximity to forested areas. In this context, domesticated pigs adopt a diurnal pattern, foraging in the forest during the day and returning to their enclosures at night. Some households also incorporate wild piglets into their herds, due to its ready availability, resilience to diseases, desirability as a meat source, and recognized medicinal attributes (Namusisi et al., 2021). Consequently, this deliberate inclusion amplifies the probability of dynamic interactions occurring between wild boars, domestic swine, and human communities. These interactions underscores the heightened risk of pathogen transmission between wild boars and the human population within Uttarakhand's densely populated districts. Although documented instances of disease transmission from wild boars to humans in Uttarakhand remain limited. A report of multiple outbreaks of 'Human Trichinosis' were reported in the Tehri and Pauri Garhwal districts of Uttarakhand during 2009-2011. These outbreaks were attributed to the consumption of undercooked wild boar meat contaminated with larvae of the nematode parasite *Trichinella sp.*, (Sethi et al., 2010). The endemic presence of Swine flu, a member of the influenza virus group in pigs, continues to be a concern, with annual reports of cases. A higher incidence of patients are reported from urban areas of Uttarakhand, owing to congested infrastructure and suboptimal hygiene conditions, at the pig rearing (Pandita et al., 2021). These findings underscore the need for heightened vigilance and proactive measures to mitigate the risk of zoonotic disease transmission in this dynamic ecosystem.

This oversight poses a heightened risk of both zoonotic and non-zoonotic diseases for populations residing in close proximity to slums and informal settlements. Such settlements can potentially serve as epicentres for infectious disease outbreaks, particularly due to the presence of roaming pigs, which can increase the likelihood of zoonotic disease transmission. One aspect often overlooked in urban development planning by the government is public health policy. The recent enforcement of one health policy might improve the control of zoonotic diseases in the future (Mackenzie et al., 2019). Over the past thirty years, it has become increasingly evident that the majority of newly emerging zoonotic infectious diseases have their origins in animals, particularly wildlife (Taylor et al., 2001). Moreover, it is clear that the main catalysts for their emergence are linked to human activities. These activities encompass various factors such as alterations in ecosystems and land use, the intensification of agriculture, urbanization, and the expansion of international travel and trade (Daszak et al., 2001). To mitigate these risks, strict monitoring of pig herds and their foraging activities is imperative. A broad understanding of the interactions between wildlife, livestock, and human populations, coupled with meticulous surveillance, should be a central focus of the Uttarakhand government to prevent the emergence and spread of diseases. Such proactive measures are essential in averting the potential spill over of pathogens from the wild reservoirs (Wu et al., 2012 and Hassell et al., 2017). These strategies play a critical role in safeguarding public health by mitigating the risks associated with zoonotic disease transmission in urban ecosystems.

The introduction of exotic pig breeds into commercial farming in Uttarakhand is a venture marked by both challenges and opportunities. Uttarakhand's diverse topography, ranging from plains to mountainous terrain, poses a significant challenge in terms of the adaptability of exotic pig breeds to the unique environmental conditions. The success of such integration hinges on meticulous management practices to ensure the health and productivity of these breeds across varied landscapes. Exotic pig breeds may have different meat flavors and characteristics compared to the local breeds traditionally favoured in Uttarakhand. Overcoming this challenge involves strategic marketing efforts and consumer education to promote the qualities of exotic pig meat and align it with the preferences of the local population.

The economic viability of exotic pig farming in the region depends on the willingness of consumers to embrace and pay premium prices for products associated with these breeds. Another challenge is that these breed may require more inputs and management than the local and hybrid pigs, such as feed, water, housing, and veterinary care, which may increase the cost of production and reduce the profitability of the pig rearer. It may pose a risk of introducing new diseases or genetic traits that may affect the local and hybrid pigs, as well as the biodiversity and ecosystem of the region. Another aspect discuss here is the introduction of exotic pig breeds within the present herd, which can have both direct and indirect effects on local and wild pig populations. These effects are multifaceted and can vary depending on several factors, including ecological, genetic, and socio-economic considerations. Here, we discuss the potential effects of introducing exotic pigs on local and wild pig populations. Introducing exotic pig breeds may lead to direct crossbreeding with local and wild pig populations. This can result in hybridization and the formation of new genetic variations (Chaweewan et al., 2023). The extent of gene flow and the impact on the genetic integrity of local and wild populations depend on the reproductive dynamics and mating behavior between exotic and local/wild pigs. Exotic pigs may introduce new diseases or parasites to local and wild pig populations. Conversely, local or wild pigs might transmit their diseases to exotic breeds. The exchange of pathogens can have significant health implications, potentially leading to disease outbreaks and affecting the overall population dynamics. Exotic pig breeds might also exhibit different foraging and rooting behaviors compared to local or wild pigs, leading to changes in vegetation and soil structure. This, in turn, can influence the habitats of local and wild pig populations and impact the broader ecosystem dynamics (Nogueira-Filho et al., 2009). The introduction of exotic pig breeds may also influence local pig-rearing practices and traditional knowledge and can have socio-economic consequences, affecting the livelihoods of communities relying on local pig breeds It is essential to consider ecological, genetic, and socio-economic factors to assess the overall impact and develop management strategies that balance the potential benefits and risks associated with the coexistence of exotic and indigenous/wild pig populations.

On the flip side, integrating exotic pig breeds offers numerous opportunities. Addressing the challenges and capitalizing on the opportunities requires a multifaceted approach. Targeted breeding programs focused on enhancing the adaptability of exotic pig breeds to Uttarakhand unique climate and terrain are indispensable. Concurrently, collaboration with local agricultural extension services and educational initiatives becomes essential for raising awareness about the benefits of exotic pig farming among local farmers. The successful integration of exotic pig breeds into Uttarakhand commercial farming landscape demands a delicate balance between adapting to local conditions and aligning with market preferences. This comprehensive understanding of the socio-economic landscape, geographical distribution, and pig husbandry practices embraced by these pig rearers, shedding light on the ecological implications that emanate from these practices within Uttarakhand. This can serve as a foundational basis for informed decision-making and policy formulation aimed at sustainable pig farming development in the region.

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CHAPTER 3

ECONOMIC TRAITS OF PIGS: A COMPARATIVE STUDY OF BREEDS AND CROSSBREEDS

3.1 SUMMARY

India, home to 9.06 million pigs, constitutes 1.16% of the global pig population (20th Livestock Census data). Pig farming, primarily small-scale and unorganized, is a significant source of income, especially in tribal areas. Indigenous pig breeds, constituting 79.03% of the population, possess unique traits like heat tolerance and disease resistance. To enhance economic traits of indigenous pig, researchers in India employ crossbreeding with exotic pig breeds, aiming to combine the strengths of both parent breeds. Various crossbred varieties have been developed, each with specific traits contributing to improved growth rates, feed efficiency, and disease resistance. In Uttarakhand, a comparative economic analysis was conducted on locally adapted and exotic/crossbred pig breeds. The study aimed to assess economic viability and sustainability, hypothesizing that while improved breeds may exhibit higher productivity, the associated costs could impact overall viability. The study surveyed 28 households across three districts, examining economic traits such as age at first farrowing, litter size, farrowing interval, and weight at market age. Local crossed with wild pigs emerged as the top performers, exhibiting advantages in various criteria such as reproduction, feed efficiency, and disease resistance. The market value of local x wild pigs was higher, attributed to enhanced genetic traits, superior carcass characteristics, and specific demand-supply dynamics.

In conclusion, the study sheds light on the economic dynamics of pig farming in Uttarakhand, emphasizing the significance of locally adapted breeds and the potential challenges in leveraging exotic breeds for economic gains.

3.2 INTRODUCTION

India has a total of 9.06 million pigs, which is about 1.16% of the global pig population (20th Livestock Census data). The highest pig population is found in eastern and north-eastern states (72.21%), followed by northern southern (10.68%), central (7.64%), northern (6.79%) and western India (2.69%). The state of Assam has the highest number of pigs (2.10 million), followed by Jharkhand, Meghalaya, and West Bengal. As per a report, the Northeast region in India has the potential to emerge as a key hub for pork exports. This is attributed to the substantial demand for pork from neighbouring countries like Myanmar and Bhutan, as well as Southeast Asian nations including Vietnam, the Philippines, Singapore, and Hong Kong (Sharma, 2021). In 2021, India exported $5.97M of pig meat. The main destinations of India exports on Pig Meat were Bhutan, Laos, Turkey, Maldives and United Arab Emirates (Simoes and Hidalgo, 2011).

Pig farming in India is mainly a small-scale, unorganized activity, which is an important source of income and livelihood for many communities, especially in the tribal belt of the country (Kumaresan et al., 2009) as they have no means to undertake scientific pig farming with improved foundation stock, proper housing, feeding and management. Among the various livestock species, piggery is most potential source of meat production and more efficient feed converters after the broiler chicken. Pig farming presents a host of advantageous features, making it a lucrative venture. Pigs exhibit exceptional feed conversion efficiency, surpassing most meat-producing animals except for broilers. Their versatile dietary habits allow them to convert a wide range of feed, including grains, forages, and damaged feeds, into valuable and nutritious meat. With a short generation interval and prolific reproduction, sows can be bred in 8-9 months and yield 6-12 piglets per farrowing, ensuring a consistent supply (Moanaro et al., 2011). Pig farming also requires modest investments in buildings and equipment, making it accessible to a broader range of farmers. Pigs are distinguished by their high meat yield, nutrient-rich pork, and the ability to store fat rapidly, meeting demands in poultry feed and traditional medicine. The utilization of pig manure as an effective fertilizer adds to the sustainability of this farming practice. With quick returns and a robust demand in both domestic and export markets for various pig products, including pork, bacon, ham, sausages, and lard, pig farming can emerge as a promising and economically viable venture in India. About 79.03% of the pig population in India is contributed by indigenous and non-descript pigs. Although the growth rates and feed conversion ratio of indigenous pigs is less compared to exotic or crossbred pigs (Kumar et al., 1990; Gaur et al., 1997), they have unique features like heat tolerance, disease resistance, early sexual maturity and ability to produce meat with less fat. Due to their smaller size, their requirement for feed and space is much less than the exotic and crossbred pigs, thus making them suitable for rearing by poor people with meagre resources (Kadirvel et al., 2023). They are well adapted to survive under most adverse conditions of nutrition and management (Naskar et al., 2013). There are 13 indigenous registered pig breeds recognized by the National Bureau of Animal Genetic Resources. Exotic pig breeds have been imported to improve the production performance and quality of pig meat, but they face challenges such as feed availability, infrastructure, management, bio-security, and socio-cultural barriers (Thomas et al., 2021). The pig industry in India is undergoing transformational changes due to the global market and the domestic demand. India struggles with an insufficient domestic production of pork to satiate the escalating demand, primarily attributed to religious constraints. Despite the overall meat production in India reaching 4.1 million tonnes in 2021, (Department of Animal Husbandry and Dairying), pork constitutes a relatively minor share.

Some of the challenges faced by the pig production sector in India includes, the lack of genetically superior-quality animals that can enhance the productivity, profitability, and quality of pig production (Thomas et al., 2021). Most of the pig population is of indigenous breeds that have low reproductive and growth performance. Most of the pigs are fed on food waste, kitchen scraps, and low-quality grains, which affect their health and meat quality. Most of the pig farms are small-scale, unorganized, and unhygienic, with inadequate housing, water, sanitation, and biosecurity facilities. There is a lack of proper slaughterhouses, cold chains, and value addition units for pork products. There is also a lack of awareness, training, and extension services for the pig farmers and consumers.

Researchers in India aim to enhance the economic traits of indigenous pig breeds by implementing crossbreeding with exotic pig breeds. This strategic approach involves merging indigenous pigs with higher productivity and genetic diversity from exotic breeds. The goal is to selectively breed offspring that harness the strengths of both parent breeds, leading to improvements in growth rate, feed efficiency, litter size, carcass quality, disease resistance, and adaptability to local conditions. Various crossbred varieties have been successfully developed in India through this method. Few crossbred varieties developed in India includes Rani, Asha, Lumsniang, HD-K75, Jharsuk (T & D), Mannuthy White, TANUVAS KPM Gold, SVVU-T17 (Tirupati Varaha), Landlly (Srikanth et al., 2023) (Table 3.1). Economic trait such as Litter size at birth and Average body weight at 8 months of age has been the main trait for improvement.

Table 3.1: List of major crossbred Pig breed in India.

Crossbred varieties	Exotic	Indigenous/Desi	States
Rani	50% Hampshire	50% Ghungroo	Assam
Asha	25% Hampshire, and 50% Duroc.	25% Ghungroo,	Assam
Lumsniang	75% Hampshire	25% Niang Megha	North-Eastern Hill
HD-K75:	75% Hampshire	50% Desi	Assam
Jharsuk (T & D)	50 % Tamworth	50% Desi	Jharkhand
Mannuthy White	75 % Large White Yorkshire	25% Desi	Kerala
TANUVAS KPM Gold	75 % Large White Yorkshire	25% Desi	Tamil Nadu
SVVU-T17 (Tirupati Varaha)	75 % Large White Yorkshire	25% Desi	Andhra Pradesh
Landlly	75% Landrace	25% Ghurahh/Desi	Uttar Pradesh

Source: Srikanth et al., 2023

The primary objective of this chapter is to conduct a comparative economic analysis of locally adapted and exotic/crossbred pig breeds in Uttarakhand, with the aim of understanding the economic viability and sustainability of each. It is hypothesized that while improved pig breeds may exhibit higher growth rates and productivity, the associated costs, including initial investments and specialized feed requirements, may impact the overall economic viability. Locally adapted pig breeds, on the other hand, are expected to demonstrate better economic sustainability due to their acclimatization to local conditions and potentially lower input costs. The study aims to validate these hypotheses through analysis of the performance of breeds providing valuable insights for pig farmers and policymakers to make informed decisions regarding breed selection in this context.

3.3 METHODOLOGY

At the initiation of the research, there was a lack of comprehensive data on pig raisers and their geographical locations. To address this, local individuals actively involved in pig rearing were identified with the assistance of a field assistant. Employing the Rapid Appraisal Approach as a "survey and monitoring tool" (FAO, 2011) helped uncover additional geographic clusters in Uttarakhand where pig rearers were concentrated. Before commencing data collection, a semi-structured questionnaire (ILRI, 2011) was meticulously designed to align with the study's objectives (Appendix 1). Interviews were conducted in the local language (Hindi), and 'key informants' played a crucial role in establishing rapport with pig-rearing households. The study employed random sampling to ensure of pig.

A total of 28 households were surveyed for this study, distributed across 25 in Dehradun, 2 in Haridwar, and 1 in Nainital. Economic traits of 25 adult animals were examined during the survey period, which spanned from March to December 2019, excluding July and August due to the monsoon season. Data related to economic traits were collected, compiled, and analyzed using Microsoft Excel 2007. The identification and classification of pigs were into three groups based on their phenotype: local, local X wild, and crossbred/exotic based on coat color and the recall ability of the rearer (Figure 3.1). The breeding capacity of the sows was assessed through various indicators, including the age at first farrowing (measured in months), the number of piglets in the first litter, the subsequent number of piglets in subsequent litters, the farrowing interval (measured in months), the age at which they were sold (in years), the weight at market age, mortality rate, feed efficiency, maternal behaviour, and disease resistance. These parameters were scrutinized to evaluate and compare the reproductive performance and overall characteristics of the three distinct pig genotype groups.

Local

Local X Exotic

Exotic

Local X Wild

Figure 3.1: Classification of pigs were into three groups based on their phenotype.

3.4 OVERVIEW OF PIG HUSBANDRY PRACTICES IN UTTARAKHAND

Pig husbandry historically served as a customary pursuit by the specific community in Uttarakhand community, predominantly practiced by landless individuals engaged in miscellaneous occupations. For 100% of the surveyed breeders, pig rearing serves as an additional source of income. A higher prevalence of pig keepers was observed in the planar districts (Dehradun, Haridwar, Udham Singh Nagar), reflecting the increased population density in these areas. However, the scale of pig keeping across households consistently remained modest, with an average of 8-12 pigs (including adults and piglets) per household.

The limited scale of pig production can be attributed to a confluence of factors. These include deep-rooted social taboos, spatial constraints, changes in land use due to rapid urbanization in the districts, the reluctance of the younger generation to embrace pig farming, and suboptimal production due to constraints related to feed availability and reliance on family labour. The husbandry practise includes a combination of both farrow-to-finish and fattener production methods was observed in the surveyed households. Fattening practices were more prevalent when exotic pig breeds were raised. Pigs were primarily fed with hotel waste and kitchen refuse, following a semi-intensive husbandry system. For exotic breeds, specially formulated meals comprising purchased wheat bran, rice, maize, and Lobia meal were prepared and mixed with generous amounts of hot water, and supplemented with additional kitchen waste. After more than two years raising, pigs were sold to butcher or traders or along the road for local consumption.

3.5 RESULT

The examination of the various production parameters (Table 3.2) aimed to evaluate the breeding capacity and overall performance of each pig breed, provided us with some valuable insights into the distinct characteristics and potential applications of each pig breed. The age at first farrowing was observed to range from 7-8 months for local pigs, 5-6 months for local X wild pigs, and 9 months for exotic pigs. The number of piglets in the first litter varied, with local pigs having 5-6, local X wild pigs having 6-8, and exotic pigs having 5-6. Subsequent litters showed a range of 6-8 for local pigs, 8-10 for local X wild pigs, and 6-8 for exotic pigs. Farrowing intervals were approximately 6 months for local pigs, 5 months for local X wild pigs, and 6 months for exotic pigs. Age at selling ranged from 2.5-3 years for local and local X wild pigs, and 3-4 years for exotic pigs. Weight at market age showed variations, with local pigs weighing 50-53 kg, local X wild pigs weighing 70-80 kg, and exotic pigs also weighing 70-80 kg. Mortality rates, feed efficiency, motherly behaviour and disease resistance were categorized as per their performance levels (I, II, III) in each group. Meat flavour preferences were also specified, with local and local x wild pigs catering to local community tastes, while exotic pigs were more suitable for restaurant and institutional preferences. Among the three types of pigs compared in the Table 3.2, local x wild pigs have the best performance in most of the criteria. They are the youngest to start reproducing, have the most offspring per litter, the shortest time between litters, the highest weight when sold, the lowest mortality rate, the best feed efficiency, the best motherly behaviour, and the highest disease resistance. Local pigs are the second best in most of the criteria, except for weight at market age, where they are the lowest. Exotic pigs are the worst in most of the criteria, except for weight at market age, where they are equate with local x wild pigs.

Table 3.2: Performance of Pig breed in Uttarakhand.

Production Parameter	**Local**	**Local X Wild**	**Exotic**
Age at first farrowing (months)	7- 8	5- 6	9
Number of piglets/first litter	5- 6	6-8	5-6
Number of piglet/after first litter	6- 8	8-10	6-8
Farrowing interval (months)	6	5	6
Age at sold (year)	2.5-3	2.5- 3	3-4
Weight at market age	50- 53	70-80	70-80
Mortality *	II	III	I
Feed efficiency*	II	I	III
Motherly behaviour*	II	I	III
Disease resistance	II	I	III
Meat Flavour	Local community	Local community	Restaurant and Institution

***Rank: I- Excellent, II-Good, III- Average**

Table 3.3: Market value of pig and its product in Uttarakhand.

Pig and its products	Market value
3 month piglet	INR 2500-3000
Adult pig	3000-4000 (crossbred or exotic)
	4000-5000 (local, local X wild)
Pork	180-200/ kg
Hair	500-1000/kg

In Uttarakhand, the market values for pigs and their products vary based on different categories (Table 3.3). A 3-month piglet is priced between INR 2500 and 3000. Adult pigs command different market values depending on their breed, with crossbred or exotic pigs being valued at INR 3000-4000, while local or local X wild pigs are priced slightly higher at INR 4000-5000. When it comes to pork, the market rate ranges from INR 180 to 200 per kilogram. The market value of pig hair falls within the range of INR 500 to 1000 per kilogram. These pricing differentials reflect the diverse preferences and economic considerations within the Uttarakhand pig farming market, taking into account factors such as pig age, breed, and specific products derived from pigs.

3.6 DISCUSSION

Local pigs and local x wild pigs have an advantage over exotic pigs in terms of meat flavour, as they are more preferred by the local community. Exotic pigs are only popular among restaurants and institutions, who may have different preferences and standards for meat quality. The comparatively lower performance of exotic pig breeds in comparison to local x wild pigs can be attributed to several key factors. Exotic pig breeds are typically acclimated to temperate climates and thrive in intensive production systems, whereas local x wild pigs are better adapted to tropical and subtropical environments, excelling in extensive or semi-intensive management systems. This inherent adaptability of local x wild pigs allows them to navigate a broader range of environmental conditions, granting them a performance edge.

Nutritional and health requirements also play a pivotal role in the performance disparity. Exotic pig breeds exhibit higher nutritional and health needs, often relying on more sophisticated and controlled feeding regimes. In contrast, local x wild pigs showcase a remarkable ability to subsist on lower-quality feed, displaying resilience against diseases and parasites. This adaptability to varying feed qualities contributes significantly to their superior performance, especially in resource-constrained or less controlled environments. Regarding the reproductive and growth potential of exotic pig breeds is counterbalanced by higher mortality and stress levels, particularly when subjected to harsh conditions. Local x wild pigs, with their robust adaptability, tend to cope more effectively with environmental stressors, resulting in lower mortality rates and reduced stress levels under challenging circumstances. A crucial factor influencing performance lies in the genetic diversity and adaptive traits inherent in local x wild pigs. These breeds often possess a broader genetic spectrum and adaptive characteristics, enabling them to thrive in diverse settings. Exotic pig breeds, often selected for specific traits and susceptible to inbreeding, may exhibit limitations in their ability to confront the range of challenges presented by various environments. In summary, the interplay of environmental adaptability, nutritional resilience, stress management, and genetic diversity collectively contribute to the superior performance of local x wild pigs compared to exotic pig breeds. This underlines the importance of considering the specific environmental contexts and characteristics of each breed when evaluating their overall performance in diverse farming settings.

The increased market value of local x wild pigs compared to local pigs can be attributed to several factors. Firstly, local x wild pigs result from crossbreeding local pigs with wild boars, potentially enhancing their genetic traits and overall performance. Research findings, reveal that local x wild pigs demonstrate favourable attributes such as a lower age at first farrowing, higher piglets per litter, reduced farrowing interval, increased weight at market age, lower mortality rates, improved feed efficiency, heightened motherly behaviour, and superior disease resistance when compared to their local counterparts. These advantageous traits contribute to heightened productivity and profitability, rendering local x wild pigs more appealing to the pig rearer. Local x wild pigs also possess a superior carcass yield and low fat meat compared to local pigs, further influencing their market value. The dynamics of demand and supply also play a crucial role in elevating the market value of local x wild pigs. Geographical variations in pig population distribution, particularly concentrated in non-vegetarian tribal belts, may influence local preferences. The meat flavour of local x wild pigs makes them favored by the local community. However, challenges such as the complexities of crossbreeding, insufficient storage facilities, distant markets, a deficient marketing structure, and a lack of abattoirs may limit the availability of local x wild pigs, intensifying their market value due to the interplay of demand and supply factors (Simangaliso et al, 2021). It is also worth mentioning that The Indian wild pig (*Sus scrofa cristatus*) enjoys protected status under the Wildlife Protection Act of 1972. Designated in Schedule II of the act, this classification prohibits hunting, capturing, or killing the animal, except under specific conditions and with a valid license, hence procuring and keeping it become illegal. In conclusion, the higher market value of local x wild pigs is a result of their enhanced genetic traits, superior carcass characteristics, and the intricate dynamics of demand and supply in specific geographical regions.

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CHAPTER 4

GENETIC VARIATION AND PHYLOGENETIC RELATIONSHIP USING MITOCHONDRIAL DNA REGION

4.1 SUMMARY

The maternal lineage of the domestic swine population was traced using mitochondrial DNA control region markers. Analysis of 68 samples reveals 20 haplotypes, the signature of the Pacific Clade (D6), MTSEA (South East Asia), European (D1), and the ubiquitously distributed Chinese (D2) haplotypes are present in the domestic pig of Uttarakhand. The D3 haplotypes, which were reported in wild pigs (*Sus scrofa domesticus*) from North India, were also identified in 47 domestic samples. A unique genepool, UKD (Uttarakhand Domestic), as another lineage specific to this region has been proposed. The incorporation of an additional 15 samples originating from North Indian states, and their subsequent integration with previously published 1,518 mitochondrial D-loop sequences of *Sus scrofa*, provide a comprehensive global perspective on the geographical distribution and phylogenetic positioning of the examined specimens. The resulting dataset revealed 18 unique haplotypes, and a complex clustering patterns of porcine haplotypes with distinct phylogeographic signals. The study unveiled diverse maternal lineages among the domestic pig populations in northern Indian states situated in the foothills of the majestic Himalayas. Additionally, the study also confirmed the finding of the presence of two distinct subspecies of wild boar in India includes W1 (*Sus scrofa davidi*) and W2 (*Sus scrofa affinis*), demonstrating their migration routes and offering a strong phylogeographic signal. The haplotypes present in the UKD group align with the W1 clade, indicating the presence of the W1 maternal lineage in domestic pig samples from the region. Evidence for a separate domestication center in the Himalayan foothills and Indian sub-continent was found within Mixed Clade 5 (MC5). A novel clade (MC4) with a localized

distribution in the Chotanagpur plateau was detected, suggesting a unique population of wild boar in this region. Diverse maternal lineages among domestic pigs of Uttarakhand were observed, which may reflect human-mediated dispersal of pigs within and into the region; hence, a hypothesis linking ancient human migration with the presence of these maternal lineage clades has been proposed. Overall, the porcine haplotypes discovered in the Indian subcontinent through this study highlight its unique and significant contribution to pig genetic resources of India.

4.2 INTRODUCTION

The advent of the Neolithic period witnessed humans establishing themselves across diverse landscapes without relying on domestic animals. However, subsequent domestication of animals' facilitated significant migrations as farming societies travelled with their livestock, hence the preserved archaeological remnants and genetic imprints of both historical and contemporary domesticated animals offer a direct avenue to decipher the timelines and pathways of ancient migrations (Bellwood, 2009). The mysteries surrounding the origins of civilization, languages, and cultures are inextricably connected with the questions about the specific places, periods, and techniques of domestication. By uncovering these mysteries, we gain valuable insights into the development and transformation of early civilizations, the emergence and diversification of languages, and the evolution of various cultural practices and traditions (Stoneking et al., 2023).This concept has been validated in the case of various domestic species such as sheep (Rocha et al., 2011), goats (Peng et al., 2022), cows (Edwards et al., 2007), and notably, pigs (Larson et al., 2005; 2007; 2010) using mitochondrial marker.

Mitochondrial markers follow maternal inheritance and find utility in analysing ancient population migrations, making them suitable for studying maternal inheritance patterns, historical relatedness of the populations and track the matrilineal component of historic genetic diversity and migration routes

Pigs (*Sus scrofa*) display exceptional adaptability, blending their inherent traits and human interactions for survival across diverse environments. Their capacity to thrive in different habitats, diets, and reproductive strategies, paired with their economic and cultural significance, underscores their global prevalence and utilization.

Wild boars, belonging to the species *Sus scrofa*, have a long and ancient evolutionary history that predates the domestication of pigs by humans. Both archaeological and genetic methods have been used in recent publications to study how pigs were domesticated. Fossil evidence indicates that wild boars were present during the Early Middle Pleistocene period, around 800-900 thousand years ago. Before the initiation of pig domestication by human wild boar populations had already differentiated and established themselves in mainland East Asia and Europe (Larson et al., 2007). The process of wild boar dispersal and differentiation is thought to have occurred through natural mechanisms such as migration and adaptation to different environments over a long period of time. These processes shaped the genetic diversity and distribution of wild boar populations across Eurasia.

The domestication of pigs by humans happened independently in multiple regions, with the earliest evidence of pig domestication dating back to around 9,000-10,000 years ago in the Near East and China. Archaeozoological evidence shows that pigs were domesticated independently in western Asia (around 8500 BC) and China (around 6500 BC) (Evin et al., 2017). The domestication of pigs involved the selection and breeding of specific traits for human purposes, leading to the development of distinct domestic pig breeds (Xiang et al., 2017).

The genetic diversity and evolutionary history of wild boars and domestic pigs have intertwined to some extent, as wild boar populations have contributed genetic material to domestic pig populations through hybridization and selective breeding. However, it is important to recognize that wild boars and domestic pigs are separate entities, with wild boars representing the ancestral species from which domestic pigs were derived (Frantz et al., 2012).

By focusing on mitochondrial DNA markers, genetic studies have achieved a more precise understanding of numerous geographically distinct populations of wild boar characterized by unique genetic signatures (Giuffra et al., 2000; Larson et al., 2005; 2010). The work conducted by Larson et al. (2005) has laid a substantial groundwork for advancing the study of origins, migration, domestication, and the dispersal of *Sus scrofa* through analysis of mtDNA control region (D-loop) sequences obtained from global wild boar, domestic pig, and ancient samples.

Pigs and humans have a long history of interaction in India, dating back to the Upper Paleolithic period when they were depicted in cave paintings at Bhimbetaka (Wakankar 1973; Dubey 2014). Lipid profile studies on the diet of the ancient Harappan civilization, one of the oldest in the world, show that pigs made up about 2-3% of animal remains at Indus sites, suggesting they may have been part of the Indus people diet (Suryanarayan et al., 2021). The wild boar in northern India today are descendants of *Sus scrofa* that migrated from ISEA to Asia (Larson et al., 2005). Additionally, the genetic research provides support for separate and independent domestication pathways at the foothills of Himalayas and suggests that there was at least one population of wild boar in India that underwent domestication, from different wild populations in the region (Larson et al. 2010). Recent studies discovered the existence of two novel wild boar clades, in South Asia (Nidup 2011) and also confirm the migration route of the newly discovered wild boar clade in India from ISEA (Choi et al., 2020). However, there is no evidence to suggest that wild boars belonging to these clades have made any maternal genetic contribution to modern domestic pigs. Nevertheless, the distribution and variation of mtDNA lineages in Indian subcontinent have not thoroughly been explored, and the origin and domestication of these pigs remain poorly understood. This chapter aims to examine the maternal lineage and genetic diversity of the domestic pig raised in Uttarakhand and the second aim is to examine the distribution of porcine mtDNA lineages in Northern India from the samples collected from Uttarakhand, Jharkhand and Bihar, as well as to assess the potential of using pigs as a proxy to trace human migration, especially concerning the D3 haplotype and other distinct haplotypes found within the Indian subcontinent. This chapter established a cohesive framework outlining the origin and global path of wild boars, including their passage through the Indian subcontinent and subsequent radiation to other continents.

4.3 METHODOLOGY

4.3.1 SAMPLE COLLECTION, DNA EXTRACTION, AMPLIFICATION AND SEQUENCING

Non-invasive biological samples (hair) were collected from 68 unrelated domestic pigs from the surveyed household Uttarakhand (Appendix 2). Additional, 15 hair samples of domestic pig from northern India were also collected, specifically from Jharkhand and Bihar (Appendix 3) to give a comprehensive view of the maternal lineage of Northern India.

Genomic DNA was extracted using DNeasy Blood & Tissue kit (Qiagen, Hilden, Germany) in a final elution volume of 40–80 µL. DNA extraction products were electrophoresed on 0.8% agarose gel and visualized under UV light. The amplification of the mtDNA hypervariable control region was recorded using control region-specific primer pair (Appendix 4) (Balakrishnan et al., 2003). The reactions were performed in 20µl volumes containing 10-20 ng of extracted genomic DNA. PCR master mix contained: 1× PCR buffer (Applied Biosystems), 2.0 mM $MgCl_2$, 0.2 mM of each dNTP, 2 pmol of each primer, and 5U of Taq DNA polymerase. The PCR conditions for both the primer were as follows: an initial denaturation for 5 minutes at 95°C, followed by 35 cycles at 95°C for 45 seconds, 55°C for 45 seconds and 72°C for 1minutes 30 second seconds, with a final extension of 72°C for 10 minutes. The PCR products were electrophoresed on 2% agarose gel and visualized under UV light.

Positive amplicons were treated with Exonuclease-I and Shrimp alkaline phosphatase (USB, Cleveland, OH) for 15 min each at 37ºC and 80ºC, respectively to remove any reaction residues. The purified fragments were sequenced directly in Genetic Analyzer 3500XL from forward primers set using a BigDye v3.1 Kit (Applied Biosystems).

4.3.2 SEQUENCE DATA ANALYSIS

In total 425 bp of mtDNA control region were obtained which were confirmed using the BLAST tool on NCBI (http://ncbi.nlm.nih.gov/). The generated sequences obtained were clean and edited with SEQUENCHER® version 4.9 (Gene Codes Corporation, Ann Arbor, MI, USA) and corresponds to position 15442 to 15867 bp in the complete porcine mtDNA (Accession no. AJ002189) (Ursing & Arnason 1998).

4.3.3 PHYLOGENETIC AND POPULATION STRUCTURE ANALYSIS OF SAMPLES FROM UTTARAKHAND

The mitochondrial control region sequences were aligned using the CLUSTAL W algorithm (Thompson et al., 1994) in program MEGA X (Kumar et al. 2018) and alignment was checked by visual inspection and was submitted to GenBank National Centre for Biotechnology Information (NCBI) GenBank (OP382063-82). Diversity indices like polymorphic segregating sites (S) Haplotype diversity (hd), nucleotide diversity (π), and pairwise genetic differentiation (F_{ST}) among the Uttarakhand, Asian and European clusters were calculated using program DnaSPv.5 (Librado et al., 2009). Demographic history mismatch distribution and population expansion were also studied using DnaSPv.5 software under the assumption of neutrality estimation of D (Tajima 1989) test statistics, which use the information of the mutation frequency (segregating sites), Fu-Fs statistics (Fu 1997), which uses information from the haplotype distribution and Harpending's raggedness (r) index (Harpending 1992), based on the mismatch distribution. For all statistics, the coalescent algorithm implemented in DnaSP were run to 1000 permutation to estimate the probability of obtaining values.

The spatial relationship among the haplotypes was reconstructed using the median-joining network in PopART v.1.7 (Leigh et al., 2015). Phylogenetic relationships among the sequences were reconstructed using the partial fragment of the control region based on the Bayesian approach as implemented in program MrBayes v.3.2 (Ronquist et al. 2003). The most appropriate model (GTR+I+G) for nucleotide substitution was selected based on the Bayesian Information Criterion (BIC) values using program jModelTest v.2.1.3 (Posada 2008). Two MCMC chains of 10 million simulations sampling at every 5,000 generations were performed. The output tree topologies were edited using FigTree v.1.4.2 (http://tree.bio.ed.ac.uk/sofware/fgtree/). Warthog (Phacochoerus africanus; NC_008830) was used as outgroup. The nomenclatures described by Larson et al. 2005 with six clades (D1 to D6) including the new clade (MTSEA) proposed by Tanaka et al. 2008 were used as the reference for clade/ haplogroup notation. Thus including 72 sequences from their studies in order to accommodate most of the major porcine haplotypes (Appendix 5).

4.3.4 PHYLOGENETIC AND PHYLOGEOGRAPHIC ANALYSIS OF NORTHERN INDIA

The 83 mitochondrial control region sequences of domestic pigs (68 Uttarakhand and 1 Bihar and 14 Jharkhand) were combined with 1518 published *Sus scrofa* mitochondrial D-loop sequences (NCBI). Phylogenetic analyses of northern India were conducted using a 417-bp fragment. These sequences were aligned using the CLUSTAL W algorithm (Thompson et al., 1994) in program MEGA X (Kumar et al., 2018) and alignment was checked by visual inspection and was submitted to GenBank databases.

The spatial relationship among the haplotypes was reconstructed using the median-joining network in PopART v.1.7 (Leigh et al., 2015). Phylogenetic relationships among the sequences were reconstructed using the partial fragment of the control region based on the Bayesian approach as implemented in program MrBayes v.3.2 (Ronquist et al., 2003). The most appropriate model (GTR+I+G) for nucleotide substitution was selected based on the Bayesian Information Criterion (BIC) values using program jModelTest v.2.1.3 (Posada 2008). Two MCMC chains of 10 million simulations sampling at every 5,000 generations were performed.

The output tree topologies were edited using FigTree v.1.4.2 (http://tree.bio.ed.ac.uk/sofwa re/fgtree/). Warthog (*Phacochoerus africanus*; NC_008830) was used as outgroup. The nomenclatures described by Larson et al., 2005 with six clades (D1 to D6) were used as the reference for clade/ haplogroup notation in order to accommodate most of the major porcine haplotypes.

4.4 RESULTS

4.4.1 HAPLOTYPE DISTRIBUTIONS AND PHYLOGENETIC ANALYSIS OF SAMPLES FROM UTTARAKHAND

20 haplotypes (H1 to H20) were observed from 68 individuals of Uttarakhand. All the haplotypes were aligned with respect to the representative haplotypes of a North Indian origin wild pig under accession number AY884675 (Table 4.4) these haplotypes were segregated by 36 polymorphic sites which represented substitution mutation. Five haplotype (H1, H3, H4,

H5, H12) occurred in multiple pigs, and the other fifteen were found only once in the 68 animals. 141(140 +1 outgroup) sequences were used to perform the phylogenetic tree and median-joining network analysis. Network analysis supported the phylogenetic tree and revealed strong genetic structuring among Uttarakhand pig haplotypes, where different haplogroups could be observed (Figure 4.1, Figure 4.2). Broadly Asian ancestry (90%) was observed and eight haplotypes (H1, H2, H11, H12, H13, H14, H17, H20) which accounted for 69.12% (47/68 individuals) of the total sampled, nested under the D3 haplogroup. The haplogroup D3 has been reported from wild boars and domestic pigs in northern India and Bhutan (Tanaka et al., 2008). Three Haplotypes H10, H16, and H18 (4.41%, 3/68 individuals) distributed in the D2 haplogroup which is dispersed worldwide and found particularly among Chinese pig breeds and has some relationship with Asian pigs, as well as with East Asian wild boars (Scandura et al., 2011; Fang et al., 2006; Wu et al., 2007). Two haplotypes H7, H8 (2.94%, 2/68) revealed the presence of ancestry in the D1 haplogroup (European clade), harbouring maternal lineages from the European breed, Large White. H3, H4, H6 (16.18%, 11/68) and H5 (2.94%, 2/68) has not been reported in the previous studies but it has a close association with D6 (Pacific Clade) and MTSEA haplogroup respectively, and fall in the same clade in the phylogenetic tree. Pacific clade consisted of domestic or feral pigs from Oceania. The haplogroup MTSEA (Mitochondrial Southeast Asian) consists of both wild and domestic samples restricted to Indo-Burma Biodiversity Hotspots (IBBH). Three haplotype H9, H15, and H19 (4.41% 3/68) does not fall under any of the proposed haplogroups and formed a separate and prominent cluster (named as UKD) in the network. It possessed a unique nucleotide polymorphism at sites C120T, A142G, T179C, A315G, G401A, two transversion substitutions at the base G81C and T400A and insertion at 137 with A.

4.4.2 GENETIC DIVERSITY AND PHYLOGENY OF SAMPLES FROM UTTARAKHAND

In Uttarakhand domestic pigs, the study revealed a haplotype diversity (SD) of 0.763±0.048 and a nucleotide diversity of 0.013±0.00181 (Table 4.1). The genetic differentiation coefficient (Fst) between the Uttarakhand pig population and Asian pigs was 0.25, while it was 0.60 with European pigs (Table 4.2).

Overall, the analysis showed negative and non-significant Tajima's D (-0.87, $p>0.01$) and Fu's Fs (-2.74, $p>0.01$) test results for neutrality (Table 4.3). The mismatch distribution (Figure 4.3) displayed a ragged and multimodal pattern, while the Harpending's raggedness index was positive and non-significant ($r=0.047$), results from the simulation based on the coalescence process.

To explore the hypothesis of population expansion, mismatch distribution was also examined for the dominant D3 haplogroup found in Uttarakhand. The haplotypic and genealogical relationships, as depicted in the network, exhibited star-like patterns (Figure 4.1). A smooth and unimodal mismatch distribution (Figure 4.4) was observed, along with a significant and negative Tajima's D (-1.78721, $p<0.01$) and Fu's Fs (-7.416, $p<0.01$) test results for neutrality and the high and non-significant value of Harpending's raggedness index ($r=0.0916$).

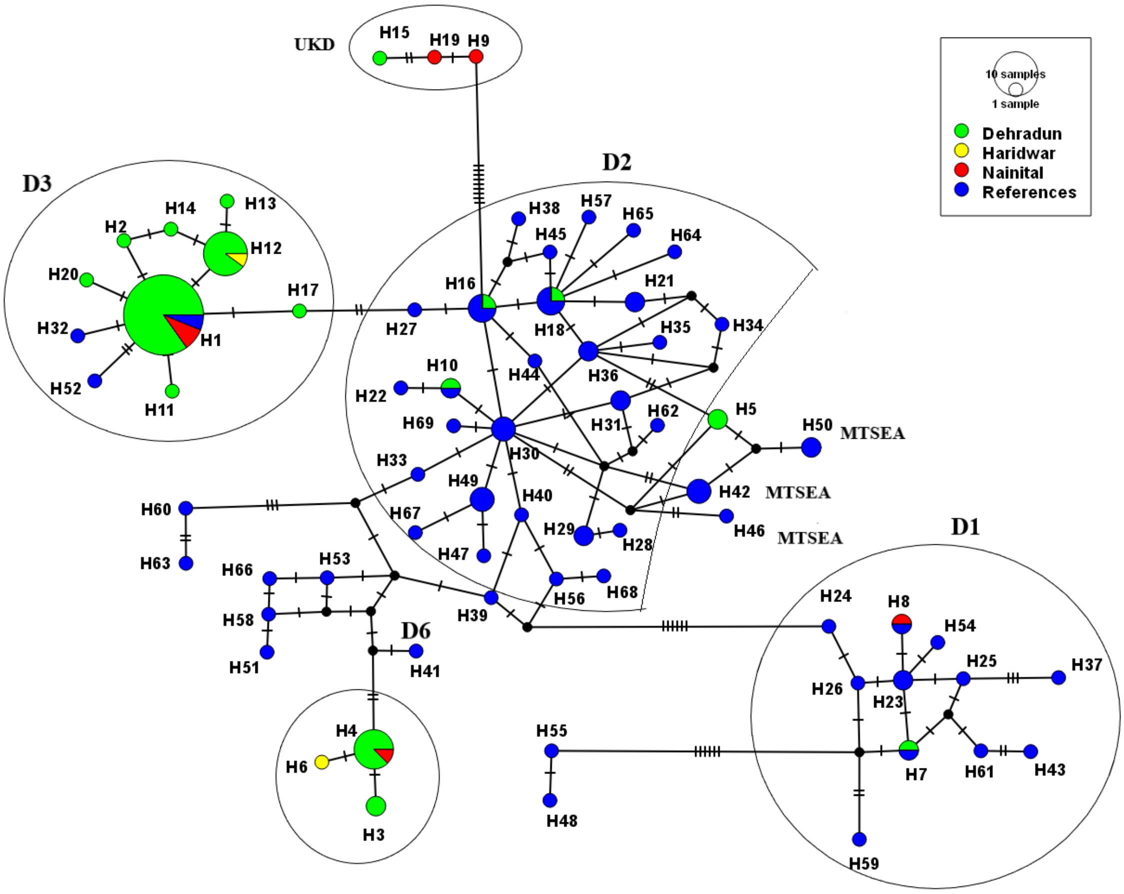


Figure 4.1: Relationship and clustering of 20 (H1-H20) Uttarakhand pig haplotypes 49 with representative haplotypes (H21-H69) mtDNA control region haplotypes (marked). Geographical locations of samples are given in colour and node size is proportional to the frequency of the corresponding haplotypes. Small black dots are median vector (mv) representing hypothetical sequences that were not detected in this study. Each bar on the branch indicates one nucleotide substitution (or an insertion/ deletion).

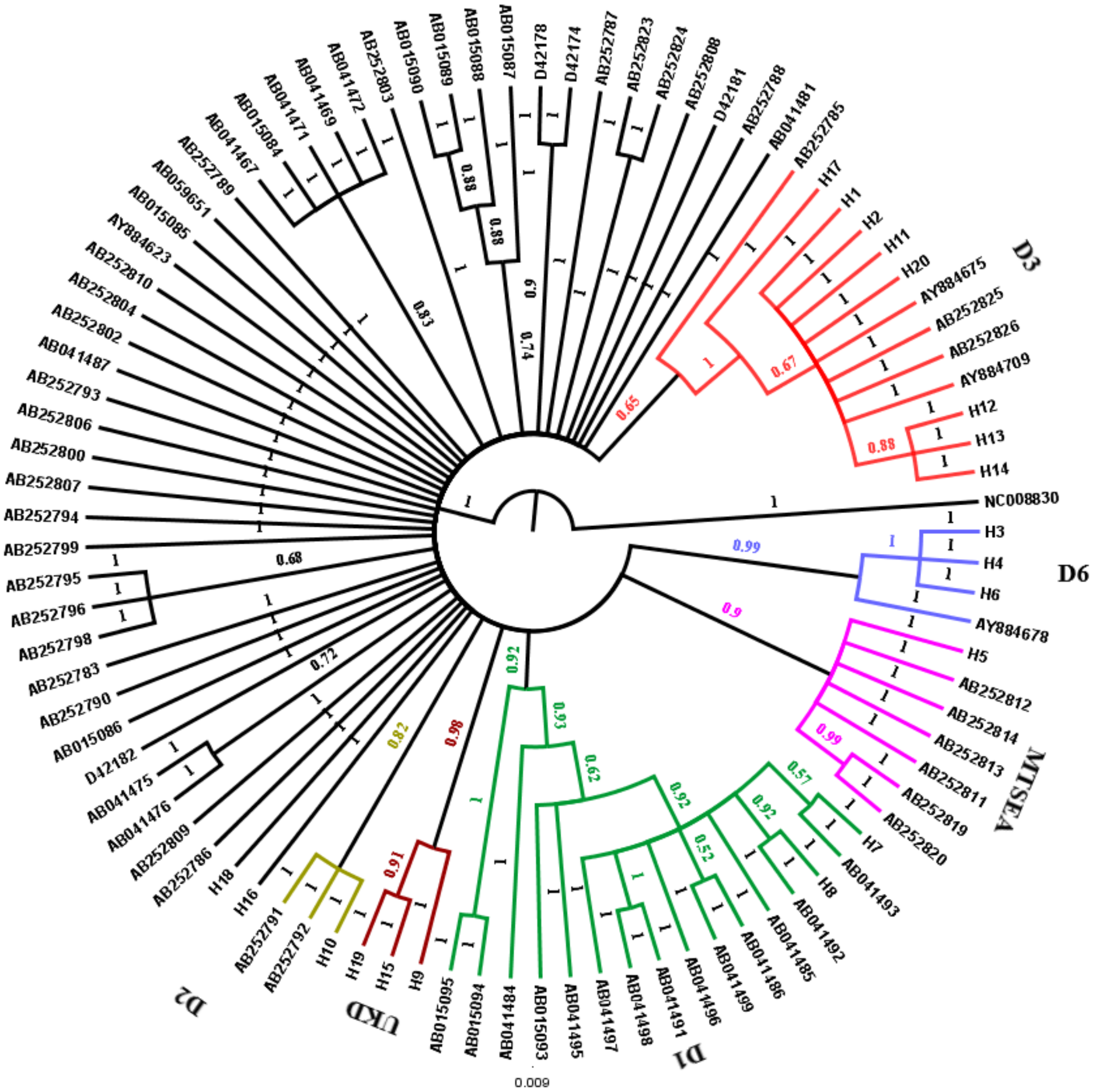


Figure 4.2: Phylogenetic relationships of Uttarakhand domestic pigs obtained from the Bayesian analysis of mitochondrial control region sequences (425 bp). Bayesian Posterior Probability (BPP) values are provided at respective nodes with *Phacochoerus aethiopicus* (NC_008830) used as outgroup and other reference haplotype sequences. Uttarakhand domestic pigs revealed to encompass founder sources from four different geographic origins excluding the D3 and UKD which is endemic to foothills of Himalaya and a novel unique clade respectively.

Table 4.1: Genetic diversity indices for domestic pigs of Uttarakhand and domestic and wild pigs of Asian and European populations (reference sequences).

Region	***n***	***H***	***vs***	***P***	***S***	***K(SD)***	***hd***	***π***
Uttarakhand	68	20	36(32/4/4)	31	5	5.492(0.974)	0.763(0.048)	0.013(0.002)
Asian	60	44	41	29	12	5.271(0.223)	0.988(0.005)	0.013(0.001)
European	12	11	19	6	13	4.621(0.981)	0.985(0.040)	0.011(0.003)
Total	140	75	56	66	30	7.172(11.4)	0.935(0.016)	0.017(0.001)

n= no.of sequences H= no. of Haplotype vs= No. of polymorphic sites (transitions/transversions/ insertions or deletions) P=Parsimony informative sites S= Singleton variables site K (SD) = Average number of nucleotide differences hd=haplotype diversity (SD)
***π*= nucleotide diversity (SD)**

Table 4.2: Genetic differentiation coefficient (*Fst*).

	Fst
Uttarakhand vs Asian	0.24471**
Uttarakhand vs European	0.59748**

***** p < 0.001. ** p < 0.01. * p < 0.05 ns=non-significant**

Table 4.3: Neutrality's tests of all the samples Uttarakhand population and D3 haplogroup.

Neutrality's tests	**Uttarakhand**	**D3**
Tajima's D	-0.87(ns)	-1.78721 *
Fu's Fs statistic	-2.74(ns)	-7.416**

***** p < 0.001. ** p < 0.01. * p < 0.05 ns=non-significant**

		Nucleotide Position																																		
		#	*	#	#	#	#	#	#	#	#	*	#	#	#	#	#	#	*	*	#	#	#	#	#	#	*	#	#	#	#	#	#	#	#	*	#	#
	Cluster	10	17	33	49	80	81	102	117	120	124	137	139	142	147	152	153	175	176	178	179	208	234	235	273	288	295	300	315	317	318	353	381	384	385	399	400	401
AY884675	D3	T	G	G	C	C	G	C	A	C	A	-	T	A	T	G	T	T	T	C	T	T	T	T	T	G	C	T	A	C	T	G	A	T	C	T	T	G
H1	D3	.	.	.	.	.	.	.	.	.	.	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H2	D3	.	.	.	.	.	.	.	.	.	.	-	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H11	D3	.	.	.	.	.	.	.	.	.	.	-	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H12	D3	.	.	.	A	.	.	.	.	.	.	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H13	D3	.	.	.	A	.	.	.	.	.	.	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.
H14	D3	.	.	.	A	.	.	.	.	.	.	-	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H17	D3	.	.	.	.	.	.	.	.	.	.	-	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H20	D3	C	.	.	.	.	.	.	.	.	.	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H7	D1	.	.	.	.	T	.	T	T	.	G	-	C	.	C	A	C	.	.	.	.	.	.	.	.	A	.	C	.	.	.	.	.	C	.	.	.	.
H8	D1	.	.	.	.	T	.	T	T	.	G	-	C	.	C	A	C	.	.	.	.	.	.	.	C	A	.	C	.	.	.	.	.	C	.	C	.	.
H10	D2	.	.	.	.	T	.	.	.	.	.	-	.	.	.	.	C	C	.	.	.	.	.	.	C	.	T	.	.	T	.	.	.	.	.	.	.	.
H16	D2	.	.	.	.	T	.	.	.	.	.	-	.	.	.	.	C	C	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.
H18	D2	.	.	.	.	T	.	.	.	.	.	-	.	.	.	.	C	C	.	.	.	.	.	C	.	.	.	.	.	T	.	.	.	.	.	.	.	.
H3	D6	.	.	A	.	T	.	.	.	.	.	-	.	.	.	.	C	.	.	.	.	C	.	.	C	.	.	.	.	T	C	.	G	.	T	.	.	.
H4	D6	.	.	A	.	T	.	.	.	.	.	-	.	.	.	.	C	.	.	.	.	C	.	.	C	.	.	C	.	T	C	.	G	.	T	.	.	.
H6	D6	.	.	A	A	T	.	.	.	.	.	-	.	.	.	.	C	.	.	.	.	C	.	.	C	.	.	C	.	T	C	.	G	.	T	.	.	.
H5	MTSEA	.	A	.	.	T	.	.	.	.	.	-	.	.	.	.	C	C	C	.	.	.	.	C	C	.	.	.	.	T	.	.	.	.	.	.	.	.
H9	UKD	C	.	.	.	T	C	.	.	T	.	A	.	G	.	.	C	C	.	.	C	.	.	.	.	A	.	.	G	T	.	.	.	.	.	.	A	A
H15	UKD	C	.	.	A	T	C	.	.	T	.	A	.	G	.	.	C	.	.	.	C	.	.	.	.	A	.	.	G	T	.	A	.	.	.	.	A	A
H19	UKD	C	.	.	.	T	C	.	.	T	.	A	.	G	.	.	C	C	.	.	C	.	.	.	.	A	.	.	G	T	.	A	.	.	.	.	A	A

Table 4.4: Variable positions among haplotypes of the partial mitochondrial DNA control region (425 bp) found in this study. Dots (.) indicates matches with the nucleotide sequence GenBank accession number AY884675 (Main cluster of D3). Nucleotide positions are numbered according to our sequence alignment. Variable Sites 36/425 , # Parsim-info 31/425, Conserved 389/425, *Singleton 5/425

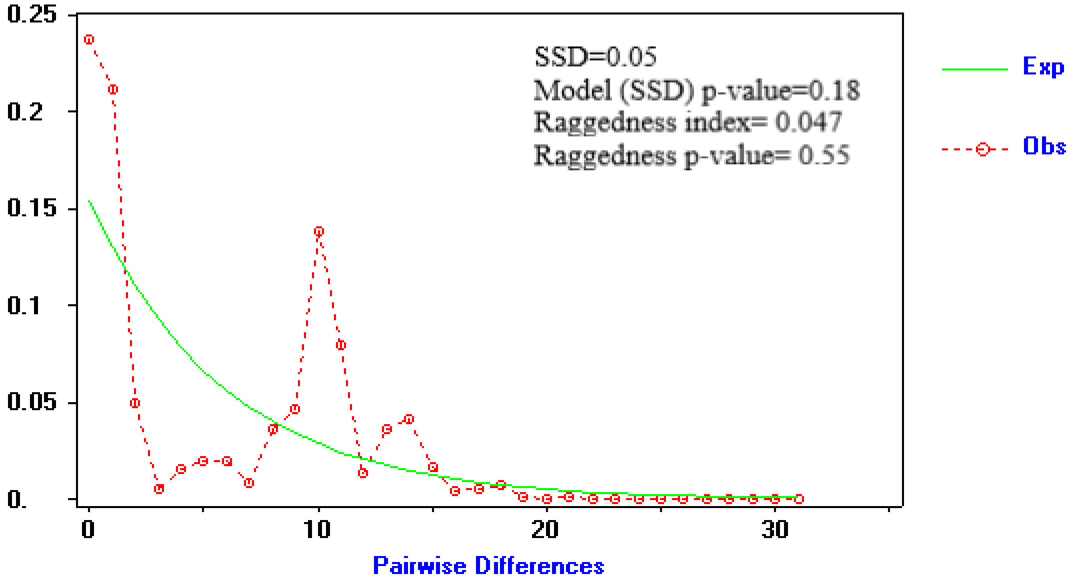


Figure 4.3: Mismatch distributions of mitochondrial DNA sequences of the Uttarakhand domestic pigs (68 sequences) based on pairwise nucleotide differences.

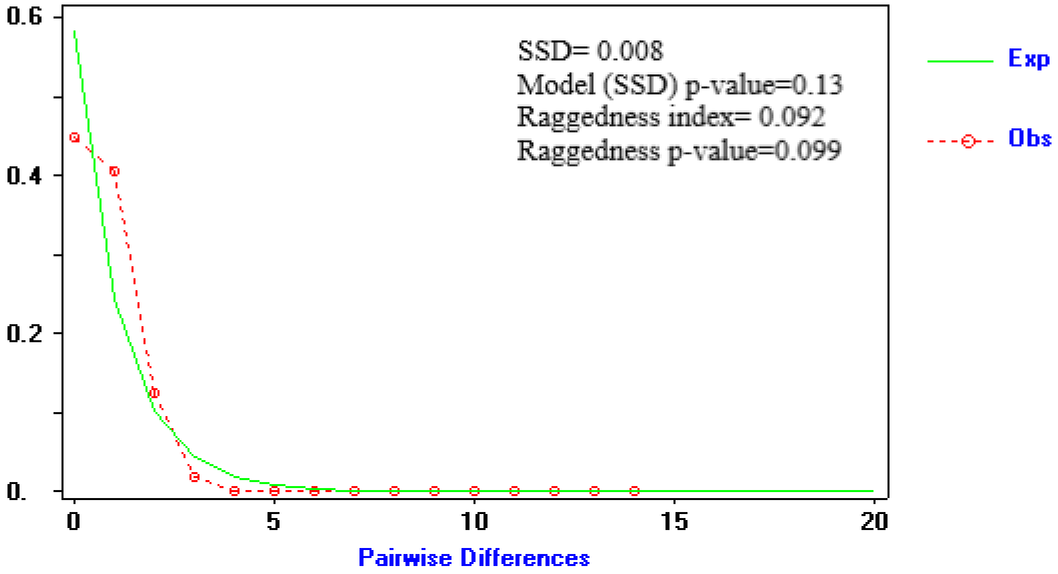


Figure 4.4: Mismatch distributions of mtDNA sequences of the Uttarakhand domestic pigs sequences and other reference sequences (Uttarakhand=47, References=4) that form D3 cluster based on pairwise nucleotide differences.

PHYLOGENETIC AND PHYLOGEOGRAPHIC ANALYSIS

4.4.3 HAPLOTYPE DISTRIBUTION AMONG THE STUDIED SAMPLES INCLUDING SAMPLES FROM NORTHERN INDIA

The analysis of combined global published mitochondrial control region sequences of *Sus scrofa* with 83 sampled sequences identified 18 haplotypes, focusing on a 417 bp fragment. 3 of 18 haplotypes in were represented by a single sequence while others were found in at least two animals (Table 4.5).

Table 4.5: DNA samples used and haplotype generated in this study.

Haplotype	Sample ID	no. of samples	Haplogroup	Location
H2, H7, H11	UK63DP, UK30DP, JH1DP, UK62DP	4	W1(UKD)	Uttarakhand, Jharkhand
H5, H6, H16	UK12DP, UK67DP, UK2DP, UK3DP, UK42DP, UK48DP, UK49DP, UK5DP, UK61DP, UK6DP, UK40DP	11	D6	Uttarakhand
H9	UK47DP, UK55DP	2	-	Uttarakhand
H14	UK7DP	1	D2	Uttarakhand
H17	UK53DP	1	-	Uttarakhand
H18	BH1DP, JH10DP, JH11DP JH12DP, JH13DP, JH14DP, JH2DP, JH3DP, JH4DP, JH5DP, JH6DP, JH7DP, JH8DP, JH9DP	14	MC4	Bihar Jharkhand
H1,H3, H8,H10,H13	UK10DP, UK11DP, UK13DP, UK14DP, UK15DP, UK16DP, UK17DP, UK18DP, UK19DP, UK1DP, UK20DP, UK21DP, UK22DP, UK23DP, UK25DP, UK41DP, UK43DP, UK44DP, UK45DP, UK46DP, UK4DP, UK50DP, UK51DP, UK52DP, UK54DP, UK57DP, UK58DP, UK59DP, UK60DP, UK65DP, UK66DP, UK68DP, UK8DP, UK26DP, UK27DP, UK28DP, UK29DP, UK31DP, UK32DP, UK33DP, UK34DP, UK35DP, UK36DP, UK39DP, UK24DP, UK38DP, UK37DP	47	MC5(D3)	Uttarakhand
H4	UK56DP	1	-	Uttarakhand
H12,H15	UK9DP, UK64DP	2	MC2(D1)	Uttarakhand

4.4.4 CLUSTERING OF HAPLOTYPES

Based on Bayesian phylogenetics, these were classified into six major clades, each further divided into sub-clades (Figure 4.5). It indicated two distinct sub-clades (W1 and W2) within the initial clade (W), consistent with previous research (Larson et al., 2005; Nidup 2011). W1 includes domestic samples from Uttarakhand that was designated as UKD haplogroup and Jharkhand, sharing haplotypes with wild pig samples of northern/central India, Nepal, and Pakistan. W2 consists of wild haplotypes from northern/central/southern India, and Sri Lanka.

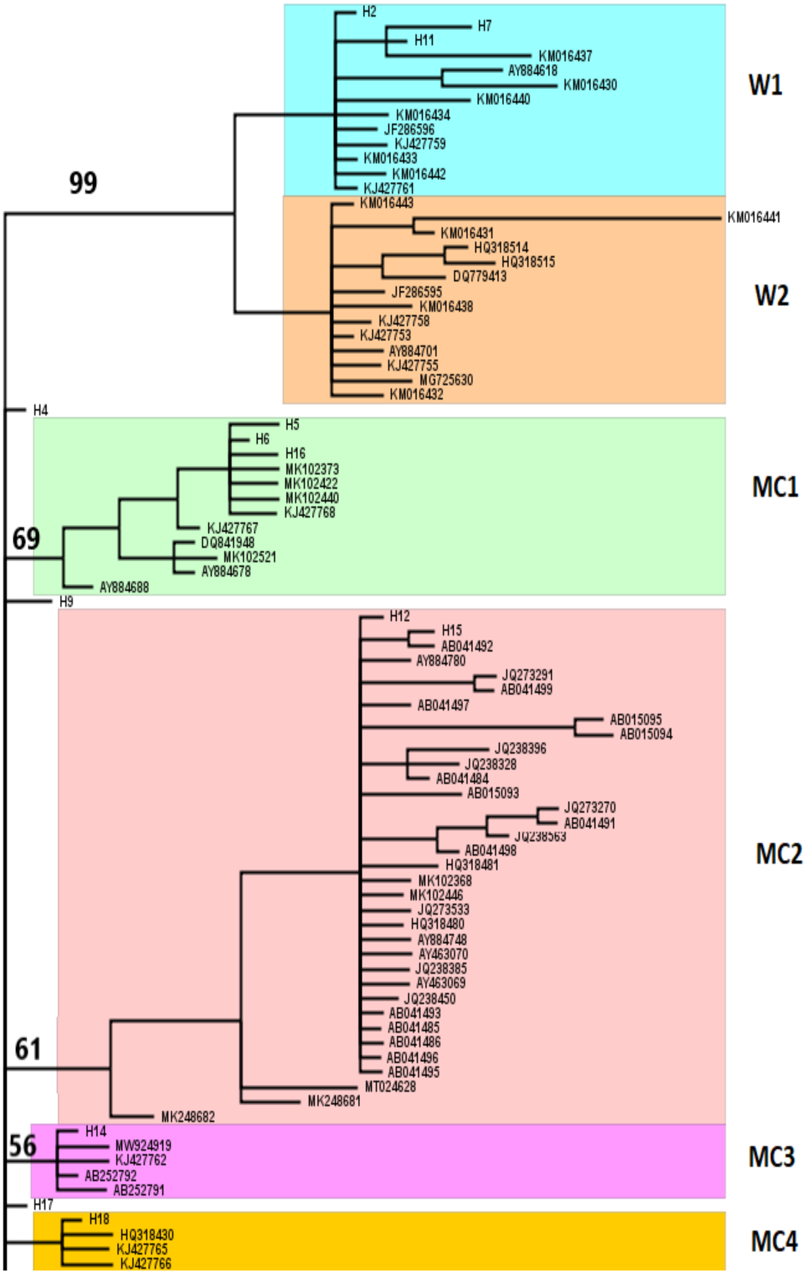
The consensus Bayesian tree differed from earlier research mainly in the placement of the wild clade (W) which lies within the continental clade. The position of the remaining clades in the tree showed similarity to previous findings (Larson et al., 2005; 2010; Tanaka et al., 2008).

Clade MC1 includes domestic pig haplotypes from northern India, north-eastern India, and the Philippines, as well as a wild pig haplotype from Indonesia. This clade contained 11 Uttarakhand samples and incorporated the reference sequence of lineage D6.

Clade MC2 includes two Uttarakhand samples and European domestic pig breed haplotypes. It shared sister clades with Indian wild and domestic Andaman pigs and domestic Nicobari pigs. The reference sequences, of the D1 lineage were present within this clade.

Clade MC3 comprises one sequence from Uttarakhand and includes domestic pig sequences from Asian countries such as the Philippines, Indonesia, Vietnam, and northern India. This clade falls within the D2 lineage.

It revealed a unique clade, MC4, comprising one sample from Bihar, 13 out of 14 domestic pig samples from Jharkhand, wild pigs from Bhutan, eastern India, and domestic pigs from central India. This novel clade does not align with any proposed reference sequences. The wild pig sequence from Bhutan, found within this clade, was previously identified as a new haplotype (W12) (Nidup 2011).



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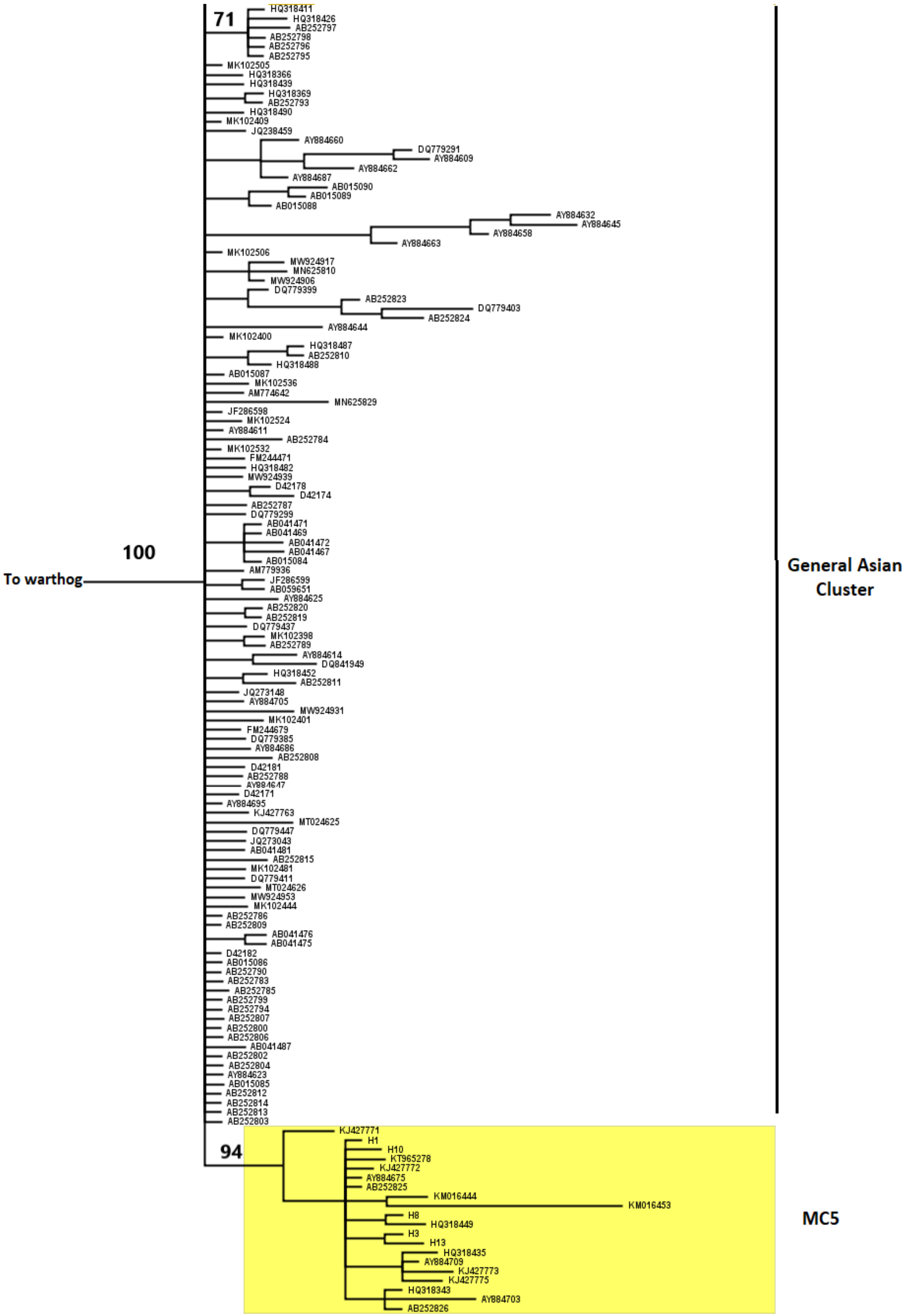

Figure 4.5: Phylogenetic relationships of North Indian (Uttarakhand, Bihar and Jharkhand) domestic pigs obtained from the Bayesian analysis of mitochondrial control region sequences (417 bp). Bayesian Posterior Probability (BPP) values are provided at respective nodes with *Phacochoerus aethiopicus* (NC_008830) used as outgroup and other reference haplotype sequences.

The general Asian clade identified in this study is synonymous with the basal ISEA clade (Larson et al., 2005). It is characterized by a lack of clear geographical structure, as haplotypes from different regions are grouped together within compact clusters of median joining network. Most of the samples within this clade are rooted in the basal branch, indicating their ancient origins. The middle portion of the clade, as depicted in Figure 4.5, shows a large polytomy of clades and individual branches, representing samples primarily from various Asian countries such as Indonesia, Myanmar, the Philippines, Japan, Malaysia, Thailand, Cambodia, and Laos.

Three haplotypes from the studied samples formed the basal branch of the tree H4, H9 and H17. H4 and H17 were widely distributed and associated with diverse populations, supporting their broad geographic presence. H4 served as a common ancestor connecting Clade W and Clade MC1. H9 was a unique haplotype found only in Uttarakhand, bridging Clade MC1 and MC2. H17 connected Clade MC3 and the distinct Clade MC4.

A significant proportion (47 out of 68) of the samples from Uttarakhand belonged to the MC5 clade, previously known as D3. This clade includes sequences from domestic pigs of Bhutan, Nepal, and indigenous registered breeds of India, such as Doom, Mali, and Niang Megha, which are native to Assam, West Bengal, and Meghalaya, respectively. Museum specimens of wild sequences from India and Bhutan were also included in this clade.

Most clades had more than 50% posterior probability support. The W clade and D3 clade had a good posterior probability (>90%) support and the newly discovered (MC4) has 71% posterior probability support, hence this give us the confidence of the reliability of clades.

Figure 4.6 shows a visual representation of the distribution of sequences belonging to the W1, W2, and MC4 clusters according to the specified regions.

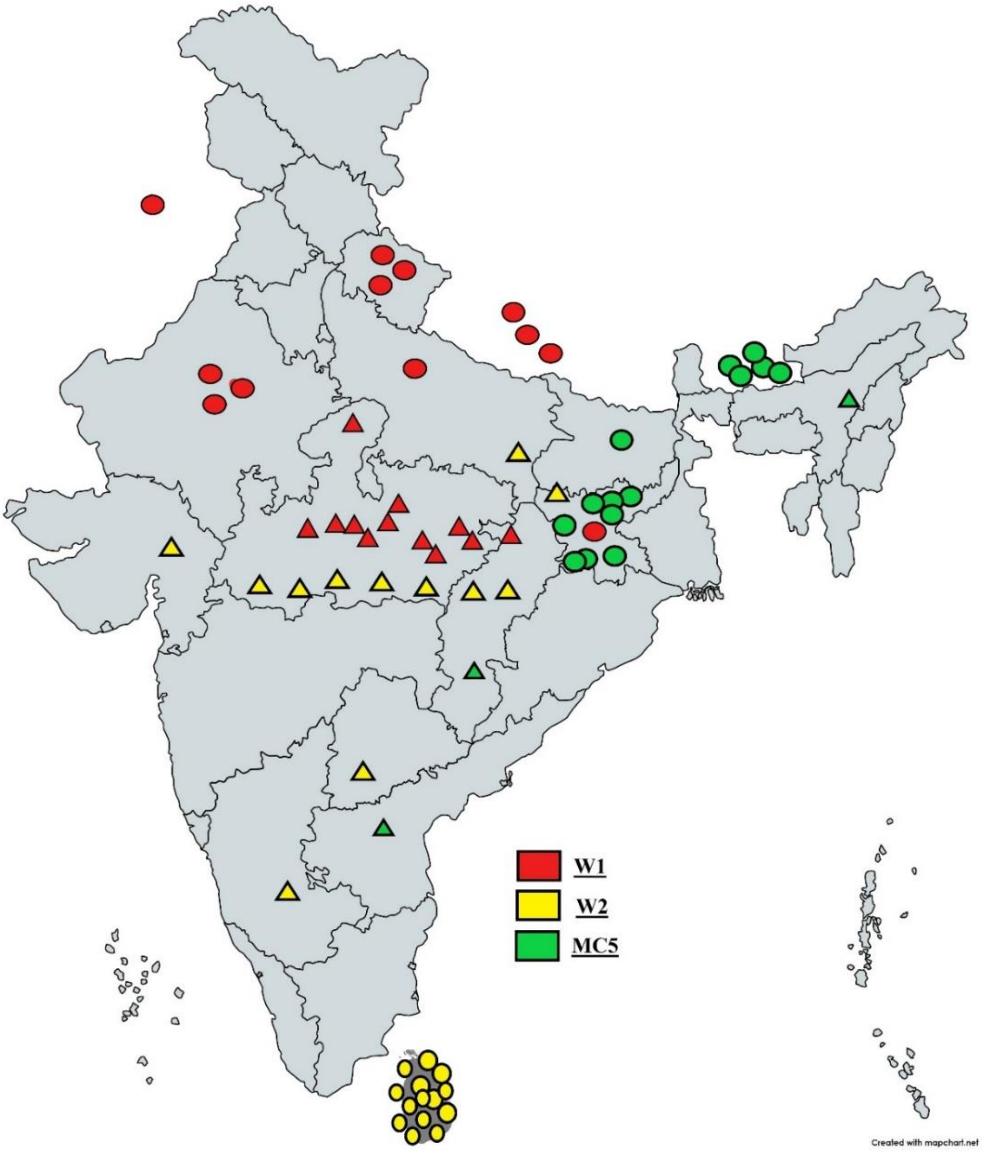

Figure 4.6: mtDNA control region haplotypes distribution amongst wild and domestic pigs in Indian subcontinent. Coloured triangles represent haplotypes with general location while circles represent haplotypes with specified states.

4.4.5 MEDIAN JOINING NETWORK ANALYSIS

The MJN analysis exhibited a similar clustering pattern to the Bayesian tree (Figure 4.7). It confirmed the distinct separation of the W (including W1 and W2), MC1 (D6), MC2 (D1), MC3 (D2), and MC5 (D3) clusters from the remaining clades, consistent with the phylogenetic tree. Notably, the newly discovered MC4 clade was observed within the general Asian cluster. Small black dots on the analysis represent median vectors (mv) representing hypothetical unsampled sequences that were not detected in this study.

The haplotypes H4, H9, and H17 were found within the Asian clusters and demonstrated a widespread distribution throughout continents.

Overall, the MJN analysis reinforces the findings of the phylogenetic tree, highlighting the distinct clustering of various clades and providing additional insights into the relationships and distributions of different haplotypes.

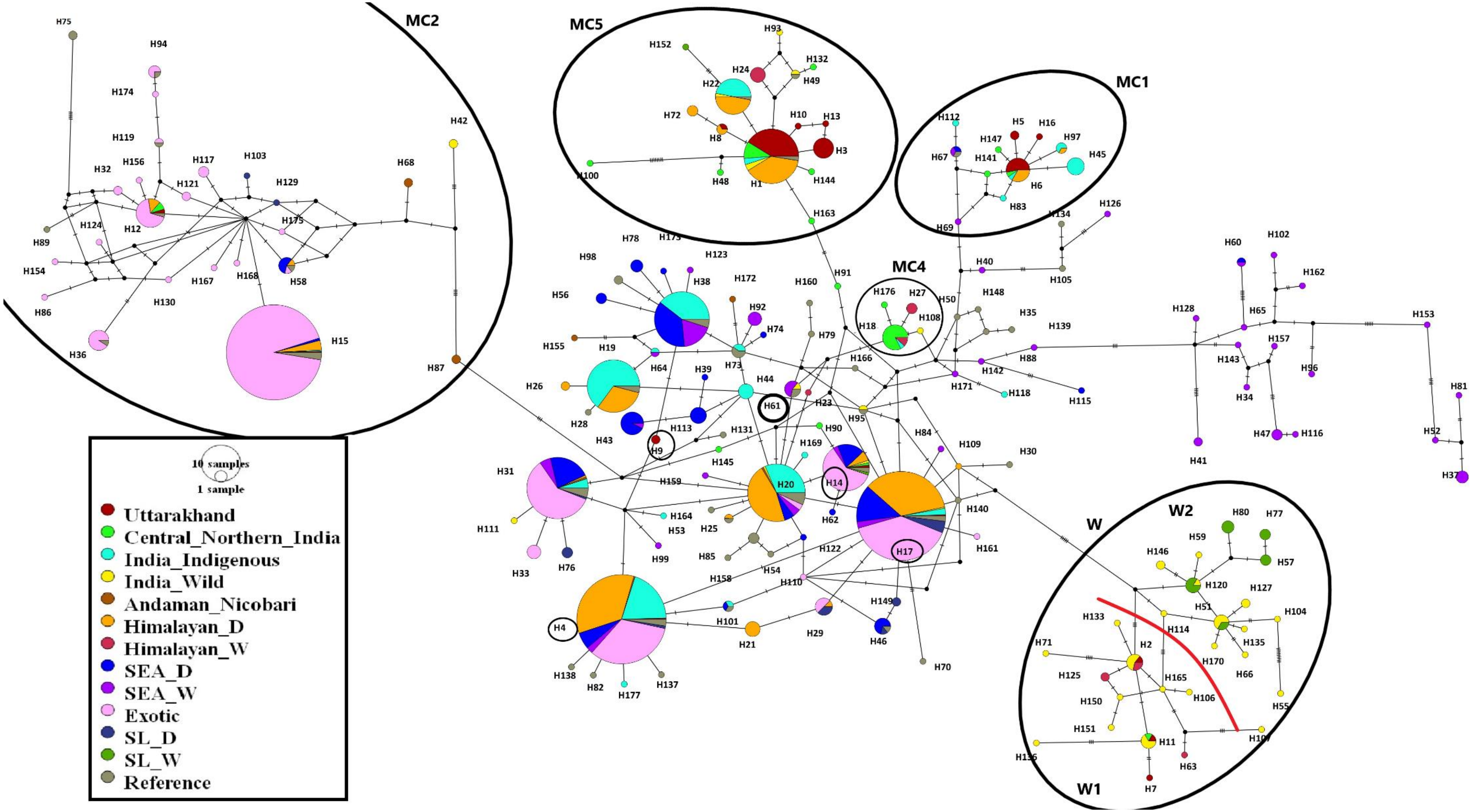

Figure 4.7: Relationship and clustering of 18 haplotypes (H1-H18) of Northern Indian States with 158 global sequences haplotypes (H20-H176) of mtDNA control region (marked). Geographical locations are given in colour and node size is proportional to the frequency of the corresponding haplotypes. Small black dots are median vector (mv). Each bar on the branch indicates one nucleotide substitution (or an insertion/ deletion).

4.5 DISCUSSION

4.5.1 MITOCHONDRIAL DNA LINEAGE IN UTTARAKHAND

This study provides insight on genetic structure and diversity, and population demography of domestic pigs raised in an Indian State located at the Himalayan foothills thus lay emphasis on further understanding of the widely researched pig domestication events at this geographic location. The dominant haplotype observed in the Uttarakhand cluster in D3 lineage that included both native wild boar and domestic pigs from India and Bhutan. Network analysis also revealed that most of the haplotype of Uttarakhand that lie in D3 cluster descended from one or a few ancestral types and the population has undergone a recent expansion. We confirm the presence of D3 lineage in the Indo-Gangetic Plain at the base of the mountains of the Himalayas which have shared haplotypes with Bhutan, and northern India (Tanaka et al., 2008). Further in this study, we reported the first signature of the Pacific Clade (D6), MTSEA, D1 (European) and the ubiquitously distributed D2 haplotypes in the domestic pig of Uttarakhand. These findings could potentially shed light on human-induced maternal gene flow via translocations, which have a higher influence on the genetic make-up of the current pig populations of Uttarakhand. The heterogeneity of haplotypes may be the relict of historical pig translocations due to the strategic location of Uttarakhand at the foothills of the Himalayas. This is facilitated by the historical role of north-eastern India, such as Assam, Manipur, and Mizoram, (LPAI, 2022) as a migration corridor between Southeast Asia and other regions that have served as connecting points, allowing for the exchange of cultures, languages, genes, and ideas throughout history (Reddy et al., 2007). The haplotype diversity as well as nucleotide diversity were high which can also be attributed to the presence of its haplotype in various haplogroup. The Uttarakhand pig population shows moderate differentiation from Asian populations and high differentiation from European populations. The ragged and multimodal pattern of mismatch distribution (Figure 4.3) suggests that the population has undergone irrelevant demographic expansion or may have been stable over a long period of time. Positive and non-significant Harpending's raggedness index confirmed the mismatch distribution of the studied populations. Therefore, the reflected mismatch distribution may be a signature of the presence of different haplogroups suggesting no relevant demographic fluctuation has occurred over a long time and multiple colonization has occurred possibly due to the presence of subgroups with different demographic histories rather than demographic stability. Overall, negative and non-significant Tajima D, Fu’s Fs test of neutrality (Table 4.3) values also suggest no demographic expansion or bottleneck.

Mismatch distribution was also calculated for the dominant D3 haplogroup present in Uttarakhand to investigate the hypothesis of population expansion. The haplotypic and genealogical relationships represented in the network showed star-like patterns (Figure 4.1) which indicates that the D3 haplotype has gone a recent expansion. A smooth and unimodal mismatch distribution (Figure 4.4) shows that most alleles descend from one or a few ancestral types. A significant and negative Tajima D and Fu’s Fs test of neutrality, along with the high and non-significant value of Harpending’s raggedness index (Figure 4.4) likely support a scenario of demographic expansion experienced in the past.

4.5.2 MITOCHONDRIAL DNA LINEAGES IN NORTHERN INDIA

The amalgamation of additional published data and samples from North Indian states collectively provides a comprehensive global perspective on the phylogenetic positioning of the studied samples of northern Indian states and enriched our understanding of the broader genetic context and evolutionary relationships. Our phylogenetic analysis revealed that domestic pigs raised in Uttarakhand exhibit the presence of multiple maternal lineages (D1, D2, D3, and D6 as designated by Larson et al., 2005),

4.5.3 PRESENCE OF DIFFERENT HAPLOGROUP OF *Sus scrofa* SPECIES IN UTTARAKHAND

The Indian subcontinent has played a crucial role as a migration corridor, facilitating movements between Southeast Asia and other regions. The region has witnessed numerous migrations, both incoming and outgoing, with migration routes often traversing through north-eastern India, including Assam, Manipur, and Mizoram, which serve as connecting points to Southeast Asia (LPAI, 2022). This geographical pathway has facilitated the exchange of cultures, languages, genes, and ideas between these regions throughout history.

The concept of a migration corridor is supported by the presence of genetic and linguistic connections between populations in the Indian subcontinent and Southeast Asia. Research exploring genetic markers and linguistic similarities has provided substantial evidence of shared ancestry and connections between specific populations in these regions, reinforcing the concept of a migration corridor (Reddy et al., 2007). These findings highlight the historical interactions and exchanges that have taken place, contributing to the cultural and genetic diversity observed in both regions.

The concept of a migration corridor, where human movements have played a crucial role in facilitating the spread of genetic diversity among pig populations across various geographical areas. This migration corridor has enabled the exchange and mixing of genetic material, resulting in the observed diversity and distribution of pig haplogroups. Therefore, the presence of different haplogroups in Uttarakhand can be attributed to the human-mediated migration of porcine mitochondrial DNA in these regions.

In a phylogenetic tree, the MC1 lineage branches into multiple subclades, indicating the presence of distinct lineages that have evolved from a common ancestor. These subclades may arise due to factors such as genetic variation and environmental pressures, leading to divergence in characteristics or traits among the organisms. The presence of the haplotype of

Ghungroo pig breed in the subclade with H5 and H6 haplotypes of Uttarakhand domestic breed suggests its distinctiveness as an indigenous pig breed in West Bengal. The widespread distribution of the Ghungroo pig indicates human-mediated dispersal, likely facilitated by the close geographical proximity between West Bengal and Meghalaya.

The H9 haplotype, positioned near the MC1 lineage in the basal portion of the phylogenetic tree, serves as a connecting link between the MC1 and MC2 lineages. Despite having only two samples from Uttarakhand and not sharing any haplotype with other sequences, its presence in the basal branch signifies its significance in unravelling the evolutionary history of the connecting clades. The rarity of this haplotype or its limited sampling suggests that further research is needed to fully comprehend its role and distribution among the pig populations under investigation.

The median joining network analysis of haplotypes H4 and H17 reveals a star like pattern, suggesting a recent population expansion. The diffuse nature of the network also indicates the possibility of multiple domestication events within the general Asian cluster, rather than just a single lineage. These haplotypes, found in the basal portion of the phylogenetic tree, hold ancestral significance and are believed to have migrated from the ISEA. Haplotype H4, with its wild counterparts in Vietnam and Malaysia, is widely distributed across different continents. It forms a basal haplotype that serves as a connection between the W clade and the MC1 lineage. Notably, a domestic sample from Uttarakhand is associated with this haplotype. Similarly, haplotype H17, whose wild counterparts are found in Vietnam, also occupies a basal position in the phylogenetic tree.

The presence of these haplotypes in basal portion of the phylogenetic tree suggests their involvement in ancient migration events and supports the hypothesis of multiple domestication events within the general Asian cluster. Further investigation is necessary to gain a comprehensive understanding of the genetic history and migration patterns associated with these haplotypes.

4.5.4 HYPOTHESIS ABOUT THE PROBABLE ORIGIN AND DISPERSAL OF THE CLADE MC5

Interestingly, we discovered that the haplotypes in the MC5 clade (similar to D3 lineage) exhibits a widespread distribution and are not only prevalent in Northern India (including Uttarakhand) and Central India but also extend to the north-eastern states such as Assam, Tripura, and Meghalaya. A considerable number of domestic pigs from Bhutan and Nepal share identical or closely related haplotypes with this haplogroup. The presence of these shared haplotypes suggests potential interactions and gene flow between wild and domestic populations in these areas and a star-like pattern in the median joining network, indicating geographic expansion. The haplotypes within this clade also shared the haplotype with museum samples of wild boars from Northern India (Kashmir, West Bengal, and Chattisgarh). These findings supports the existence of an independent centre of "cryptic domestication" in the foothills of the Himalayas and the Indian subcontinent. Previous studies have reported the presence of wild pig lineage *S. s. cristatus* in the Himalayas, including Northern Indian states such as Jammu and Kashmir, central states, and West Bengal (Larson et al., 2005; Tanaka et al., 2008). This indicates a historical presence of wild boar populations in these regions, which likely played a role in the domestication process of pigs in the area.

Moreover, MC5 clade, which comprises the haplotypes found in the wild pig museum samples from Jammu Kashmir and the ancient sample from Nepal reported by Nidup (2011), provides valuable insights into the origin, evolution and migration of this lineage. It is worth noting that the ancient sample from Nepal, estimated to be approximately 1500 years old, indicates that the ancestral history and geographic distribution of the MC5 clade date back more than 1500 years. This information suggests that the genetic characteristics and lineage of the MC5 clade have roots in a time period that predates the estimated age of the ancient sample. It does strongly support the idea that the MC5 wild haplotype did not enter domestic pigs recently. The ancient pig sample from Nepal shows that wild boars and domestic pigs shared maternal lineages, indicating historical genetic exchanges and hybridization between these populations.

One potential hypothesis regarding the origin and dissemination of this particular clade (MC5) suggests its association with the ancient Indus River Valley Civilization, commonly known as the Harappan Civilization This civilization thrived approximately 5,300 to 3,300 years ago in present-day Pakistan and north-western India. However, around 4,500 years ago, the Harappan Civilization experienced a decline attributed to environmental changes, prompting the migration of its people towards the eastern regions near the Himalayan foothills (Malik, 2020). In these new areas, they established small agricultural communities while preserving their cultural practices. One of these practices might have been the consumption of wild pigs (the domestication status of pigs during the Harappan civilization remains uncertain and unclear). This was supported by the presence of pig bones, which make up about 2-3% of the animal remains found at Indus sites provides evidence supporting this assumption (Chase, 2014). Furthermore, the analysis of lipid residues found in ceramic pots from Harappan sites has confirmed the presence of pork residues (Suryanarayan et al., 2021).

Based on this evidence, we can speculate that the MC5 haplotype originated from the Harappan Civilization and spread to the Himalayan region through migration. However, this speculation requires more genetic and archaeological data to confirm.

The W1 clade encompasses samples from various regions such as northern and central India, Nepal, and Pakistan, aligning with the geographic distribution of the MC5 clade. Currently, there is no evidence suggesting any maternal genetic contribution from W1 wild boars to the modern domestic pigs of that region. However, the presence of three specific haplotypes (H2, H7, H11)/UKD haplogroup observed in domestic pig samples from Uttarakhand and Jharkhand raises the possibility of wild pig farming practices in these regions. It is plausible that wild pigs were captured, domesticated, and raised alongside domestic pigs, either in herds or as individual piglets, due to their easy availability. In fact, during our fieldwork in Uttarakhand, we have even observed two instances of such practices. It is worth noting that the subspecies *Sus scrofa davidi*, occurring in northwest India, Pakistan, and south-eastern Iran, has been confirmed (Groves et al., 2008, Choi et al., 2020) in this study. Hence, the presence of a distinct subspecies of wild boar with a strong phylogeographic signal is confirmed within this clade. We suspect that the W1 clade represents this subspecies, *Sus scrofa davidi.*

The results of the study reveal a strong coexistence between the two subspecies of wild pigs, namely *Sus scrofa cristatus* and *Sus scrofa davidi*, in the foothills of the Himalayas and northern India. These findings imply a close association and integration between the two subspecies, indicating potential interactions between them in this particular region.

The second divergent clade, W2, provides confirmation of Nidup's 2011 findings regarding a distinct mitochondrial DNA (mtDNA) clade. This clade includes the Sri Lankan wild pig as well as wild pigs from northern, central, and southern India, which most likely correspond to the subspecies *Sus scrofa affinis* (Groves 2008). The wild boar population in Sri Lanka can potentially be recognized as another authentic subspecies, but it has not contributed maternal genetic material to modern domestic pigs.

The mitochondrial DNA (mtDNA) of Sri Lankan village pigs differs significantly from that of their wild counterparts (Nidup 2011). Interestingly, the genetic composition of Sri Lankan village pigs shows a closer resemblance to immigrant pigs from diverse regions around the world, primarily falling within the broader Asian cluster depicted in our tree (Figure 4.6). However, to gain a clearer understanding of the migration patterns and genetic makeup of these pigs, additional samples from South Indian states of India would be valuable. Further research in this area can provide more insights into their genetic heritage and potential migration routes.

4.5.5 ORIGIN AND MIGRATION OF TWO DISTINCT CLADES OF WILD PIG LINEAGE

The presence of two distinct clades of wild pig lineage (W1and W2) in India was also supported by the median joining network. The phylogenetic tree reveals that the H4 haplotype, which is widespread and found in wild pigs from Malaysia and Vietnam, occupies a basal position of the phylogenetic tree connecting clade W to clade MC1. This suggests that the H4 haplotype was present in the common ancestor of both clades and indicates that the earliest lineages of *S. scrofa* originated in island Southeast Asia (ISEA) (Figure 4.8). The haplotype's presence in the Indian subcontinent after dispersal from ISEA suggests that it is an ancient form predating the divergence of the two clades. Consequently, the H4 haplotype provides valuable insights into the evolutionary history and dispersal of this wild pig lineage (Choi et al., 2020, Larson et al. 2005).

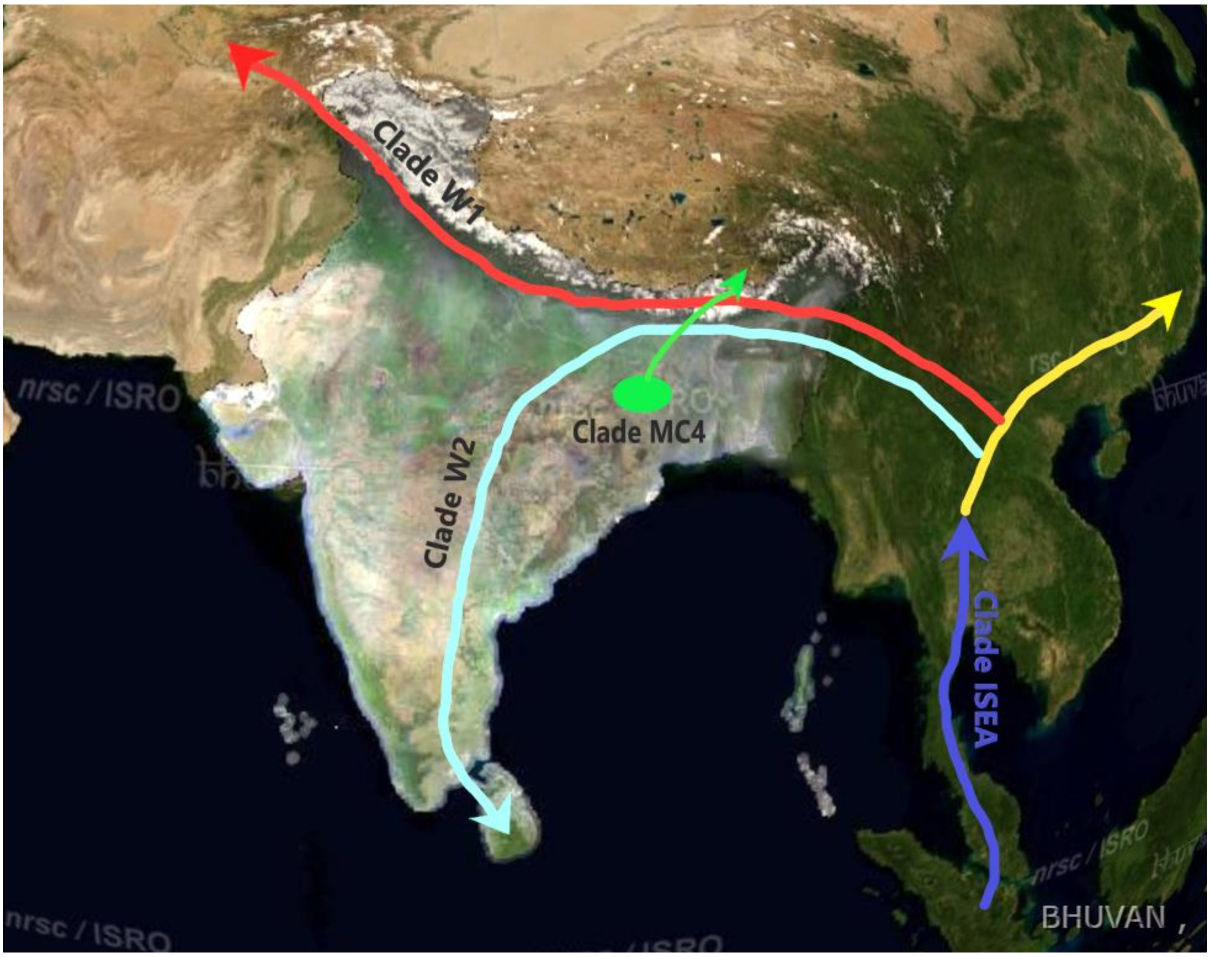

Figure 4.8: Suggested migration routes of wild boar in Indian Subcontinent

4.5.6 HYPOTHESIS ABOUT THE PROBABLE ORIGIN AND DISPERSAL OF NEWLY DISCOVERED CLADE MC4

A new lineage of wild pig, called MC4, has been found in India, besides the previously known wild pig clades. This lineage includes all the samples from Bihar and Jharkhand in eastern India and Central India. Interestingly, the MC4 lineage also contains haplotypes of wild pigs from Bhutan. The sequences from Bhutan in the MC4 lineage belong to a new haplotype of wild pig, which was earlier classified as the W12 clade in Nidup's 2011 study. The W12 clade (Nidup 2011), represents the D5 haplogroup (Larson et al. 2005), shared haplotypes with wild boars from Myanmar, Bhutan and the nearby Indian state of Sikkim. However, our MC4 clade does not include samples from Myanmar and Sikkim.

The median-joining network analysis reveals that the D5 haplotype (H61) is distinct and includes wild pig samples from Myanmar, Thailand, and Sikkim, but not from Bhutan. Notably, this haplotype was observed as a descendant haplotype originating from the widely distributed H20 haplotype. The H20 haplotype was found in wild pig populations in India, Malaysia, and Vietnam and belongs to the D2 group within the haplogroup. This finding aligns with Larson's suggestion that all these lineages have emerged from the ISEA (Indonesia and Southeast Asia) haplogroup.

The available data concerning the newly discovered wild pig lineage, MC4, provides intriguing evidence suggesting the possibility of cryptic domestication or introgression of maternal genetic material from the MC4 wild boar lineage into the local domestic pig population within the respective regions. The samples collected from Jharkhand initially represented a domestic species. However, with the recent increase in the development of the piggery sector in Jharkhand, driven by government initiatives, and the generation of crossbreeds between indigenous pigs of Jharkhand and the wild there has been an observed increase in maternal gene flow.

The MC4 lineage occupies a basal position in the phylogenetic tree and, although it falls within the general Asian cluster in the network analysis and exhibits a star-like pattern. Further sampling is required in this region to better understand the unique population characteristics and establish its distinctiveness.

The MC4 clade includes the haplotype of wild and domestic pigs that share a common maternal ancestor mainly found in Eastern India and Bhutan. The two wild samples from Bhutan in this clade are from the southwestern while other samples are from south-eastern and south central part of Bhutan. This clustering offers a distinct perspective on the domestication events in this region. The inclusion of the wild pig haplotype from the southwestern region of Bhutan into this clade affirms the statement that this region of pig in Bhutan has undergone gene exchange with East Indian wild boars (Tanaka et al., 2008). Network analysis shows this haplotype lies in Asian cluster with a distinct circle of haplotype emerging from the Asian cluster. It also shows the presence of number of black circles, which represent the missing haplotypes and further sampling in these locations can confirm the presence of another lineage of wild pigs in India.

We propose the presence of this clade in the region based on multiple lines of evidence. One such line of evidence stems from the linguistic diversity found in the Indian subcontinent, which encompasses four major linguistic groups: Austro-Asiatic, Dravidian, Indo-European, and Tibeto-Burman. These groups are believed to have migrated to the region at different time periods (Gadgil et al., 1997). Among these linguistic groups, the Austro-Asiatic family is considered the oldest and exhibits the highest level of divergence. Notably, the Austro-Asiatic family can be further classified into three distinct sub-families, namely (1) the Austro-Asiatic tribal communities, (2) Khasi-Khmuic, which includes the Khasi tribes confined to the Khasi and Jantia hills of Meghalaya, and (3) Mon-Khmer, which settled in Southeast Asia.

The Mundari branch of the Austro-Asiatic tribal communities primarily resides in the vicinity of the Chotanagpur plateau, where the presence of clade MC4 was detected. This geographical region encompasses parts of Odisha, Jharkhand, Madhya Pradesh, West Bengal, and north-eastern Maharashtra. These communities inhabit neighbouring areas characterized by similar habitats, including hills and forests (Kumar *et al.* 2003). The Mundari populations have a traditional lifestyle centred on hunting and gathering. They predominantly inhabit regions unsuitable for agricultural practices, indicating the continuation of their ancestral way of life. Therefore, the hypothesis suggesting their migration during the Neolithic era, when agricultural communities expanded, seems unlikely (Thangaraj et al., 2005).

The analysis of mtDNA in the present-day Mundari populations indicates that their ancestors migrated to India during the Pleistocene era (Kumar et al., 2007). This finding aligns with the archaeological evidence that suggests human presence in India since the early Paleolithic era (Lal BB, 1956). This evidence of the past show that the haplotype H18 of the pig is concentrated in this region and as these human communities have maintained their ancestral ways of subsistence hence the migration of this linguistic community is limited. Hence, the haplotype of the pig raised by this community is only restricted to eastern India and Bhutan.

4.5.7 INFLUENCE OF WILD BOAR ON DOMESTIC PIG IN UTTARAKHAND

At present, the lack of sufficient ancient and modern boar samples from Uttarakhand hinders the provision of evidence for a local center of domestication or the introgression of wild genes into domestic populations. However, given the socio-cultural and economic significance of wild boar to various communities, as well as their frequent interaction with local pigs, we cannot dismiss the possibility of maternal genetic introgression. It is important to note that the introgression of paternal genes from wild boar to local pigs cannot be completely ruled out either, especially due to the scavenging system of pig farming in the area. In this farming practice, domestic sows or gilts often have opportunities to mate with wild boars from nearby village forests, potentially leading to the introgression of nuclear genes without concurrent changes in mitochondrial DNA. Microsatellite data also reveals the presence of such introgression. Thus, while the evidence suggests the existence of genetic exchange between wild boars and local village pigs, further research is needed to elucidate the extent and mechanisms of this introgression.

4.5.8 GENETIC INFLUENCE OF EUROPEAN PIGS IN THE LOCAL PIG POPULATION IN UTTARAKHAND

Out of the 68 samples collected from individuals, only 2 samples showed evidence of maternal lineage with European mitochondria. This indicated a relatively low level of introduction and introgression of exotic breeds and their paternal nuclear genes into the local pig population. It is interesting to note that despite the increasing preference for exotic pig breeds and crossbreeds among pig breeders, driven by changes in consumer taste preferences, the observed level of introgression remains minimal. In Uttarakhand, breeds such as Landrace, Yorkshire, and their hybrids are being raised (Breed survey 2013). The presence of exotic pigs can be seen in the local herds. Consequently, there is a possibility of paternal introgression of nuclear genes from European breeds into the local pig population. Microsatellite evidence in chapter 5 supports this possibility, indicating potential paternal contamination of the local pigs with nuclear genes from European breeds. However, the observed introgression remains relatively low.

Overall, this chapter highlight the diverse maternal lineages present in domestic pigs in Uttarakhand and their genetic connections with both wild boars and domestic pigs across various regions, suggesting a complex history of domestication and population expansion in the Himalayan foothills and the Indian subcontinent. This observation provides evidence for the Indian subcontinent acting as a migration corridor, facilitating the movement of wild boars between Southeast Asia and the Indian subcontinent. The presence of multiple maternal lineages in Uttarakhand signifies the historical connectivity and exchange of genetic material between these regions, highlighting the significance of this area in the broader context of wild boar dispersal and evolutionary dynamics.

This chapter provides conclusive evidence of the existence of at least four distinct mitochondrial lineage populations of the wild boar in Northern India and Central Provinces.

These populations are likely associated with three different subspecies of wild boars, *S. s. cristatus, S. s. davidi,* and *S. s. affinis*. This revealed that the wild pig populations in the Indian subcontinent have undergone genetic differentiation, indicating the presence of unique genetic variations. These variations can be attributed to factors such as adaptation to local habitats, historical isolation, and genetic drift. The study also reveals the presence of a significant concentration of wild boars in eastern and central India, specifically in the Chotanagpur plateau. This finding highlights a distinct population of wild boars in this region, suggesting a localized distribution and potential ecological significance. The concentration of wild boars in the Chotanagpur plateau adds to our understanding of the regional dynamics and conservation efforts for wild pig populations in India.

To gain a comprehensive understanding of the genetic diversity and population dynamics of wild boar in India, further research is imperative. In particular, future investigations can focus on exploring additional lineages of wild boar, especially in South India. Expanding our knowledge in these areas will be crucial for a more comprehensive understanding of wild boar populations in India.

Therefore, while the analysis supports the possibility of introgression, it emphasizes the need for comprehensive investigations that include additional lines of evidence, such as archaeological and fossil records, to gain a more thorough understanding of the domestication history and genetic dynamics of domestic pigs in the Himalayan region.

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CHAPTER 5

GENETIC VARIATION USING NUCLEAR MICROSATELLITE MARKERS

5.1 SUMMARY

Genetics play a crucial role in animal production and can significantly impact the quality and productivity of breeds. However, there is a growing concern regarding the potential loss of genetic diversity in naturally adapted animal breeds due to habitat loss, inbreeding, and selective breeding. This can lead to reduced adaptability to changing environments and increased susceptibility to diseases. This chapter assessed the genetic diversity of domestic pigs raised in Uttarakhand, an Indian state situated at the Himalayan foothills. Genotypes of 67 animals were analyzed, using 13 microsatellite loci. The observed (Ho) and expected (He) heterozygosities were 0.83±0.02 and 0.84±0.01 respectively. The average polymorphic information content (PIC) values 0.83±0.01 indicate the highly informativeness of the marker. The overall mean FIS value considering all the microsatellite markers was low (F = 0.04, P<0.01). Seven loci deviated from Hardy-Weinberg equilibrium (HWE) at a significant level ($p < 0.05$). Two clusters were identified, indicating overlapping populations. The low differentiation and significant F_{ST} value ($F_{ST} = 0.09$, $P < 0.001$) support these findings. These results suggested that the traditional management practices in Uttarakhand have allowed for genetic mixing and the sharing of genetic material among pig populations. This can contribute to increased genetic diversity but may also result in the loss of distinct genetic characteristics or breed purity of the local breeds, if not carefully managed.

5.2 INTRODUCTION

According to the Food and Agriculture Organization (FAO), animal genetic resources for food and agriculture (AnGR) provide crucial options for the sustainable development of livestock production. Erosion of within-breed diversity can be a problem even in breeds whose total population size remains large. The state of Uttarakhand occupies the northern part of India and lies on the southern slope of the world's youngest mountain system, "the Himalayas". It shares its international boundaries with Tibet in the north and northeast, and Nepal in the east and southeast (UBB 2002). Mountainous terrain makes animal husbandry practices the backbone of the state’s economy.

Pigs are found throughout India but are mainly raised by certain ethnic groups due to the influence of the caste system. However, they are still an important animal genetic resource used for their economic, sociocultural, and nutritional purposes. This is particularly true in areas where pig rearing is culturally and socially accepted, regardless of whether they live in urban or rural areas.

The local swine meat is an essential ingredient in many traditional dishes in India (Govindasamy, 2018) and is very popular within select communities. This highlights the important role that pigs play in the food culture and traditions of certain communities and also offering income and livelihood to the communities that raise them.

While policies are in place to improve the performance of pig farming in India, its overall contribution to the farmed species at the national level is still relatively low at 1.69% (20th Livestock census 2019). This could be due to a variety of factors, including cultural and social factors that limit the practice of pig farming to certain communities as well as limited access to resources and markets. The majority of the pig population in India is composed of indigenous breeds, which comprise 76% of the total population. The fact that these breeds are so prevalent underscores the importance of preserving India's genetic resources and supporting sustainable animal husbandry practices. The major concentration of pig population comes from the eastern and north eastern regions of India, which accounts for around 63% of the total population (20th Livestock census 2019). Over millennia, the journey from pig domestication to extensive natural selection for agro ecological adaptation has established the existence of many indigenous pig breeds in India. Presently, thirteen breeds have been characterized and officially recognized as indigenous registered breeds, which are mostly from the eastern states of the country (www.nbagr.res.in). Indian indigenous pigs are an integral part of the swine germplasm, not only because of their high phenotypic diversity, unlike most other domesticated species, but also because of the availability of wild ancestry. Breed characterization and cataloguing of a diverse population has gained momentum in recent years in India. To date, thirteen indigenous pigs (Agonda Goan, Ghoongroo, Niang Mega, Tenyi Vo, Nicobari, Doom, Nowak, Purnea, Banda, Manipuri Black and Wak Chambil have been characterized and have accorded the status of indigenous registered breeds (ICAR- National Bureau of Animal Genetic Resources), which are mostly from the eastern states of the country.

The new animal husbandry practices are altering the diversity of animal genetic resources by generating novel genetic types that are not fitted to the production system and these changes can have both positive and negative impacts on animal genetic diversity (Kantanen et al., 2015). Genetic diversity plays a pivotal role in upholding the endurance of animal populations against environmental challenges, including climate change, emerging diseases, resource constraints, and evolving market demands (FAO 2007; FAO 2011). This diversity is shaped by factors like population size, historical context, ecological dynamics, and adaptability. It is a vital source of traits that drive species enhancement, survival, and adaptation (Phocas et al., 2016). On the one hand, the development of new breeds or strains can help to improve animal productivity and contribute to food security. On the other hand, the loss of traditional breeds or strains through replacement or displacement can lead to a reduction in genetic diversity, which is essential for maintaining the resilience of animal populations to environmental and other stresses. (FAO 2007; FAO 2011). Recent advancements in molecular biology and genetics have made cost-effective breed characterization possible. Molecular markers, such as nuclear microsatellites are often used to assess genetic diversity.

Microsatellites are inherited from both parents, enhancing genetic diversity and displaying significant polymorphism. Their efficacy lies in studying intra-population genetic variations and inter-individual relationships within a breed (Nidup, 2011). Therefore, understanding the genetic diversity and phylogeny of indigenous pigs can help in developing conservation strategies, designing effective breeding programs, and formulating management policies (Notter, 1999). Over the past several years, Indian pig breeds diversity has been studied in detail (Kaul 2001; Behl et al., 2005; Zaman et al., 2013a, b; 2014a, b, c, d; Sahoo et al., 2015, 2016, 2017; Boro et al., 2019). The present chapter focused on population genetic structure to address the following research questions (a) what is the level of genetic structuring in of domestic pigs currently raised in an Indian State (Uttarakhand) located at the Himalayan foothills (b) what is the genetic diversity of domestic pigs kept by the pig breeders. Hence it will generate baseline genetic information to devise a suitable strategy for conserving biodiversity and ensuring sustainable utilization of swine genetic resources in the region.

5.3 METHODOLOGY

5.3.1 SAMPLE COLLECTION

In total, 67 non-invasive biological (hair) samples were collected from the pig rearer household that raised non-descript pigs (Appendix 1). There were 53 samples from Dehradun, 2 samples from Haridwar, and 7 samples from Nainital (Figure 5.1). These pigs have not been formally selected for any commercial production traits and are raised by specific group of community in Uttarakhand. Unrelated animals (2–3 per household) were selected and sampled because the surveyed households did not have a well-structured system of recording breed and breeding system practices.

Coarse hair with large follicles were obtained from the top of the neck. To collect hair roots, 10-15 hairs were grasped close to the skin and pulled out. After ensuring that hair roots were not covered with faecal matter or dirt, samples were placed in a labelled zip lock and immediately sealed to minimize contamination. The samples were preserved at -80°C until DNA extraction.

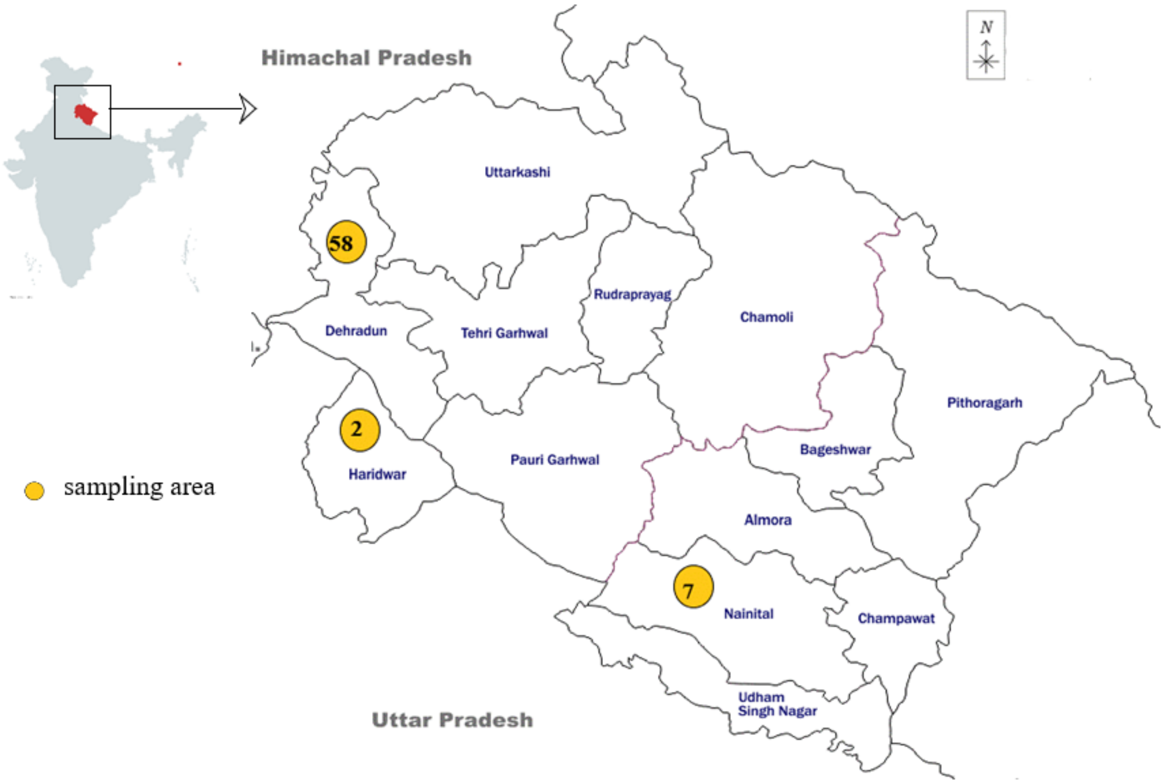


Figure 5.1: Sampling areas in Uttarakhand (Number within each circle indicates number of pigs sampled from each district).

5.3.2 DNA EXTRACTION

The genomic DNA (gDNA) was extracted from the samples using DNeasy Blood & Tissue kit (Qiagen, Hilden, Germany) in a final elution volume of 40–80 μL. DNA was stored in -20°C until further processing.

5.3.3 SELECTION AND SCREENING OF MARKERS

We used 14 microsatellites loci (Table 5.1) for assessing the genetic variability in the domestic (67 samples) population of pig raised in Uttarakhand. Microsatellites were chosen based on their ease of scoring, location and informativeness. All other markers were recommended by FAO and the International Society for Animal Genetics (FAO 2011). Six sets of multiplex panels were carefully assembled based on amplicon size and labelled fluorescent dyes of loci. To avoid ambiguity and amplification errors, for each sample, three multiplex PCR were carried out in 10 μl reaction volumes containing 5 μl of QIAGEN Multiplex PCR Buffer Mix (QIAGEN Inc.), 0.2 μM labeled forward primer (Applied Biosystems), 0.2 μM unlabelled reverse primer, and 20–100 ng of the template DNA. Conditions of PCR amplification were: 95°C (15 min), then 8 cycles at 95°C (30 s) / 62°C (90 s) /72°C (30 s), followed by 14 cycles of 95°C (30 s)/ 57–50°C (decreased by 0.5°C per cycle for 90 s) /72°C (30 s), again followed by 12 cycles of 95°C (30 s)/ 52°C (90 s) /72°C (30 s) and then final extension at 60°C for 30 minutes.

The reliability of reactions was monitored using positive and negative controls. Alleles were resolved in an ABI 3500XL Genetic Analyzer (Applied Biosystems) using the LIZ 500 Size Standard (Applied Biosystems). Within this temperature range and conditions 13 markers were successfully amplified (except SW632).

5.3.4 NUCLEAR MICROSATELLITE VARIATION

The alleles were scored using the program GeneMarker v2.7.4 (SoftGenetics, LLC) with combined automated allele scoring and validated through visual inspection (Hulce et al., 2011). The presence and frequency of null alleles were estimated using the Micro-Checker 2.2.3 software and Brookfield 1 (Oosterhout et al., 2004).

The likelihood of two unrelated individuals and siblings sharing the same genotype (probability of identity, PID and probability of identity for siblings, PID_{sibs}), the probability of identity with both the unbiased equation for small sample size (PID), the equation for siblings (PID_{sibs}), and estimate of genotyping errors [i.e. allelic dropout (ADO) and false alleles (FA)] from replicate genotyping were executed in Gimlet v.1.3.3 (Valiere, 2002).

The overall summary statistics comprising of the number of alleles per locus, observed heterozygosity, expected heterozygosity, and list of private alleles with its frequencies were calculated in GenAlEx V.6.3. (Peakall, 2012). The inbreeding coefficient (Fis) per locus were calculated with the FSTAT 2.9.3.2 software (Goudet, 2002).The matrix of genetic distances (Nei M 1972) between the samples were calculated with the software GenAlEx V.6.3. and used to construct a Neighbor-Joining (NJ) tree with MEGA 11 (Tamura et al., 2021). The phylogenetic tree was constructed with the FigTree application version 1.4.2 (Rambaut, 2014) using a JAVA application environment. Individual identity confirmation and The Polymorphic Information Content (PIC) values were estimated in the program CERVUS v.3.0.7 (Kalinowski et al., 2007).

A Fisher's exact test was performed to determine possible deviations of the markers from the Hardy–Weinberg equilibrium (HWE) (Guo et al., 1992) and Linkage disequilibrium for all marker pairs was estimated by G test (log likelihood) parameter using GenePop software version 1.2 (Raymond et al., 1995; Rousset, 2008).

Allele richness per locus and private allele richness in the individual samples was calculated using a rarefaction approach implemented in HP-Rare v1.1 (Kalinowski, 2005).

5.3.5 POPULATION GENETICS STRUCTURE AND GENETIC DIFFERENTIATION

We inferred the population genetic structure using the Bayesian approach implemented in Structure v2.3.4 (Pritchard et al., 2000). Structure is a systematic model-based Bayesian clustering approach that uses allele frequencies at each locus to infer the population structure. The analysis was performed for 1–8 clusters (K). For each K, ten iterations were run under the admixture model with correlated allele frequencies and sampling location as a priori. The LOCPRIOR (sampling location as a priori) model performs well when no clear signal of a structure is detected or when there is low genetic differentiation, limited loci or a limited sample size (Hubisz et al., 2009). The simulations were run for 50,000 burn-in and 500,000 Markov chain Monte Carlo iterations (MCMC). This setting produced consistent estimations that were not significantly altered by a longer burn-in or Markov chain Monte Carlo (MCMC). The optimum number of K was inferred using the likelihood distribution L(K) and the delta K (Evanno, 2005), which were estimated using the web version of Structure Harvester, v0.6.94 (Earl et al., 2012). The ADMIXTURE results were visualised using the CLUMPAK software (Kopelman 2012). Additionally, we used the DAPC, a multivariate nonmodel-based approach, to identify and describe genetic clusters (Jombart, 2010). The optimal number of the clusters was estimated based on the lowest associated BIC. The analysis was performed using the adgenet in R studio.

The GENETIX 4.05 software (Belkhir et al., 2004) package was used to spatially plot clusters and individuals based on the allele frequencies of all loci and a correspondence analysis in which the Chi-square distances served to judge the proximity of the clusters.

The result generated by Structure software v2.3.4 (Pritchard et al., 2000) assisted in defining the number of populations this help us to estimate the proportions of the total genetic variance arising from within and between the identified genetic groups of pigs populations, using analysis of hierarchical molecular variance (AMOVA) in Arlequin software version 3.5.1.3. (Excoffier and Lisher 2010).

5.3.6 BOTTLENECK DETECTION

A bottleneck analysis was performed to check whether the sampled pig population in Uttarakhand underwent a recent bottleneck by using the Bottleneck v1.2.02 software (Piry et al., 1999). The estimation of demographic changes was done using qualitative HET approach implemented in Bottleneck v1.2.02. The HET approach assumes that in a recently reduced population, an excess of the gene diversity under Hardy–Weinberg equilibrium is expected relative to gene diversity under mutation-drift equilibrium. One-tailed Wilcoxon test was used to determine the presence of a significant number of loci with excess heterozygosity. The estimates were calculated under an infinite allele model (IAM), two-phase model (TPM), and stepwise mutation model (SMM). The TPM tends to be the most appropriate mutation model for microsatellite loci (Di Rienzo et al.,1994) The TPM was carried out at 95 % probability (variance at 15), and the simulations were run for 10000 iterations.

Table 5.1: Microsatellite markers with their chromosomal location and primer sequences with annealing temperature and allele range.

Sl.no.	Oligo name	Chromosomes	Sequence 5' to 3'	Annealing Temp.	Universal primers	Allele Range (given) (bp)	Allele Range (bp) observed
1	S0155-F	1q	D-TGTTCTCTGTTTCTCCTCTGTTTG	55	M13 FAM	142-162	156-186
	S0155-R		AAAGTGGAAAGAGTCAATGGCTAT				
2	S0226-F	2q	D-GCACTTTTAACTTTCATGATACTCC	55	M13 FAM	180-210	190-236
	S0226-R		GGTTAAACTTTTNCCCCAATACA				
3	SW72-F	3p	D-ATCAGAACAGTGCGCCGT	55	M13 FAM	97-114	116-136
	SW72-R		TTTGAAAATGGGGTGTTTCC				
4	IGF1-F	5	D-GCTTGGATGGACCATGTTG	55	M13 FAM	193-209	203-235
	IGF1-R		CATATTTTTCTGCATAACTTGAACCT				
5	SW122-F	6	D-CAAAAAAGGCAAAAGATTGACA	55	M13R HEX	106-128	117-139
	SW122-R		TTGTCTTTTTATTTTGCTTTTGG				
6	SW632-F	7	D-TGGGTTGAAAGATTTCCCAA	55	M13R HEX	148-178	163-189
	SW632-R		GGAGTCAGTACTTTGGCTTGA				
7	S0178-F	8	D-TAGCCTGGGAACCTCCACACGCTG	60	M13R HEX	101-128	125-147
	S0178-R		GGCACCAGGAATCTGCAATCCAGT				
8	SW911-F	9	D-CTCAGTTCTTTGGGACTGAACC	60	M13R HEX	149-173	164-198
	SW911-R		CATCTGTGGAAAAAAAAAGCC				
9	SW830-F	10	D-AAGTACCATGGAGAGGGAAATG	50	M13R HEX	168-203	191-224
	SW830-R		ACATGGTTCCAAAGACCTGTG				
10	Swr1941-F	13	D-AGAAAGCAATTTGATTTGCATAATC	55	T7 TET	202-224	224-250
	Swr1941-R		ACAAGGACCTACTGTATAGCACAGG				
11	SW857-F	14	D-TGAGAGGTCAGTTACAGAAGACC	55	T7 TET	141-159	158-242
	SW857-R		GATCCTCCTCCAAATCCCAT				
12	S0355-F	15	D-TCTGGCTCCTACACTCCTTCTTGATG	50	T7 TET	244-271	249-299
	S0355-R		TTGGGTGGGTGCTGAAAAATAGGA				
13	S0218-F	X	D-GTGTAGGCTGGCGGTTGT	55	T7 TET	158-205	169-207
	S0218-R		CCCTGAAACCTAAAGCAAAG				

D = Dye labelled.

5.4 RESULTS

5.4.1 GENOTYPING QUALITY AND ERROR RATES.

The average amplification success rate of the polymorphic loci was 97.23% for all the pig samples of Uttarakhand. The average allelic dropout (ADO), false allele (FA) and null allele frequency across polymorphic loci were below 5% in the populations (Table 5.2). The occurrence of large allele dropout was not detected in our data. All samples amplified the 13 tested microsatellite loci successfully. We observe the presence of null allele at one locus (SW830). There was no evidence for scoring error due to stuttering.

Table 5.2: Amplification success and error rates calculated for polymorphic loci.

Locus	AS (%)	ADO	FA	Null Present	NA frequencies (Brookfield 1)
IGF1	96	0	0	no	-0.02
SW122	90	0	0	no	0
S0155	100	0	0	no	-0.01
S0178	100	0	0	no	0
S0226	97	0	0	no	0.02
SW632	99	0	0	no	0.02
SW72	100	0	0	no	0
SW830	100	0	0	yes	0.1
SW857	96	0	0	no	-0.01
S0355	94	0	0	no	0.03
SW911	99	0	0	no	-0.03
SWr1941	100	0	0	no	0.01
S0218	93	0.67	0	no	0
Mean	97	0.05	0	-	0.009

Amplification Success (AS); Quality Index per locus (QI); Allele Drop out (ADO); False Allele (FA) and Null Allele (NA).

5.4.2 NUCLEAR MICROSATELLITE VARIATION

All the individual samples used in the study were a distinct with no matching genotypes. The genetic diversity estimates are summarised in (Table 5.3). 171 alleles from the 13 microsatellite loci were identified in the 67 evaluated samples of the pigs from Uttarakhand. 11 samples showed private alleles richness in the genetic group. The number of alleles obtained individually inside the tested loci varied from 11 (SW122 S0178 SW72) to 17 (S0226). The effective number of alleles varied from 4.87 to 10.14 and was proportional to the values of expected heterozygosity found in loci S0155 (0.79) and IGF1 (0.90), respectively. The estimates of allelic richness obtained in studied genetic group, showed again that the locus S0155 presented the lowest value (5.50) and locus IGF1 the highest one (7.99). The mean number of alleles (Na) was 13.15 and the mean number of effective alleles (Ne) was 6.65. The mean observed heterozygosity (Ho) in the population was 0.83 (0.02) while the mean expected (He) was 0.84(0.01). The average observed heterozygosity were slightly lower than the expected values in the populations. The polymorphic information content (PIC) values ranged from 0.77 (S0155) to 0.89 (IGF1), with an average value of 0.83±0.01. These values indicated that all 13 markers were highly informative (>0.5), and can distinguish between individuals with different genotypes. Five of the markers (IGF1, S0155, SW857, SW911, and S0218) had negative F values, indicating that they were not fixed in the population and that there was genetic variation at those loci. For the other markers, only one (SW830) had a high F value, indicating that it was fixed in the population and that there was little genetic variation at that locus.

The overall mean FIS value considering all the microsatellite markers was low (F = 0.04, P<0.01), indicating that the population was not inbred and that there were considerable genetic variation at most of the loci tested.

Table 5.3: Genetic diversity of 67 individuals at 13 microsatellite nuclear loci.

Sl. No.	Locus	N	Na	Ne	I	Ho	He	FIS	PIC	AR
1	**IGF1**	64	14	10.14	2.44	0.94	0.9	-0.03	0.89	7.99
2	**SW122**	60	11	7.54	2.19	0.87	0.87	0.02	0.85	7.02
3	**S0155**	67	11	4.87	1.83	0.82	0.79	-0.02	0.77	5.5
4	**S0178**	67	12	5.11	1.9	0.81	0.8	0.03	0.78	5.76
5	**S0226**	65	17	6.48	2.23	0.8	0.85	0.08	0.83	6.93
6	**SW632**	66	13	6.49	2.1	0.82	0.85	0.05	0.83	6.55
7	**SW72**	67	11	5.26	1.88	0.81	0.81	0.03	0.79	5.71
8	**SW830**	67	15	5.44	2.06	0.63	0.82	0.27	0.8	6.36
9	**SW857**	64	12	6.78	2.11	0.88	0.85	-0.02	0.84	6.59
10	**S0355**	63	14	6.74	2.18	0.79	0.85	0.10	0.84	6.82
11	**SW911**	66	15	7.96	2.34	0.94	0.87	-0.06	0.86	7.47
12	**SWr1941**	67	12	5.55	2.01	0.81	0.82	0.05	0.8	6.25
13	**S0218**	62	14	8.14	2.3	0.87	0.88	-0.02	0.87	7.34
	Mean	65	13.15	6.65	2.12	0.83	0.84	0.04**	0.83	6.64
	SE	0.63	0.52	0.42	0.05	0.02	0.01	0.02	0.01	0.195

**** P<0.01**

Number of individuals sampled (N), number of alleles per locus (Na), No. of Effective Alleles (Ne), Allele Richness (AR), observed heterozygosity (Ho), expected heterozygosity (He), Inbreeding coefficient (FIS), Polymorphic information content (PIC), Shannon's Information Index (I), Standard error (SE)

Out of the 13 microsatellite loci tested, 7 loci deviated from Hardy-Weinberg equilibrium (HWE) at a significant level ($p < 0.05$). More specifically, four loci (SW830, SW857, S0355, and S0218) had p-values less than 0.001, indicating a highly significant deviation from HWE. The other three loci (S0226, SW632, and SW72) had p-values less than 0.05, indicating a significant deviation from HWE, but not as strong as the first four loci (Table 5.4).

Table 5.4: Estimation of exact P-Values by the Markov chain method.

Sl.no.	Locus	P-value	SE
1	IGF1	0.50	0.003
2	SW122	0.49	0.003
3	S0155	0.06	0.001
4	S0178	0.06	0.002
5	S0226	0.01*	0.000
6	SW632	0.03*	0.001
7	SW72	0.01*	0.000
8	SW830	0.000***	0.000
9	SW857	0.000***	0.000
10	S0355	0.000***	0.000
11	SW911	0.09	0.002
12	SWr1941	0.11	0.002
13	S0218	0.000***	0.000

*** P<0.05, ** P<0.01, *** P<0.001**

All the markers exhibited private alleles and 46 private alleles were found in the sampled population. However, only five of the markers (SW122, S0226, S0218, S0178, and SWr1941) had private alleles with a frequency higher than 5% (Table 5.5).

Table 5.5: List of private alleles with frequency higher than 5%.

Sl.no.	Locus	Allele	Frequency
1	SW122	139	0.07
2	S0226	220	0.06
3	S0218	187	0.08
4	S0218	191	0.06
5	S0178	133	0.07
6	SWr1941	234	0.12
7	S0218	201	0.06

PID (Probability of Identity) and PID (Sibs) (Probability of Identity for Siblings) are important measures of the genetic polymorphism of the markers used in individual identification (Waits et al., 2001). A set of markers with high power of discrimination enables reliable individual identification, as it reduces the likelihood of two individuals having the same set of traits by chance.

In the study, the locus IGF1 was found to be the most informative, with a PID of 1.79x10-2 and PID (sib) of 3.04x10-1(Table 5.6). This indicates that the probability of two individuals randomly selected from the population having the same genotype at this locus is relatively low. The high power of discrimination of IGF1 was consistent with its high level of polymorphism, as indicated by its high Ho value of 0.94 and high PIC value of 0.89. In contrast, the locus S0155 had the lowest PID value of 6.54x10-19 and a lower level of polymorphism, as indicated by its lower PIC value of 0.77.

These results suggested that the level of polymorphism of a marker, as measured by its PIC value, was correlated with its power of discrimination as measured by its PID value. Markers with higher PIC values tend to have higher power of discrimination and are therefore more informative for individual identification.

The 13 microsatellite loci analysed showed 78 possible combinations of linkage disequilibrium, which refers to the non-random association of alleles at different loci. Of these possible combinations, 19 combinations of markers were found to be significant, indicating that the genotypes at one locus were dependent on the genotypes at another locus (Table 5.7). This suggests that there may be some degree of physical linkage or genetic linkage between these loci.

Table 5.6: Multi-loci PID (biased) and PID (sibs) in increasing order of single-locus values (the first locus is the most informative locus).

Locus	PID (biased)	PID (sibs)
IGF1	1.79× 10-2	3.04× 10-1
S0218	4.79× 10-4	9.66× 10-2
SW911	1.28× 10-5	3.19× 10-2
SW122	3.95× 10-7	1.00× 10-2
SW857	1.52× 10-8	3.33× 10-3
S0355	5.64× 10-10	1.11× 10-3
S0226	2.21× 10-11	3.75× 10-4
SW632	9.03× 10-13	1.26× 10-4
SWr1941	4.72× 10-14	4.46× 10-5
SW830	2.43× 10-15	1.58× 10-5
SW72	1.47× 10-16	5.71× 10-6
S0178	9.31× 10-18	2.07× 10-6
S0155	6.54× 10-19	7.68× 10-7

PID=Probability of identity, PID (Sibs) =Probability of identity for siblings

Table 5.7: The linkage disequilibrium between pairs of microsatellites markers

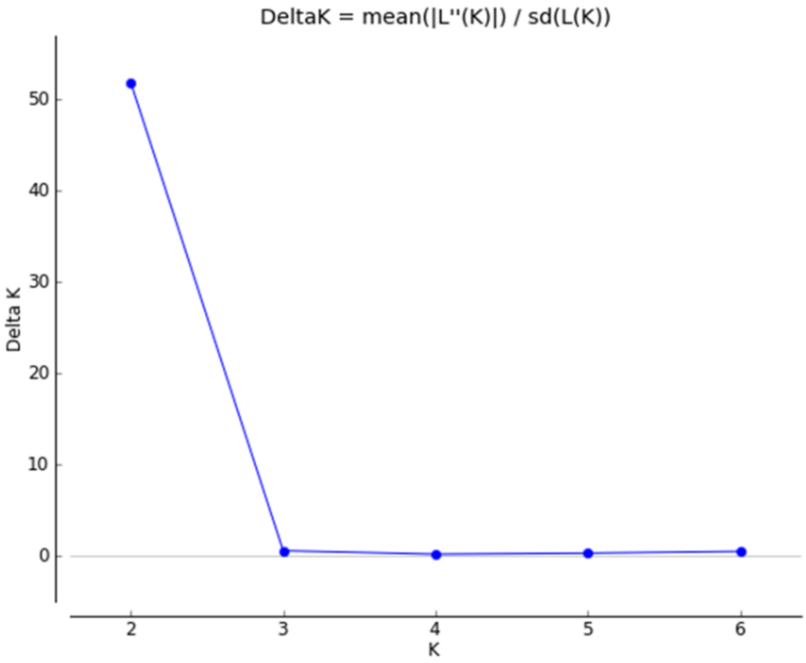
Sl.no.	Locus#1	Locus#2	P-Value	S.E.
1	IGF1	S0178	0.0305	0.0052
2	SW122	S0226	0.0034	0.0018
3	SW122	SW632	0.0159	0.0037
4	S0226	SW632	0.0011	0.0008
5	IGF1	SW72	0.0477	0.0064
6	S0226	SW72	0.0077	0.0024
7	SW122	SW830	0.0112	0.0030
8	S0226	SW830	0.0079	0.0025
9	SW632	SW830	0.0129	0.0027
10	SW122	S0355	0.0000	0.0000
11	SW632	S0355	0.0227	0.0043
12	SW72	S0355	0.0351	0.0054
13	SW857	S0355	0.0259	0.0047
14	SW632	SW911	0.0352	0.0056
15	S0355	SW911	0.0003	0.0003
16	S0226	SWr1941	0.0000	0.0000
17	SW632	SWr1941	0.0000	0.0000
18	SW72	SWr1941	0.0344	0.0054
19	SW122	S0218	0.0391	0.0056

P-Value significance = 0.05. SE standard error

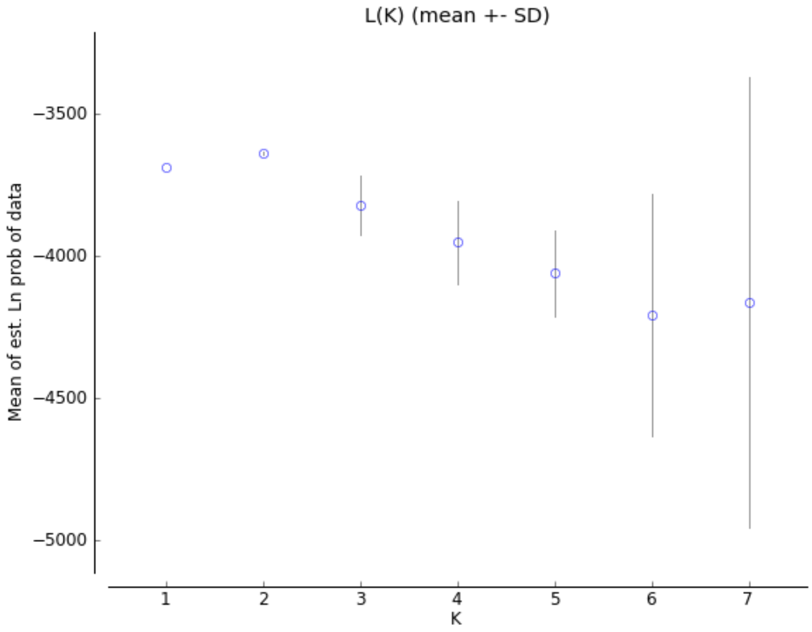
5.4.3 POPULATION GENETIC STRUCTURE AND GENETIC DIFFERENTIATION

The Bayesian approach implemented in Structure v2.3.4 grouped the 67 sampled animals into two optimum number of clusters as inferred by the likelihood distribution L (K) and delta K estimates. The highest ΔK value (ΔK = 51.77) was recorded at K=2, suggesting this value as the most probable K group fitting the data in this model of structuring (Figure 5.2). Hence, two ancestral populations characterized the randomly sampled animals with respective ancestry proportion. The observed high to moderate ancestry proportions suggested admixture events, as they indicated that some animals have ancestry from two genetically distinct populations. The first ancestry population (blue) and the second ancestry population (orange) were assigned in cluster 1 and cluster II with an average proportion of membership (q) of 0.944 and 0.056 respectively of the sampled population. Local pigs were included in Cluster I, crossbred or exotic breed pig are in Cluster II due to the close physical appearance to the exotic pig breed. The distinctness between the two clusters was maintained until K=5 (Figure 5.3).

Discriminant Analysis of Principal Components (DAPC) is a popular method in population genetics used to identify genetic clusters in a set of individuals. The Bayesian Information Criterion (BIC) is a statistical criterion used to compare the fit of different models and select the one with the best balance between goodness of fit and complexity of the model (Miller et al., 2020). The DAPC analysis identified no distinct genetic clusters associated with the lowest BIC value (Figure 5.4). This means that the data do not support the presence of discrete genetic clusters in the population under study. The two identified clusters that show an overlapping pattern with no clear clustering suggest that the genetic variation in the population is continuous and there is no clear separation between the groups (Figure 5.5).



a)



b)

Figure 5.2: The plot of a) Structure Harvester results (approximate numbers). Graph for the detection of the number of clusters (K) for observation of population group of sampled pig. b) Delta K using sampling location as a priori.

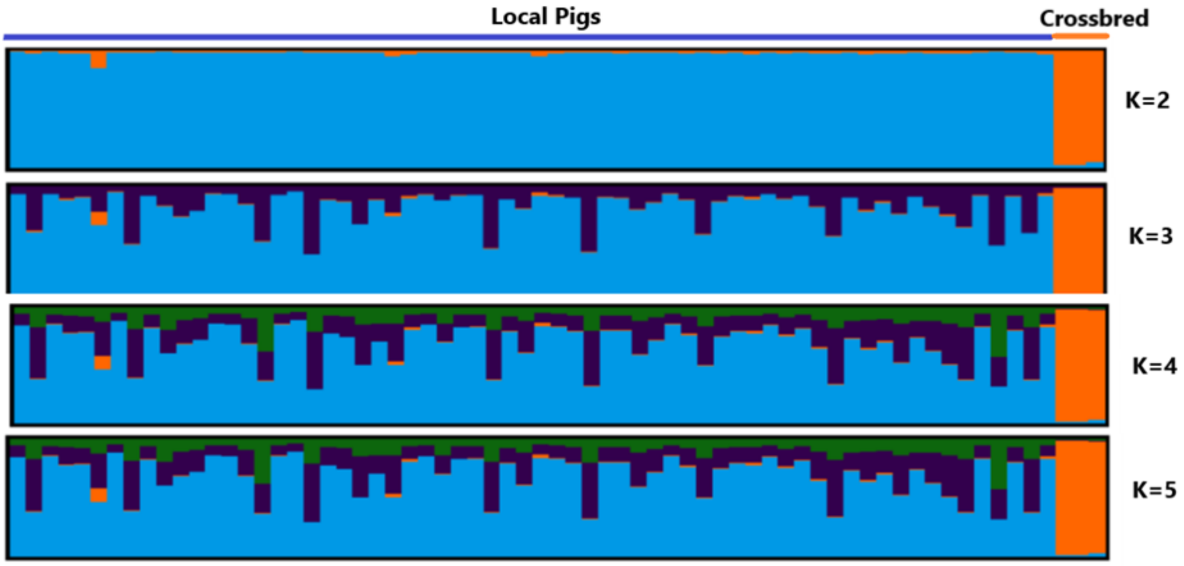


Figure 5.3: Bayesian clustering based on autosomal genotypes from the sampled pig population in Uttarakhand using the STRUCTURE software. Results for K = 2, K = 3, K=4 and K = 5 are shown. Iterations were combined and visualized using the CLUMPAK software. Colours in the bar plot depict the probability of assignment to each cluster.

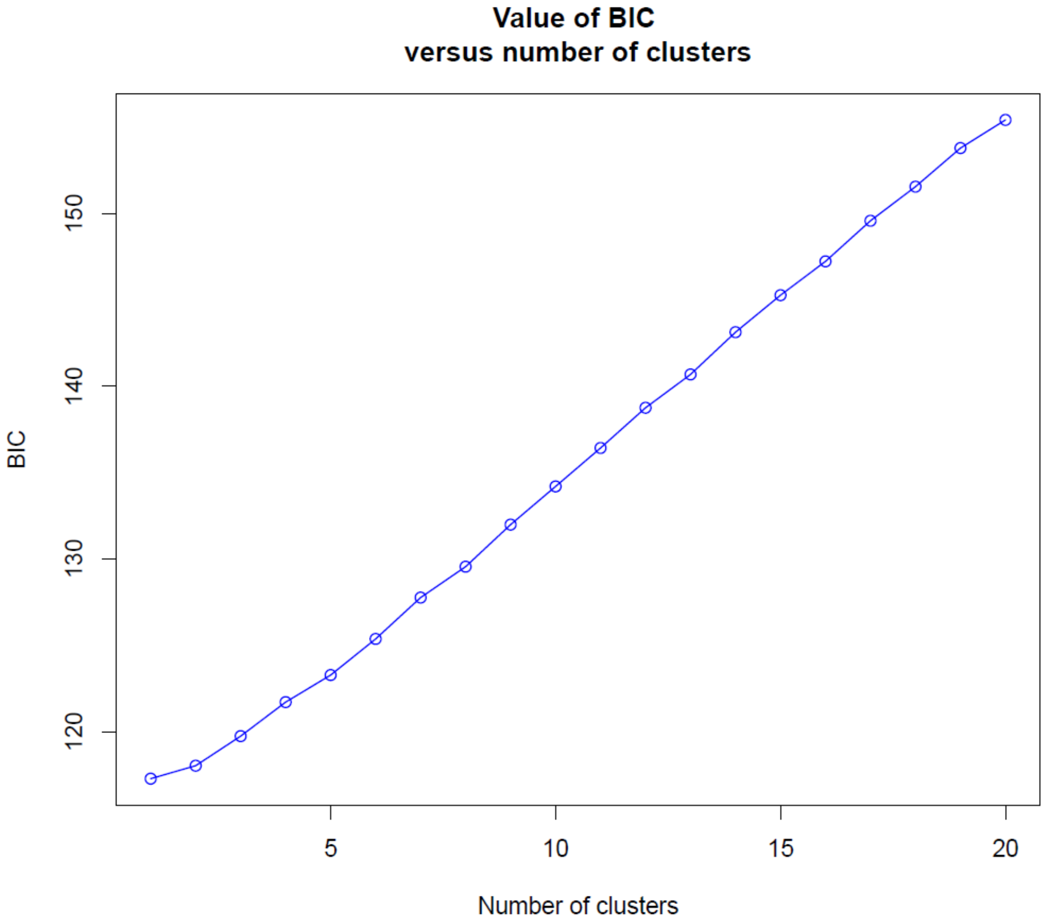


Figure 5.4: The plot of the BIC against the number of clusters.

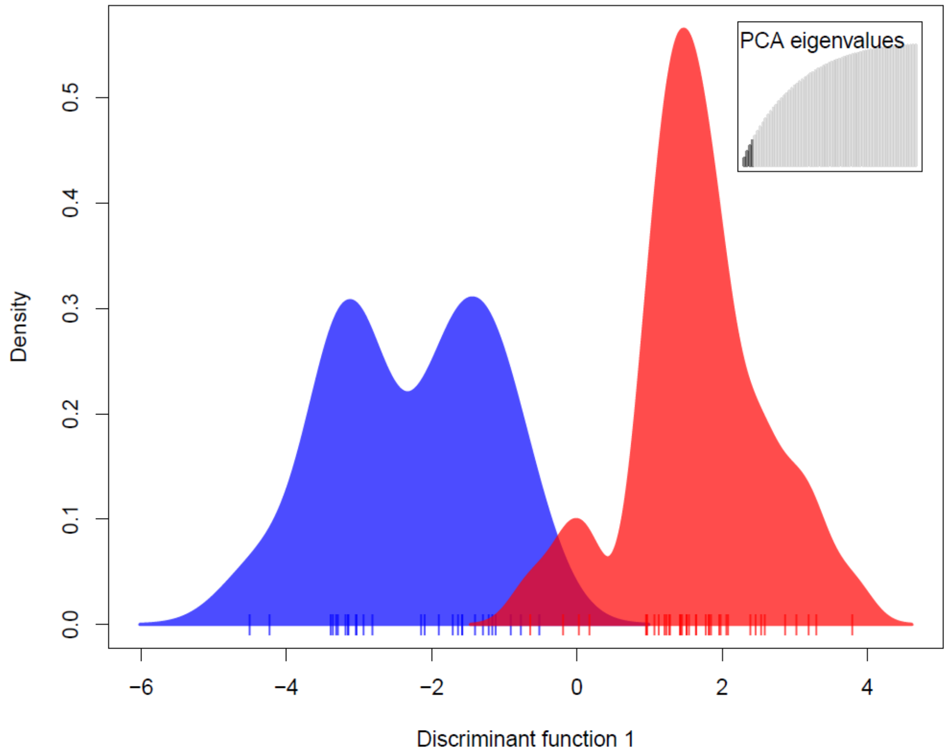


Figure 5.5: Density plot of the sampled pig individuals shows overlapping but two pre-defined groups.

Factorial Correspondence Analysis (FCA) was used to observe the associations between individuals and to evaluate probable admixtures between suggested clusters based on the frequencies of alleles at all loci in the populations.

The first three components of FCA account for 12.48% of the total variation (Figure 5.6), which means that there was a significant amount of variation, which was not explained by these components. Populations belonging to Cluster 1 and Cluster II were represented as white and red squares, respectively. Cluster I showed greater scatter, which suggests that there was more genetic variation within this cluster compared to Cluster II. The presence of admixture between the two clusters can be evaluated by examining the position of individuals on the FCA plot. If individuals from different clusters were located in close proximity on the plot, this indicates that there may be admixture between the two clusters.

It is important to note that FCA is a descriptive method and does not provide definitive evidence of admixture or genetic structure. Therefore, additional analyses such as ancestry estimation or model-based clustering may be necessary to confirm the presence of admixture and to determine the genetic structure of the populations under study.

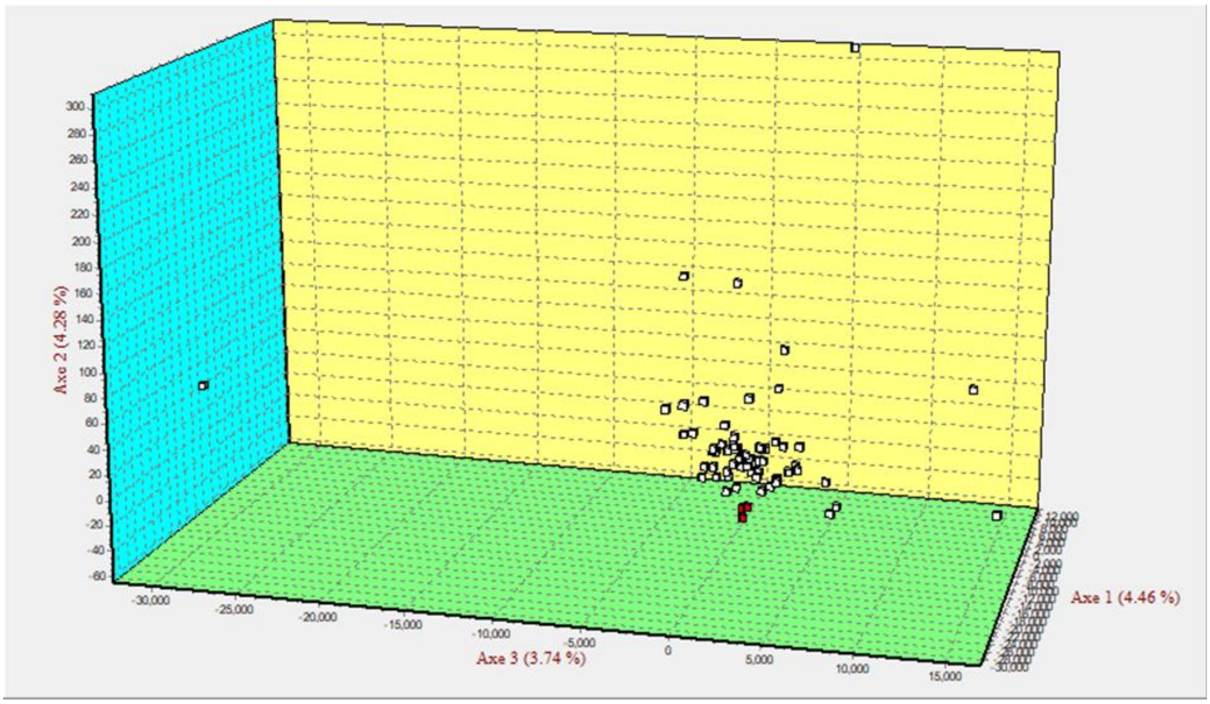


Figure 5.6: 3D clustering patterns of all individuals analyzed using 13 microsatellite markers as revealed by factorial correspondence analysis (FCA) implemented in GENETIX.

5.4.4 PHYLOGENETIC RELATIONS

Utilizing distance matrices generated from population-level allele frequencies, the neighbour-joining technique has been widely used as a population grouping tool. In population-genetic studies, a neighbour-joining(NJ) tree was treated as a type of population clustering diagram rather than a precise representation of the evolutionary history of the populations because migration and admixture occasionally produce evolutionary histories that cannot easily be described by a bifurcating tree of populations (Kopelman et al., 2013). Figure 5.7 showed a phylogenetic tree revealing the links among individuals based on genetic distances. The tree

isolated Cluster II and has a short external branch for a population on a constructed NJ-tree can imply recent admixture of the population, and that admixed populations often appear in the “middle” of a NJ-tree, on branches incident to paths connecting possible source populations. When a population appears on a short external branch of a NJ-tree, it suggests that the population had relatively little genetic diversity compared to the other populations in the tree. One possible explanation for this was recent admixture, where two or more previously separate populations have interbred and produced offspring with mixed genetic ancestry. As a result, the admixed population may appear on a short external branch, indicating that it has not had enough time to accumulate as much genetic diversity as the other populations in the tree.
Conversely, when an admixed population appears in the middle of a neighbor-joining tree, it suggested that the population had a more complex genetic history, with contributions from multiple source populations. The admixed population will be located on branches incident to paths connecting the possible source populations, reflecting the genetic mixing that has occurred.

On the other hand, Cluster I was divided into six genetic branches that were interconnected at the root of the phylogenetic tree. This suggests that there was more genetic diversity within Cluster I, and the different branches may represent subpopulations or different genetic lineages.

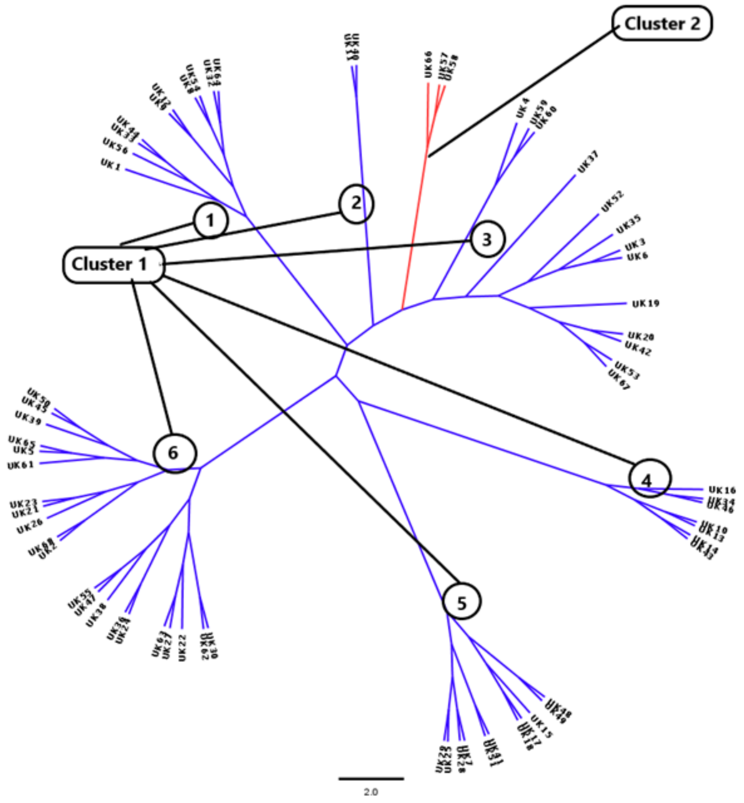


Figure 5.7: Phylogenetic tree of the pig populations showing different clusters.

5.4.5 ANALYSIS OF MOLECULAR VARIANCE

The Arlequin software version 3.5.1.3 was used to analyze the genetic variance within and between the identified genetic groups of pig populations, which were identified by the Structure software. The results showed that the majority (89.76%) of the genetic variation was found within the whole population, indicating that there is a high degree of genetic diversity within the population as a whole.

On the other hand, 8.69% of the genetic variation was observed among subpopulations, with variance components of 5.38 and 0.52, indicating that there was some genetic differentiation among the identified genetic groups. Additionally, 1.5% of the genetic diversity was explained by differences among individuals within subpopulations (Table 5.8).

The indices of fixation, FIS, FST, and FIT, were estimated at 0.02 (p=ns), 0.09 (p<0.001), and 0.10 (p<0.05), respectively (Table 5.8). FIS measures the deviation of the observed heterozygosity from the expected heterozygosity within subpopulations, while FST measures the genetic differentiation among subpopulations. FIT measures the overall level of genetic differentiation between the subpopulations and the total population. The values of FST and FIT indicate significant genetic differentiation among the identified genetic groups.

Overall, these results suggest that there was some degree of genetic differentiation among the identified genetic groups of pig populations, but there is also a high degree of genetic diversity within the population as a whole.

Table 5.8: AMOVA results partitioning the genetic variability within and among populations at three levels

Source of variation	df	Sum of Squares	Variance Components	Percentage variation	Fixation Index
Among Populations	1	11.53	0.52	8.69	FIS=0.02 ^{ns}
Among individuals within populations	63	350.78	0.09	1.55	FST=0.09***
Within individuals	65	349.5	5.38	89.76	FIT=0.10**
Total		711.81	5.998	100	

ns=not significant, * P<0.05, ** P<0.01, *** P<0.001

5.4.6 BOTTLENECK ANALYSIS

A sign test showed that the number of expected loci with heterozygosity excess was estimated at 7.9, 7.57 and 7.58 under an infinite allele model (IAM), two-phase model (TPM), and stepwise mutation model (SMM) respectively. It further showed that the number of loci observed with heterozygosity excess test (HET) was 13, 4 and 2 under IAM, TPM, and SMM respectively (Table 5.9). The difference between the observed and the expected loci with heterozygosity excess, as determined by the sign test and the Wilcoxon test was significant under IAM only, but not under TPM and SMM. However, this was not necessarily indicative of true heterozygosity excess, as the IAM is thought to be a less appropriate model for microsatellites than the SMM (Shriver et al., 1993) and TPM (Cornuet et al., 1996). A normal L-shaped allelic distribution for the populations was obtained (Figure 5.8).

Table 5.9: Bottleneck test in sampled pig population

Test		IAM	TPM	SMM
Sign test	Expected number of loci with heterozygosity excess	7.9	7.57	7.58
	Observed number of loci with heterozygosity excess	13	4	2
	p-value	0.00**	0.04*	0.00**
Wilcoxon signed- rank test	p-value(one tail for He excess)	0.00***	0.97ns	0.99ns

IAM infinite allele model, TPM two phase model, SMM stepwise mutation model, **ns=not significant, * P<0.05, ** P<0.01, *** P<0.001**

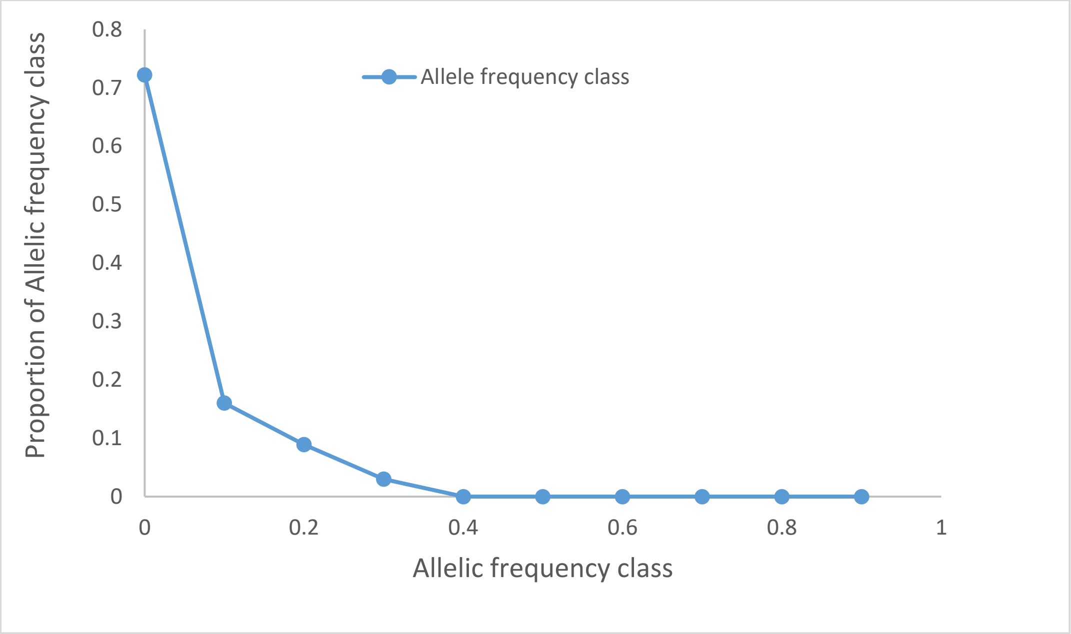


Figure 5.8: Mode-shift analysis resulting in a normal L-shaped curve

5.5 DISCUSSION

The study may have limited generalizability due to the small sample size and the difficulty in obtaining a larger sample size given the dispersed locations of pig rearers in the sampled districts and small herd sizes 3 to 7 (2-4 adult sows and 1-3 boars with piglets). The unorganized nature of the pig farming sector in Uttarakhand may contribute to a lack of information about breeding history and recognition of specific breed. This lack of information on the specific breeds of the sampled pigs may have limited the accuracy of the genetic analysis. However, the study provides a valuable first assessment of genetic diversity and relationships in pig populations in Uttarakhand, which can serve as a basis for future research on pig husbandry practices, improvement and conservation efforts. The high morphological variation in local pig breeds underscores the need for further investigation and documentation of these breeds in order to preserve their genetic diversity. This chapter advances our understanding on the genetic diversity of native and local pig populations in India.

The pig population in Uttarakhand is relatively small compared to other mammalian species used for animal production in Uttarakhand. (Figure 5.9). Moreover, the presence of wild pig populations in the region is also a factor to consider, as there is a potential for genetic exchange between wild and domestic pigs. This highlights the need for careful management of pig populations to ensure the conservation of unique genetic traits and to prevent the spread of disease.

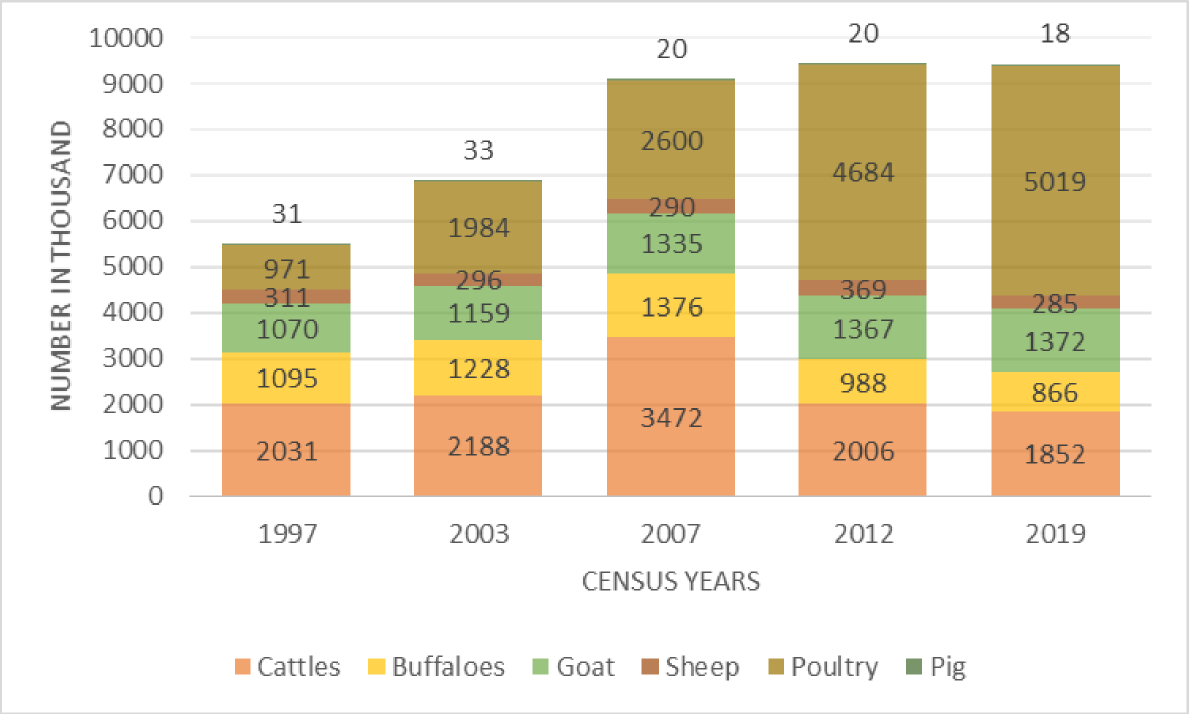


Figure 5.9: Population trend of Livestock species raise in Uttarakhand

The introduction of exotic pig breeds such as Landrace and Yorkshire in commercial pig farming systems in Uttarakhand may also have implications for the genetic diversity of local pig populations (Breed survey 2013). It is important to monitor and manage these introductions to ensure that they do not have negative impacts on the genetic diversity of local pig populations. Overall, continued research on pig breeds and their genetic diversity in Uttarakhand is crucial for supporting sustainable pig farming practices and conserving valuable genetic resources.

The number of alleles, expected heterozygosity, and PIC value observed at a locus are all important indicators of genetic variability at that locus. The fact that the total number of alleles per locus ranged from 11 to 17 with a mean value of 13.15 suggests that the microsatellite loci used in this study were sufficiently polymorphic. This is important because it allows for greater discrimination between individuals and populations and can aid in the classification and selection of microsatellites for further study on this species. The expected heterozygosity values ranging from 0.80 to 0.91 (Takezaki et al., 1996) indicated a high level of genetic diversity within the studied native pig populations. Despite of introduction of commercial breeds in pig farming, high genetic diversity in indigenous breed is a good sign. This is a positive finding, as genetic diversity is important for maintaining the health and adaptability of populations in the face of environmental change.

The PIC values ranging from 0.77 to 0.89 (Botstein et al.,1980) also suggest that the microsatellite loci used in this study were highly informative for studying genetic diversity and population structure in these pig populations. This information can be used to inform conservation efforts and breeding programs for these pig populations.

The fact that the loci used in this study are commonly used in paternity tests and for studying genetic diversity in pigs, and are recommended by the International Society for Animal Genetics (ISAG), is a positive indication of the quality of the microsatellite loci used.

This suggests that the results of this study are comparable with those from other studies using these microsatellite loci, allowing for better comparison of results and greater consistency in research findings.

When compared to other Indian pigs (Table 5.10), the level of genetic diversity within the analysed samples of Uttarakhand tended to be slightly higher than North and South Indian pigs (Behl et al., 2002; Behl et al., 2006), and significantly higher than North-eastern pig breeds (Kaul et al., 2001, Zaman et al., 2013a, b, c, d; 2014a,b; Sahoo et al., 2015, 2016, 2017, Boro et al., 2019. Compared with Asian breeds, the genetic diversities reported for the Uttarakhand pig estimated in this study were marginally higher levels to most Chinese, Tibetan, Bhutanese and Nepalese pig breed (Li et al.,2000; Fan et al., 2002, 2003, 2009 ; Yang et al. 2003, Li et al. 2004, Wang et al. 2004, Wu et al. 2021) and while several Asian breeds (Thuy et al., 2006, Nguyen et al., 2020) which have been locally conserved in small population size, showed a quite lower genetic diversity . The levels of diversity detected in the Uttarakhand pig tended to be higher than those previously reported for European breeds. This is consistent with past studies (Laval et al., 2000; San Cristobal et al., 2006, Sollero et al., 2009), which showed a lower level of diversity within most European pig breeds compared with indigenous breeds.
Furthermore, the higher mean PIC (0.83± 0.01), mean He (0.84±0.01) and mean Ne (6.65±0.42) values observed in this study compared to other Indian pig breeds, as shown in (Table 5.10), suggest that the pig populations studied in Uttarakhand have higher levels of genetic diversity than many other Indian pig breeds. Overall, the use of recommended microsatellite loci, suggest that the results of this study are reliable and informative for understanding the genetic diversity and population structure of pig populations in this region.

Table 5.10: Summary of expected heterozygosity and effective number of alleles used as estimators of genetic diversity.

Country	No. of breeds/ types (Name)	Total Pigs	Average He	Average Ne	PIC	Research Studies
Bhutan	4	196	0.78±0.02	5.43±0.50		Nidup et al., (2016)
Nepal	2	45	0.75±0.02	4.58±0.38		
India (North East) Tripura	1 Mali	40	0.57±0.01	3.16±0.33	0.53±0.266	Zaman et al., (2013)
India (North East) Nagaland	1 Votho	40	0.57±0.01	3.02±0.28	0.53±0.250	Zaman e .al, (2014)
India (North East) Assam	1 Doom	40	0.67±0.14	3.59±1.5	0.635±0.143	Zaman et al., (2014)
India (North East) Assam	1 Assam Local	30	0.71±0.18	4.38±1.8	0.67±0.18	Zaman et al, (2014)
India (North East) Mizoram	1 Zowak	40	0.54±0.19	2.71±1.47	0.48±0.212	Zaman et al., (2014)
India (North East: Meghalaya, Nagaland) Eastern: West Bengal)	3 Niang Megha Tenyi vo Ghungroo	34 30 38	0.67±0.02 0.69±0.03 0.75±0.02	0.67±0.03 0.69±0.06 0.75±0.02		Sahoo et al., 2016
India (North East: Meghalaya, Nagaland)	2 Niang Megha Tenyi vo	46 43	0.67±0.03 0.69±0.03	3.55±0.33 3.81±0.41	0.65±0.03 0.68±0.03	Sahoo et al., 2017
India (Eastern: West Bengal)	1 Ghungroo	40	0.58±0.22	3.15±0.15	0.67±0.18	Zaman et al., (2013)
India (Eastern: West Bengal)	1 Ghungroo	45	0.75±0.02	4.39±0.26	0.54±0.22	Sahoo et al., 2015
India (North)	1 Ghurrah	40	0.74±0.05	4.78±0.57	0.74±0.04	Boro et al., 2019
India (North , North East)	2 North North East	25 25	0.81±0.05 0.83±0.04	5.17 5.3		Kaul, 2001
India (South: Kerala)	Ankamali	26	0.83±0.03	5.34±0.77	0.80±0.03	Behl et al., 2005
India (Andaman)	Andaman Desi	25	0.77±0.01	5.09±0.20	0.74±0.01	De, 2013
India (Nicobar)	Nicobari	50	0.75±0.01	5.04±0.21	0.74±0.01	De, 2014
China	4	61	0.72	4.07		Li et al., 2000
China	7	380	0.59	2.82		Fan et al., 2002
China (Tibet)	1	31	0.75	4.98		Fan et al., 2003
China	3	90	0.65	3.8		
China (Tibet) China	1 17	60 967	0.87 0.84	8 7.41		Yang et al., 2003
China	10	817	0.81-0.85	5.2-6.8		Fang et al. 2009

China	10	379	0.68	4.4		Li et al., 2004
China	10	536	0.46-0.64	2.43-3.76		Wang, 2004
China	7	346	0.625			Wu et al., 2021
Vietnam	5	152	0.75	4.54		Thuy et al., 2006
Vietnam	24	1136	0.63-0.80	6.5-11.3		Nguyen et al., 2020
European	10 (commercial + native)	471	0.53	2.19		Laval et al., 2000
European	58 (commercial + native)	2737	0.56			San Cristobal et al., 2006
Brazil	5 (native + commercial)	182	0.685	8.96		Sollerro et al., 2009

The significant locus-specific deviations from Hardy-Weinberg equilibrium were observed at 7 microsatellite loci. Deviations from Hardy-Weinberg equilibrium (HWE) can occur for a variety of reasons in domestic animal populations, the common one are non-random mating, genetic drift, gene flow, selection, and mutation and in addition of these, null alleles or genotyping errors/artifacts (Pompanon et al., 2005), may also forms one of the major reasons for deviation (Waples 2015). The specific factors affecting the population may vary depending on the breed, breeding practices, and other environmental and genetic factors. The discussion of pig husbandry practices in Uttarakhand has been provided, as it is important to understand the specific factors that may affect deviations from Hardy-Weinberg equilibrium (HWE).

Pig husbandry practices as observed in the household surveyed have a range of pig herd sizes, with 2-4 adult sows and 1-3 boars with piglets being the typical range. The breeds present in the herds are a mix of indigenous, mixed parentage of indigenous crossed with exotic breeds, and putative crosses with wild pigs. The households practice amalgamation of farrow-to-finish and piglet production systems, with some also engaging in fattener production by purchasing piglets from other households.

The households predominantly follow a semi-intensive system of rearing, with natural and controlled breeding methods being the only available reproduction methods. Breeding males were either from their own or nearby farms, and sometimes wild relatives. Castration of old boars was done by all respondents. The traits like mothering ability, reproductive performance, and disease tolerance over general appearance and scavenging ability was preferred as a quality of the females that were to be kept by the owner in the herd for future production.

In the context of pig husbandry practices in Uttarakhand, deviations from HWE may occur due to non-random mating, as breeders may preferentially select for certain traits or mate closely related animals to maintain certain characteristics. Migration may also be a factor as pigs from other regions or breeds are introduced into the population which may introduce new alleles. Mutation and selection may also play a role in shaping allele frequencies in these populations, depending on the specific genetic and environmental factors at play. In the case where a null allele is present at a low frequency (i.e., <0.5), as in the locus SW830 with a null allele frequency of 0.1, the impact on HWE is likely to be small and may not be statistically significant. Hence, correction methods were applied to adjust for their presence and minimize their impact on the estimation of allele frequencies and HWE (Huang et al., 2016).

It is important to identify the specific factors contributing to deviations from HWE in domestic animal populations, as this information can inform management strategies aimed at maintaining genetic diversity and preventing the accumulation of deleterious alleles that could impact population health and fitness. It is important to realize that tests of HWP provide no information about cumulative levels of inbreeding in a population. The inbreeding coefficient (FIS) is a measure of the deviation from Hardy-Weinberg proportions (HWP) due to inbreeding, and it reflects the recent history of mating in a population (Doyle, 2014). However, FIS does not provide information about the cumulative levels of inbreeding over multiple generations is often only weakly correlated with inbreeding and fitness (Allendorf et al., 2007). The low average FIS = 0.04 recorded in this study infers the existence of a high level of heterozygosity, thus few homozygous individuals within the pig population (Hedrick 2012). This genetic diversity is favoured by the extensive breeding systems or it may be that the local pig has evolved in Uttarakhand itself. This estimate is also likely to be affected by several factors including use of a limited number of loci, closely related individuals, genetic bottleneck, gene flow and admixture (Wang et al., 2017; DeGiorgio et al., 2010).

Linkage disequilibrium (LD) is a common phenomenon in domestic animals, just as it is in other organisms. LD can arise as a result of various factors, including genetic drift, selection, population structure, Inbreeding, breeding practices and demographic history. The identification of 19 significant combinations of markers out of 78 possible combination suggests that it is a diverse populations and hence LD tends to be low (Zollner, 2008).

Table 5.11: Expected' numbers of homozygotes or heterozygotes are computed using Levene's correction in GENEPOP software

Sl. No.	Locus	Expected number of homozygotes	Observed number of homozygotes	Expected number of heterozygotes	Observed number of heterozygotes
1	IGF1	5.86	4	58.14	60
2	SW122	7.52	8	52.48	52
3	S0155	13.37	12	53.63	55
4	S0178	12.71	13	54.29	54
5	S0226	9.61	13	55.39	52
6	SW632	9.75	12	56.25	54
7	SW72	12.34	13	54.66	54
8	SW830	11.92	25	55.07	42
9	SW857	9.02	8	54.98	56
10	S0355	8.91	13	54.09	50
11	SW911	7.85	4	58.15	62
12	SWr1941	11.67	13	55.33	54
13	S0218	7.17	8	54.83	54
	Mean	9.8	11.23	55.18	53.77

The observed Heterozygosity Ho was slightly less than the expected Heterozygosity He (Ho = 0.83, He = 0.84) (Table 5.11) the observed heterozygosity (Ho) being slightly less than the expected heterozygosity (He) could be due to factors such as null alleles, Wahlund effect, assortative mating, inbreeding, or selective breeding.

It appears that inbreeding and null alleles are unlikely to be the cause of the heterozygote deficiency observed in this study. As inbreeding can affect all or most of the loci in a similar way, leading to a reduction in heterozygosity across the genome. However, in the case of studied pig in Uttarakhand, it appears that the deficit of heterozygotes was not observed at all loci (Table 5.3). The fact that less than 50% of the loci showed a significant deficit of heterozygotes suggests that the inbreeding is not affecting all parts of the genome equally. It is also interesting to note that the community avoids breeding between relatives, despite the exchange of live piglets or adult animals being a common practice. This suggests that there may be some cultural or religious factors at play that promote outbreeding and help to maintain genetic diversity within the herd.

However, other factors such as the Wahlund effect, assortative mating, or selective breeding cannot be ruled out hence it may be necessary to examine the population structure and mating patterns in more detail to determine the underlying causes of the heterozygote deficiency.

The presence of population substructure within the pig breed in Uttarakhand is likely contributing to the Wahlund effect and the observed heterozygote deficiency. Pig rearers in Uttarakhand have been practicing a form of crossbreeding that involves using indigenous breeds as well as introducing exotic breeds and possibly wild pigs to create a mixed population, which favour the creation of a continuous pool of genes responsible for the observed genetic diversity, likely contribute to the population substructure within the breed. Since the animals were sampled from 25 small herds, which likely include a mixture of individuals from more than one breeding unit as well as mixed-age individuals, the observed heterozygote deficiency could be a consequence of the Wahlund effect, which can lead to a reduction in heterozygosity and an increase in homozygosity. Overall, the combination of breeding practices, population substructure, and the Wahlund effect is likely contributing to the observed heterozygote deficiency in the pig breed in Uttarakhand. Understanding these factors is important for developing effective breeding and conservation strategies for the future.

In the case of the pig breeding practices in Uttarakhand, the selection of breeding males and females based on certain traits can lead to positive assortative mating and a reduction in heterozygosity, contributing to the heterozygote deficiency observed in the study. Male pigs are carefully selected by the pig rearer to avoid using males from their own herd. Instead, the breeding males are often sourced from nearby farms or even wild relatives. Old boars are castrated to prevent inbreeding. For females, the pig rearer selects individuals based on desirable traits such as good mothering ability, reproductive performance, disease tolerance, and scavenging ability.

A high frequency of private alleles with frequencies above 0.01 indicates that there was significant genetic differentiation among individuals within the studied pig populations, which could be due to factors such as selection pressure or migration. This could have implications for the population's ability to adapt to changing environmental conditions or to maintain genetic diversity over time.

2.5.1 POPULATION GENETIC STRUCTURE, DIFFERENTIATION

Population genetic structure present in the sampled pig population was supported by the results of the Structure analysis. The presence of two distinct clusters within the population suggests that there are genetic differences between these groups, which could be due to factors such as geographic isolation, genetic drift, or selective breeding practices.

The DAPC analysis also supported the presence of population clusters, but with a different approach that does not rely on population genetics models. The observed low level of differentiation and admixed population structure suggests that there were widespread breeding practices in which pigs of mixed parentage were kept in the herd. This was likely due to the increasing demand for exotic pig meat, which was driving breeders to cross local breeds with exotic breeds to improve their size and meet market requirements.

However, these practices could also threaten the sustainability of local pig breeds by leading to genetic erosion and the loss of adaptive traits that are important for the local environment. Additionally, the movements of animals through transhumance and/or trade could facilitate gene flow and introgression of genes from exotic breeds into local pigs, further complicating the genetic structure of the population.

The factorial correspondence analysis (FCA) plots exhibited very closely placed but distinct sets of points for the clusters II, which was corroborated by clustering analysis ($K = 2$) that indicated two distinct genetic groups but with mix of individuals. The representation of FCA and NJ tree also support the DAPC and structure findings.

A low level of population differentiation in pigs was identified from the same geographical region, this is supported by a significant FST value ($Fst = 0.09$, $P < 0.001$) indicating genetic differentiation among populations, as well as a heterozygosity deficit observed in individual samples that suggests a possible Wahlund effect resulting from admixture of individuals from different populations. These results suggested that the traditional management practices of pig genetic resources in Uttarakhand have contributed to genetic mixing and the sharing of genetic material among populations.

The bottleneck was detected under the IAM model only using the Wilcoxon test for heterozygous excess, which may be due to a small number of loci analyzed (Cornuet et al., 1996) or insufficient sample size. The normal L-shaped allelic distribution obtained in the Bottleneck test suggests that the population has not experienced a recent bottleneck or reduction in population size. It was possible due to the high levels of relatedness and gene flow among individuals, which may have helped to maintain sufficient levels of genetic diversity. However, it is important to note that bottleneck detection can be influenced by various factors such as timing, duration, magnitude, gene flow, pre-bottleneck genetic variability, mating system, sample size, and the number of loci analysed (Peery et al., 2012) . These factors should be carefully considered when interpreting the results of bottleneck tests to avoid drawing inaccurate conclusions about the population's history.

Based on the microsatellite diversity and differentiation, this Chapter provides a valuable first assessment of this study area and adds to the existing knowledge on the genetic diversity of indigenous/local pig populations in India. Genetic variability indicators, including the number of alleles, expected heterozygosity, and PIC value, demonstrate that these loci are sufficiently polymorphic and highly informative. Despite the introduction of commercial breeds in pig farming, the high expected heterozygosity reflects a significant level of genetic diversity, which plays a crucial role in preserving the health and adaptability of populations in response to environmental changes. The information obtained from these indicators can be utilized to inform conservation efforts and implement effective breeding programs for the pig populations.

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CHAPTER 6

SUMMARY

Modern agriculture's heavy reliance on a limited number of animal species for meat, dairy, and other products has led to the endangerment or extinction of their wild ancestors (FAO, 1995). Domesticated pigs *(Sus scrofa domesticus)* stand out due to the continued presence of their wild ancestor, the wild boar (*Sus scrofa*), globally. This unique coexistence is attributed to factors such as the wide distribution of wild boars, their adaptability, high reproductive capacity, cultural significance, and conservation efforts (Bosse et al., 2019). The wild boar *(Sus scrofa*) historically occupied a vast Eurasian range; the subspecies *Sus salvanius*, known as the pygmy hog, once widespread in the Himalayan foothills, is now confined to Assam's Manas National Park; and the Indian wild pig, *Sus scrofa cristatus*, inhabits sub-Himalayan regions like the Shiwalik and Tarai areas in northern India.

The FAO Databank reveals concerning trends, with approximately 28% of domestic mammalian breeds at risk of extinction. The pig category comprises 625 documented breeds, and crucial action is needed to safeguard genetic diversity as 70 pig breeds have already gone extinct (FAO, 2022). The importance of this conservation imperative is underscored by the potential loss of a substantial portion of breeds that remains inadequately understood (FAO, 2022). The Food and Agriculture Organisation (FAO) introduces a global initiative for breed preservation, emphasising genetic markers as a means to protect breeds with exceptional characteristics for the longevity of agriculture and cultural diversity (Ajmone et al., 2023).

Since the 1800s, there has been a prolific development of numerous new pig breeds through systematic crossing and artificial selection, driven by consumer demand and government requirements (Jones, 1998). Presently, the diversity is exemplified by over 566 local domestic pig breeds globally, with Asia alone contributing up to 232 local breeds (FAO, 2022).

Intensive artificial and natural selection, coupled with environmental factors, has led to marked phenotypic modifications in domestic pigs, including behavioural traits, body composition, disease resistance, reproductive characteristics, and coat coloration. While pigs *(Sus scrofa domestica*) play a crucial role as a significant meat source globally and hold cultural importance, challenges such as market demands for leaner fatteners and shifts in agricultural practices have led to the dominance of a few high-yielding breeds, threatening the survival of diverse local pig populations and putting some breeds at risk of extinction.

India provides a unique case, recognising and characterising thirteen indigenous pig breeds with remarkable phenotypic diversity, originating mainly from the eastern states of the country (www.nbagr.res.in). These Indian indigenous pigs play a distinctive role in the swine germplasm, incorporating elements of wild ancestry. The global trend of favouring high-yielding breeds has repercussions for the preservation of traditional breeds in India, some of which now face extinction. A significant threat to wild populations arises from genetic swamping, where interbreeding between domesticated and wild populations leads to the dilution or loss of unique genetic characteristics in the wild species (Rutherford et al., 2019). Species like wild yaks, Bactrian camels, wild pigs, and jungle fowl are at risk of genetic swamping. The resulting hybrids may carry genes adapted to domestication conditions but not well-suited for survival in the wild, compromising the distinct traits and behaviours crucial for the survival of the wild population (Redford et al., 2018). Conservationists and researchers are concerned about the reduced fitness, adaptability, and overall viability of wild populations, emphasising the need for a balance between the conservation of wild populations and sustainable domestication practices. Managing and mitigating the risks associated with genetic swamping is imperative to preserving the integrity and survival of both domestic and wild populations.

A combination of ecological and genetic assessments is deemed necessary to evaluate pig husbandry practices, phylogeny, and genetic diversity in India, particularly in Uttarakhand. Uttarakhand's remarkable geography includes vast forestland covering 45.44% of its total area. The urban districts of Dehradun, Udham Singh Nagar, Nainital, and Haridwar collectively make up 23.32% of this forest cover (State Forest Reports). However, rapid urbanisation has led to extensive deforestation, mainly due to land development and construction. This has increased the chances of interactions between wild boars, humans, and domestic animals. The environmental and ecological challenges of pig rearing in the hilly and forested regions of Uttarakhand increase the risk of disease outbreaks and conflicts with wildlife and humans (Riedel et. al., 2012; Pietrosemoli et. al., 2020). As a result, a majority of pig farming activity was concentrated in the planer or partially planer districts of Uttarakhand, including districts such as Udham Singh Nagar, Dehradun, Haridwar, and Nainital. Moreover, the traditional dietary preferences of the majority of the population in Uttarakhand are influenced by cultural and religious factors and tend to lean towards non-pork protein sources. The intricate history of pig domestication and the interplay between wild boars and domestic pigs emphasise the necessity for a comprehensive approach. This approach considers ecological, economic, and social factors to safeguard pig populations, ensuring a balanced coexistence between agriculture and wildlife (Wang et al., 2023).

Incorporating molecular techniques into the study of domestic pig breeds and their interactions with ecosystems can enhance precision and science-based management practices, contributing to biodiversity conservation and sustainable husbandry. The result of this research gave us an overview of the spatial distribution, socio-economic status, pig husbandry methods of rearers, and the impact of exotic pig breeds on indigenous breeds and the region's ecology.

Molecular characterization, utilising tools like mtDNA and microsatellites, provides valuable insights into the evolutionary history and genetic diversity of pig breeds in a specific region. This research evaluated the genetic diversity, population structure, and maternal lineage of domestic pig breeds on a regional scale. These molecular techniques, well-established in understanding genetic diversity and population dynamics, contribute to informed conservation and management strategies, facilitating the coexistence of agriculture and wildlife (Hohenlohe et al., 2021; Giuffra et al., 2000; Fang et al., 2005; FAO, 2017).

The survey and sampling were done in the planar (Dehradun, Haridwar, and Nainital) districts of Uttarakhand, a mountainous state of India that lies in the foothills of the Himalayas, where rapid urbanisation and continuous migration make us reconsider the necessity of an organised promotion of the piggery sector due to its multiple ecological implications. 28 households were reached by adopting a rapid appraisal approach and interviewed using a semi-structured questionnaire. 68 non-invasive biological samples were collected from the herd of the household survey for molecular analysis.

Pig farming has a profound caste-based and cultural association in India. In Uttarakhand, pigs are raised predominantly by the specific community contributing to their livelihood. Unlike other livestock species in the state, the piggery sector is unorganised, and pig farming is not sufficient to meet the increasing demand for pork. Analysis showed that 60.7%, 10.7%, and 21.4% of the households raised pigs near seasonal streams (slum settlements), residential areas within the house premises, and near the forest, respectively. Moreover, 28.6% of pig rearer households' maintained breeding boar of feral origin, and 96.94% followed a semi-intensive pig rearing system; hence, human settlement is staying in close proximity with pigs (domestic and wild) and other domestic animals. The discussion reveals that the limited contribution of the piggery sector in Uttarakhand is influenced by geographical, cultural, economic, and infrastructure-related factors. The hilly terrain and temperate climate pose challenges for pig farming, with concentrations in plain districts. Traditional dietary preferences, capital-intensive nature, and inadequate government support hinder sector growth. Pig breed types involve 65 identified pigs, with 32 classified as exotic/crossbred and 33 as indigenous. Crossbreeding of local sows with wild boars is widespread. Production and management practices indicate that 85.7% follow a farrow-to-finish approach, with 96.94% adopting a semi-intensive pig rearing system. Natural and controlled breeding techniques are predominant, and 100% of respondents practice castration of old boars. The decline in indigenous pig breeds, despite adaptability, signals genetic diversity loss. The low work participation rate within the pig rearer community presents an opportunity for targeted support to enhance pig rearing. The semi-intensive system, prevalent among small-scale producers, offers economic stability. However, the rise in slaughtering activities and challenges in market access complicate the sector's dynamics. The study also emphasises the need for monitoring pig herds, understanding wildlife interactions, and implementing measures to safeguard public health in urban ecosystems. Lastly, the introduction of exotic pig breeds poses potential ecological, genetic, and socio-economic implications, emphasising the importance of context-specific management strategies.

A comparative economic analysis was also conducted on locally adapted and exotic/crossbred pig breeds to assess economic viability and sustainability, hypothesizing that while improved breeds may exhibit higher productivity, the associated costs could impact overall viability. Examining economic traits such as age at first farrowing, litter size, farrowing interval, and weight at market age. Local pigs crossed with wild pigs emerged as the top performers, exhibiting advantages in various criteria such as reproduction, feed efficiency, and disease resistance. The market value of local and wild pigs was higher, attributed to enhanced genetic traits, superior carcass characteristics, and specific demand-supply dynamics.

The maternal lineage of the domestic swine population was traced using mitochondrial DNA control region markers. Analysis of 68 samples reveals 20 haplotypes, the signature of the Pacific Clade (D6), MTSEA (South East Asia), European (D1), and the ubiquitously distributed Chinese (D2) haplotypes are present in the domestic pig of Uttarakhand. The D3 haplotypes, which were reported in wild pigs from North India, were also identified in 47 domestic samples. A unique genepool, UKD (Uttarakhand Domestic), as another lineage specific to this region, has been proposed. The incorporation of an additional 15 samples originating from North Indian states and their subsequent integration with previously published 1,518 mitochondrial D-loop sequences of *Sus scrofa* provide a comprehensive global perspective on the geographical distribution and phylogenetic positioning of the examined specimens. The resulting global dataset revealed 18 unique haplotypes and a complex clustering pattern of porcine haplotypes with distinct phylogeographic signals. The study unveiled diverse maternal lineages among the domestic pig populations in northern Indian states situated in the foothills of the majestic Himalayas. Additionally, the study also confirmed the presence of two distinct subspecies of wild boar in India, W1 (*Sus scrofa davidi*) and W2 (*Sus scrofa affinis*), demonstrating their migration routes and offering a strong phylogeographic signal. The UKD haplotype was positioned within the W1 clade, which includes *S. s. davidi*, a wild boar subspecies native to South Asia. This finding suggests a unique lineage specific to Uttarakhand, indicating a regional influence on the genetic diversity of pig populations.

Evidence for a separate domestication centre in the Himalayan foothills and Indian subcontinent was found within Mixed Clade 5 (MC5). The Himalayan region, including Northern Indian states like Chhattisgarh and West Bengal, is home to the wild boar subspecies

S. s. cristatus. Extensive genetic analysis has uncovered a fascinating association between domestic pigs in the Himalayan region, including those from Bhutan and Nepal, and this wild boar subspecies. This connection is evidenced by shared or closely related haplotypes, indicating a close integration within this maternal lineage.

A novel clade (MC4) with a localised distribution on the Chotanagpur plateau was detected, suggesting a unique population of wild boar in this region. Diverse maternal lineages among domestic pigs in Uttarakhand were observed, which may reflect human-mediated dispersal of pigs within and into the region; hence, a hypothesis linking ancient human migration with the presence of these maternal lineage clades has been proposed. Overall, the porcine haplotypes discovered in the Indian subcontinent through this study highlight their unique and significant contribution to domestic pig genetic resources.

Further analysis of genetic diversity in the collected samples was done to check the pig population health, adaptability and level of genetic mixing of samples that have different maternal lineage. 67 samples were successfully genotyped using 13 microsatellite loci, as recommended by the Food and Agriculture Organisation. The study found that the domestic pigs in Uttarakhand have a high level of genetic diversity, as indicated by the observed (Ho) and expected (He) heterozygosities of 0.83±0.02 and 0.84±0.01, respectively. The average polymorphic information content (PIC) values of 0.83±0.01 also indicate the high informativeness of the markers. Further, the domestic pigs in Uttarakhand have a low level of inbreeding, as indicated by the overall mean FIS value of 0.04 ($P<0.01$), which suggests that the animals are randomly mating within the population. However, some loci deviated from Hardy-Weinberg equilibrium (HWE) at a significant level ($p < 0.05$), which may indicate the presence of selection, genetic drift, or non-random mating at these loci. The domestic pigs in Uttarakhand have a low level of genetic differentiation, as indicated by the two clusters identified by the structure analysis, which showed overlapping populations. The low differentiation and significant FST value (Fst = 0.09, $P < 0.001$) support these findings. These results suggest that the traditional management practices in Uttarakhand have allowed for genetic mixing and the sharing of genetic material among pig populations, including the distinct wild boar maternal lineage that was found in this study. This can contribute to increased genetic diversity but may also result in the loss of distinct genetic characteristics or breed purity in the local breeds if not carefully managed.

This study provides a basic understanding of the pig population trends, the socio-economic landscape of pig rearers, their geographical distribution, and pig farming practices in Uttarakhand. This knowledge can serve as a pilot study that can be replicated in larger pig population of other districts of Uttarakhand. This can form a basis for informed decision-making and policy formulation to promote sustainable pig farming in the region. Moreover, it is crucial for the government’s piggery development policies in Uttarakhand to place significant emphasis on indigenous pig breeds and initiate a comprehensive breed study in Uttarakhand, using appropriate scientific tools to assess morphometric traits and production parameters. This can provide valuable insights and inform evidence-based policy decisions in the realm of piggery development within the region. This study thus adds a valuable dimension to the existing scientific literature on this topic.

The study also sheds light on the economic dynamics of pig farming in Uttarakhand, emphasising the significance of locally adapted breeds and the potential challenges of leveraging exotic breeds for economic gains. Before planning for a holistic piggery development programme in Uttarakhand, the government may consider these findings. In Uttarakhand, dairy farming dominates the livestock sector, and pig farming faces stiff competition for resources and investments. Currently, there are only two government-run pig farms in Uttarakhand, but they have remained non-functional for several years. Due to its unorganised nature, the piggery sector faces challenges related to market access, veterinary services, and distribution channels, which further compound the difficulties faced by the sector in the state. The declining piggery sector in Uttarakhand is also influenced by rapid urbanization, shifting land use patterns, and the traditional semi-intensive management of pig farming. Despite not being a major economic driver, the piggery sector remains important for the livelihoods of pig farmers and their communities.

The indigenous pig breeds show impressive adaptability in various landscapes. The decreasing numbers of these breeds jeopardise local pig population genetic diversity. While planning for piggery development, the association with the wild pig present in the state may not be ignored, and this approach may consider ecological, economic, and social factors to safeguard both domestic and wild pig populations, ensuring a balanced coexistence between pig husbandry practices and wildlife.

The domestic pig populations in Uttarakhand also show a unique maternal lineage, named UKD, which aligns with the W1 clade that includes *S. s. davidi*. This indicates a close genetic relationship and potential interbreeding between domestic and wild pig populations in the region.

This approach may consider ecological, economic, and social factors to safeguard both domestic and wild pig populations, ensuring a balanced coexistence between pig husbandry practices and wildlife.

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APPENDICES

APPENDIX 1: HOUSEHOLD QUESTIONNAIRE

Date of the survey **Village/block/district**

HEAD OF THE HOUSEHOLD NAME (Sex/ Age/experience year/ religion/no. of years of schooling/ can read and write/ ethnic affiliation/ who is managing day to day farm activities)

GPS COORDINATES: X (Easting) [____________________] Y (Northing) [_______________]

3. Livestock inventory.

Livestock		**Number owned**	**Who owns them (code)**	**Reasons for rearing(code-2max)**	**Number kept**	**If number kept is different from the no. owned, why? (code-2 max)**
Chicken						
Goat						
Pig	**Local**					
	Cross and exotic					
Cattle	**Local**					
	Cross and exotic					
Other specify						

Name (first name)	**Relationship with head**	**Age (yrs)**	**Sex**	**Vegetarian?** **1= Yes** **0= No**	**Primary Activity**	**Secondary activity (if applicable)**	**Number of years of schooling**
1							
2							
3							
4							

Details of the farmers house.

No. Of. Rooms / construction type of floor and roof/water supply/electricity supply/sewage facility/

Household assets:

Does the household keep pig? [   ] (1=Yes, 0=No).

Breeds code	**Place of purchase of the breed**	**Total no. kept**	**Hog (castrated male)**	**Boar (mature male pig)**	**Shoat (young male pig)**	**Sow (mature female pig)**	**Gilt (young female pig)**	**Piglet (newly born pigs)**	**Reasons of keeping (code- 2 max)**

Pig code: 1= Village pig, 2=Wild pig, 3=Wild crossed with village pig, 4=Village crossed with commercial pig, 5=Commercial pig, 6=other, specify

Breed preference- pigs:

Traits	Is this trait important to You? **1= Yes, 0=No**	**Breed 1, indicate code: _____**	**Breed 2, indicate code: _____**	**Breed 3, indicate code: _____**	**Breed 4, indicate code: _____**
Reproduction					
Age at maturity					
High conception rate					
Pre weaning survival rate					
Litter size					
Production					
Birth weight					
Weight at weaning					
High growth rate					
Carcass weight					
Meat quality					
General traits					
Docile					
Large body size					
Disease resistance					
Other, specify					
Overall ranking					

Type of Production Systems

Animal type	**Breed code**	**Management system**				**Housing system**		**If specific housing indicate**		
		Spring	**Summer**	**Rainy**	**Winter**	**Day**	**Night**	**Construction cost**	**When built**	**Used for other breed/ species**

Production parameters for pig

Breeds code	Age of first Farrowing	Farrowing interval	No of piglets per farrow	Conception rate	No died (in last 12 months)		Weight at maturity (kg)		Age of culling (old age) in years
					young	adults	male	female	

Feeding practices

		Own farm-feed type during				Purchased? 1=Yes, 0=No	If purchased-12 months for pig			
Species	Breed	Spring	Summer	Rainy	Winter		Quantity	Unit	Price/unit	Where
Pig										

Watering practices

			Main water sources during (code)				**Who collects water most regularly? (code)**	**Costs (local currency). Put 0 if no cost.**
Animal type	**Breed**	**How often do you give Water? (code)**	**spring**	**summer**	**rainy**	**winter**		
Pig								

Did you use animal health services in the last 12 months? [___]=YES [___]=NO (tick) fill the table:

What are the 2 most frequent animal health problems that affect your herd/ flock (list most important one first)?

Animal species	Pig	
	Disease 1	Disease 2
Disease/ condition code		
Which breed is more affected?		
If disease unknown what are the symptoms (2 maximum)		
Total no. of disease events in last 12 months		
Outcomes: 1=Died, 2=survived, 3=slaughtered		
Do you vaccine? 1= yes, 0=No		

Who takes care of the animals?

Grazing animal/Feeding animals/Feed preparation/Watering animal/

Caring sick animal/Animal shed cleaning/Marketing of live animals/Marketing of livestock products

Does the household hire labour who spend time on for pig activities? [ ] (Yes=1, 0= No) if yes fill the table

What are the main Breeding Strategies you follow fill the information

	Pig			
Breed code				
Breeding method				
If natural breeding, reasons for using it				
Source of breeding males				
Source of breeding females				
Who makes decisions on breeding males?				
If natural and controlled breeding, objective of controlling				
If natural and controlled breeding, how do you control?				
For pig, total number of conceptions during past one year (per female)				
Age when breeding male is replaced (years)				
Reasons for replacing the breeding male (code)				

List down the 3 main problems of breeding for each species (breed wise)

	Breed	**Problem 1**	**Problem 2**	**Problem 3**
Pig				

Do you have any costs related to breeding pig? [ ] (1=Yes, 0=No) yes fill the table

Marketing of livestock and livestock products of Pig: do you sell pig ? sale in last 12 months

Breed	Animal type	Number of pig sold	Price by animal	Weight in kg	To whom did you sell?	Where did you sell?	Reason for sale	Any “services” provided by buyer?	Who sold	Who got the money

Do you sell pig meat and other pig products? [___]=YES [___] =NO (tick)

Products: 1= Pig Meat, 2=Pig Manure, 2= Pig Skin, 3= Pig Fat, 4= other, specify

Do you have any grading method when selling? [___]=YES [___] =NO (tick)

Breed	Products	Grading mechanisms used	When do you use it?	Any external Involvement?	Since when do You use it? (year)

Do you plan to increase the amount of some or all livestock/ livestock products? [___]=YES [___]=NO (tick) If Yes, fill in the table

Breed	Products	Reason for wanting to produce or sell more	How will you achieve it? (3 max)

If NO. Why? [ ] [ ] [ ] (3 top reasons, ranked)
1= other more profitable enterprise available 2= too risky 3= not enough feed 4= poor animal health 5= no market 6= low price 7= breeds not appropriate 8= other, specify

Mode of payment and price determination
Is there any occasion when you're not paid cash? [___]=YES [___]=NO (tick)
Breeds
Products: 1= live animals, 2= meat, 3= eggs, 4= fat, 5= other, specify
Mode of payment: 1= cash advance, 2= delayed payment – after how many days? 3= other, specify
Buyer: 1= fellow farmer, 2=traders, 3=butcher, 4= cooperative 5= other, specify

Is there any occasion when price is not paid on prevailing market price? [ ] =Yes [___]=NO (tick) If yes provide the details

What are the current sources of getting information about the breeding, feeding, production, marketing and any other schemes and techniques?

Breed code
Source of information: 0= none, 1= Radio, 2=TV programmes, 3= Exhibition, 4=Open days or field days, 5= NGOs convening, 6=Govt. Institutes, 7=Other organizations,8= published material, 9= family and friends, 10= fellow farmers, 11= Other (specify)
Training needs: 1=Feeding/ feed preparation, 2=Breeding, 3=Health, 4=General management, 5=Production in general, 6=Marketing, 7=Business management, 8=Leadership skills, 9=Any other (specify)

Have you (or any other member of the household) received any training in the last 5 years

[ ] (Yes=1, No=0) If Yes, fill the table:

Indicate the use and availability livestock extension services (Project or NGO's, Private Practitioners, Cooperative/ farmer group)

APPENDIX 2: LIST OF SAMPLES WITH LOCATIONS USED IN THE STUDY

Sl.no.	Sample ID	District	Sample Type	Breed	Sex
1	UK1DP	Dehradun	Hair	Domestic	male
2	UK2DP	Dehradun	Hair	Domestic	male
3	UK3DP	Dehradun	Hair	Domestic	male
4	UK4DP	Dehradun	Hair	Domestic	male
5	UK5DP	Dehradun	Hair	Domestic	female
6	UK6DP	Dehradun	Hair	Domestic	female
7	UK7DP	Dehradun	Hair	Domestic	female
8	UK8DP	Dehradun	Hair	Domestic	female
9	UK9DP	Dehradun	Hair	Domestic	male
10	UK10DP	Dehradun	Hair	Domestic	female
11	UK11DP	Dehradun	Hair	Domestic	male
12	UK12DP	Dehradun	Hair	Domestic	female
13	UK13DP	Dehradun	Hair	Domestic	male
14	UK14DP	Dehradun	Hair	Domestic	female
15	UK15DP	Dehradun	Hair	Domestic	female
16	UK16DP	Dehradun	Hair	Domestic	male
17	UK17DP	Dehradun	Hair	Domestic	female
18	UK18DP	Dehradun	Hair	Domestic	unknown
19	UK19DP	Dehradun	Hair	Domestic	female
20	UK20DP	Dehradun	Hair	Domestic	male
21	UK21DP	Dehradun	Hair	Domestic	male
22	UK22DP	Dehradun	Hair	Domestic	male
23	UK23DP	Dehradun	Hair	Domestic	female
24	UK24DP	Dehradun	Hair	Domestic	female
25	UK25DP	Dehradun	Hair	Domestic	male
26	UK26DP	Dehradun	Hair	Domestic	male
27	UK27DP	Dehradun	Hair	Domestic	female
28	UK28DP	Dehradun	Hair	Domestic	female
29	UK29DP	Dehradun	Hair	Domestic	unknown
30	UK30DP	Dehradun	Hair	Domestic	unknown
31	UK31DP	Dehradun	Hair	Domestic	male
32	UK32DP	Dehradun	Hair	Domestic	female
33	UK33DP	Dehradun	Hair	Domestic	female
34	UK34DP	Dehradun	Hair	Domestic	female
35	UK35DP	Dehradun	Hair	Domestic	female
36	UK36DP	Dehradun	Hair	Domestic	male
37	UK37DP	Dehradun	Hair	Domestic	female
38	UK38DP	Dehradun	Hair	Domestic	female
39	UK39DP	Haridwar	Hair	Domestic	female

40	UK40DP	Haridwar	Hair	Domestic	male
41	UK41DP	Dehradun	Hair	Domestic	male
42	UK42DP	Dehradun	Hair	Domestic	female
43	UK43DP	Dehradun	Hair	Domestic	female
44	UK44DP	Dehradun	Hair	Domestic	male
45	UK45DP	Dehradun	Hair	Domestic	female
46	UK46DP	Dehradun	Hair	Domestic	female
47	UK47DP	Dehradun	Hair	Domestic	male
48	UK48DP	Dehradun	Hair	Domestic	male
49	UK49DP	Dehradun	Hair	Domestic	male
50	UK50DP	Dehradun	Hair	Domestic	female
51	UK51DP	Dehradun	Hair	Domestic	female
52	UK52DP	Dehradun	Hair	Domestic	male
53	UK53DP	Dehradun	Hair	Domestic	female
54	UK54DP	Dehradun	Hair	Domestic	female
55	UK55DP	Dehradun	Hair	Domestic	male
56	UK56DP	Dehradun	Hair	Domestic	male
57	UK57DP	Dehradun	Hair	Domestic	male
58	UK58DP	Dehradun	Hair	Domestic	male
59	UK59DP	Nainital	Hair	Domestic	female
60	UK60DP	Nainital	Hair	Domestic	female
61	UK61DP	Nainital	Hair	Domestic	female
62	UK62DP	Nainital	Hair	Domestic	female
63	UK63DP	Nainital	Hair	Domestic	female
64	UK64DP	Nainital	Hair	Domestic	male
65	UK65DP	Dehradun	Hair	Domestic	male
66	UK66DP	Dehradun	Hair	Domestic	male
67	UK67DP	Dehradun	Hair	Domestic	male
68	UK68DP	Nainital	Hair	Domestic	female

Note: Sample ID UK31DP didn’t have any positive amplification success for any Microsatellite marker hence only 67 samples were used.

APPENDIX 3: ADDITIONAL SAMPLES FROM BIHAR AND JHARKHAND

Sl. No.	Sample ID	State	Sample type	Breed	Sex
1	BR1DP	Bihar	Hair	Domestic	female
2	JH1DP	Jharkhand	Hair	Domestic	female
3	JH2DP	Jharkhand	Hair	Domestic	female
4	JH3DP	Jharkhand	Hair	Domestic	female
5	JH4DP	Jharkhand	Hair	Domestic	female
6	JH5DP	Jharkhand	Hair	Domestic	female
7	JH6DP	Jharkhand	Hair	Domestic	female

8	JH7DP	Jharkhand	Hair	Domestic	female
9	JH8DP	Jharkhand	Hair	Domestic	female
10	JH9DP	Jharkhand	Hair	Domestic	female
11	JH10DP	Jharkhand	Hair	Domestic	female
12	JH11DP	Jharkhand	Hair	Domestic	female
13	JH12DP	Jharkhand	Hair	Domestic	female
14	JH13DP	Jharkhand	Hair	Domestic	female
15	JH14DP	Jharkhand	Hair	Domestic	female

APPENDIX 4. PRIMERS PAIRS FOR MITOCHONDRIAL CONTROL REGION AMPLIFICATION

Forward Primer (Cerv.tPro: 5’ -CCACYATCAACACCCAAAGC-3’)

Reverse Primer (CervCRH: 5’- GCCCTGAARAAAGAACCAGATG-3’)

APPENDIX 5: REPRESENTATIVE GLOBAL PIG HAPLOTYPE SEQUENCES USED TO INFER PHYLOGENETIC AND NETWORK HAPLOTYPES ANALYSIS.

Accession number	**Breed/status**	**Type**	**Haplogroup**
AB041484	Berkshire	European/Domestic	D1
AB041485	Berkshire	European/Domestic	D1
AB041486	Duroc	European/Domestic	D1
AB041491	Large white	European/Domestic	D1
AB041492	Large white	European/Domestic	D1
AB041493	Large white	European/Domestic	D1
AB041495	Landrace	European/Domestic	D1
AB041496	Landrace	European/Domestic	D1
AB041497	Landrace	European/Domestic	D1
AB041498	Landrace	European/Domestic	D1
AB041499	Landrace	European/Domestic	D1
AB015093	Yucatan miniature pig	European/Domestic	D1
AB041467	Japanese wild boar	Asian/Wild boar	D2
AB041469	Japanese wild boar	Asian/Wild boar	D2
AB041471	Japanese wild boar	Wild boar	D2
AB041475	Jinhua	Asian/Domestic	D2
AB041476	Jinhua	Asian/Domestic	D2
AB041481	Moncai	Asian/Domestic	D2

AB041487	Duroc	Domestic/European	D2
AB059651	European wild boar	European/Wild boar	D2
AB015084	Japanese wild boar	Asian	D2
AB015085	Japanese wild boar	Asian	D2
AB015086	Japanese wild boar	Asian	D2
D42174	Japanese wild boar	Asian/Domestic	D2
D42178	Japanese wild boar	Asian/Domestic	D2
D42181	Japan	Asian/Domestic	D2
D42182	Japan	Asian/Domestic	D2
AB252783	Myanmar native pig	Domestic/Asian	D2
AB252785	Myanmar native pig	Domestic/Asian	D2
AB252786	Myanmar native pig	Domestic/Asian	D2
AB252787	Cambodia native pig	Domestic/Asian	D2
AB252788	Myanmar native pig	Domestic/Asian	D2
AB252789	Myanmar native pig	Domestic/Asian	D2
AB252790	Laos native pig	Domestic/Asian	D2
AB252791	Vietnam native pig	Domestic/Asian	D2
AB252792	Vietnam native pig	Domestic/Asian	D2
AB252793	Bhutan native pig	Domestic/Asian	D2
AB252794	Myanmar native pig	Domestic/Asian	D2
AB252795	Bhutan native pig	Domestic/Asian	D2
AB252796	Bhutan native pig	Domestic/Asian	D2
AB041472	Japan	Wild	D2
AB252798	Bhutan native pig	Domestic/Asian	D2
AB252799	Cambodia native pig	Domestic/Asian	D2
AB252800	Myanmar native pig	Domestic/Asian	D2
AB252802	Laos native pig	Domestic/Asian	D2
AB252803	Myanmar native pig	Domestic/Asian	D2
AB252804	Myanmar native pig	Domestic/Asian	D2
AB252806	Laos native pig	Domestic/Asian	D2
AB252807	Myanmar native pig	Domestic/Asian	D2
AB252808	Myanmar native pig	Domestic/Asian	D2
AB252809	Laos native pig	Domestic/Asian	D2
AB252810	Myanmar native pig	Domestic/Asian	D2
AY884709	Indian wild boar	Wild boar	D3
AY884675	Indian wild boar	Wild boar	D3
AB252825	Bhutan wild boar	Wild	D3
AB252826	Bhutan wild boar	Asian	D3
AB015094	Italian wild boar	Wild boar	D4
AB015095	Italuan wild boar	Wild boar	D4
AY884623	Myanmar wild boar	Wild boar	D5
AY884678	USA	Island SEA & Oceania	D6

AB252811	Bhutan native pig	Domestic/Asian	MTSEA
AB252812	Cambodia native pig	Domestic/Asian	MTSEA
AB252813	Cambodia native pig	Domestic/Asian	MTSEA
AB252814	Cambodia native pig	Domestic/Asian	MTSEA
AB252819	Laos native pig	Domestic/Asian	MTSEA
AB252820	Cambodia native pig	Domestic/Asian	MTSEA
AB252823	Cambodian wild boar	Asian	CWASIAN
AB252824	Cambodian wild boar	Asian	CWASIAN
AB015087	Ryukyu wild boar	Wild boar	
AB015088	Ryukyu wild boar	Wild boar	
AB015089	Ryukyu wild boar	Wild boar	
AB015090	Ryukyu wild boar	Wild boar	

Status of pig farming and its multi-factorial assessment in the urban districts of Uttarakhand: A case study about its sustainability and ecological implications

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Abstract

Pig farming has a profound caste-based and cultural association in India. In Uttarakhand, pigs are raised predominantly by the specific community contributing to their livelihood. Unlike other livestock species in the State, the piggery sector is unorganised and the pig farming is not sufficient to meet the increasing demand for pork. The study aims to identify the locations of the household rearing pig and its husbandry practices in the planar (Dehradun, Haridwar and Nainital) districts where rapid urbanisation and continuous migration make us reconsider the necessity of an organise promotion of the piggery sector due to its multiple ecological implications. 28 households were reached by adopting rapid appraisals approach and interviewed by using a semi-structured questionnaire. Analysis showed that 60.7%, 10.7% and 21.4% of the household raised pig near seasonal streams (slum settlements), the residential areas within the house premises and near the forest, respectively. Moreover, 28.6% of households maintained breeding boar of feral origin and 96.94% followed a semi-intensive pig rearing system; hence human settlement is staying in close proximity with pigs (domestic and wild) and other domestic animals. The possibility of the emergence of zoonotic and non-zoonotic disease and its easy transmission to humans become more prominent in the urban setting. Before planning for a holistic piggery development program in Uttarakhand, the government may consider these findings. Lastly, the location of the pig herd and its foraging areas requires to be strictly monitored for public health benefits.

Introduction

The modern world is the repercussion of a historical act of the human race, which is the paradigm shift from a hunter-gatherer to food producer based on the domestication of a few wild plants and animal species (Frantz et al. 2020). The domestication events and the sedentary settlement resulted in the formation of societies that embodied different cultures, linguistics, and food habits (Diamond et al., 2003). Unlike the other domesticated animal species, the abundance of domestic pigs (*Sus scrofa*) in a particular region is influenced by the religion, culture, and tradition for pork consumption. Except for Jews and Muslims, almost all other religions have included pork in their food regime after Pig domestication (Pappas 2013). India is a secular country with the coexistence of diverse cultures and ethnic groups and 79.8% of the total population, i.e. 966.3 million people, follows Hinduism (Census 2011).

Most of the people's cultural assumptions and customary behaviors are derived from Hinduism. In the Hindu scriptures, the animals were revered and deified according to their qualities and use. Accordingly, the boar became the symbol of food production and fertility due to its ability to dig the earth with its tusk (Krishna 2010). Furthermore, Hindu mythology also believes in the reincarnation of god Vishnu in each era to destroy evil. His third reincarnation was in the form of Varaha or wild boar, to rescue the earth from the evils of demons. Till date, many temples throughout India offer prayers to the Varah avatar of Vishnu (Krishna 2010; Dornadula 2007).

The different empires in Indian history gave recognition to the animals in their coins and Flags. The Chalukya, Kakatiya and Vijaynagara dynasties used the figure of a boar in their coins and flag to honor the animal (Krishna 2010; Math 2018).

The worship and significance of wild boar have been seen since time immemorial. Pig has been domesticated, but when the question of pig rearing comes, the scenario depends on the other aspect of Hinduism called the caste system, which is the oldest form of surviving social stratification. Its effect cannot be ignored in modern society well. In modern India, the historic social stratification in the form of caste system has been categorised by the government in the form of scheduled castes, which includes the historically weaker castes both socially and economically. The backward tribes were categorised as scheduled tribes (ST) and the disadvantaged castes as other backward castes (OBC) and all the others were included in the general caste (Sankaran et al., 2017).

According to the traditional caste system, the lower caste prefers pig rearing. Hence, in India, pigs are raised mostly by scheduled caste and tribes to maintain their social identity and traditional culture (Stevenson 1954; Banik et al. 2019). Though the upper caste avoids the eating of domestic pigs, the illegal consumption of wild boar is preferred under the caste system as it is considered a clean feeding animal (Stevenson 1954). However, wild boar is a protected species in India, and consuming its meat is a punishable offense.

The pig is considered an important livestock species in India, yet its contribution is 1.69% of the total livestock species in India (20th Livestock census 2019). The majority of the pig population in India is of indigenous breeds, which comprises 76% of the total population. The major contribution of the pig population in the country comes from the eastern and northeastern regions, which is around 63%. The highest pig population is in the State of Assam (2.10 million), followed by Jharkhand (1.28 million), Meghalaya (0.71 million) and West Bengal (0.54 million) (20th Livestock census 2019).

As very little information is available regarding the piggery sector and the effect on its growth due to urbanization in Uttarakhand. The present study aims, through the household survey method, to (1) identify the location of pig rearers in urban districts of Uttarakhand and analyse its ecological implications to the urban landscape and human settlement (2) describe the socio-economic condition and pig husbandry practices adopted by the rearer in Uttarakhand.

Uttarakhand is predominantly the mountainous State carved out of northern Uttar Pradesh as a separate state of India in 2000. It is situated on the southern slope of the world's youngest (geo-dynamically sensitive) mountain system, "the Himalayas". It makes Uttarakhand a vulnerable and disaster-prone ecosystem with frequent floods, landslides and earthquakes (UBB 2002).

The total geographic area of the State has been divided into four physiographic zones based on the mean altitude from the sea level. Haridwar and Udham Singh Nagar lie in the plain area, Dehradun and Nainital are partially plain and the other nine districts of Almora, Bageshwar, Champawat, Chamoli, Pauri, Pithoragarh, Tehri, Rudraprayag and Uttarkashi are hilly (SAP 2017).

Materials And Methods

The household survey was selected by analysing the district-wise pig population density from the livestock census data of 2012 (Fig. 1). According to the census data, Dehradun, Haridwar and Udham Singh Nagar have a large pig population and Nainital is also conducive to piggery development (19th Livestock Census 2012). These districts are also considered as major urban centers of the State. Rapid urbanisation and tremendous population growth are also seen in these districts and the existence of a large number of pigs compared to the other districts (Sati 2013).

The data about pig raisers and their location were not available when we planned to conduct this study. We contacted the local persons involved in pig rearing with the help of the field assistant. A rapid appraisal approach was adopted as a "survey and monitoring tool" to identify more geographical locations in Uttarakhand where pig rearers were localized (FAO 2011).

Before collecting data, a semi-structured questionnaire (ILRI 2011) was prepared following the study's objectives (Supplementary table: ST1). A draft of the questionnaire was pre-tested with 5 households in Dehradun and according to the requirement, responses and feedback from the respondent, the questionnaire was then refined. The final questionnaire took around 30 minutes to complete and targeted the head of the household who took care of the pig farming. Interviews were performed in the local language (Hindi) and were carried out with the assistance of the key informant and the author herself.

'Key informants' played an important resource in our survey. They help us in establishing rapport with the pig rearer household. The geographical position of each participating family was recorded using the android based software Google Map.

Pig-owning households were selected randomly and purposeful sampling was employed to ensure the interview of the family that raised pigs near the forest area was also included in the study. 28 households (HH) (25 Dehradun, 2 Haridwar, and 1 Nainital), including one family without herd size data, were surveyed for this study. The survey was conducted between March to December 2019. The survey was not performed during July 2019 and August 2019 due to the rainy season. Data related to the socio-economic status of pig rearers and the management system of pig farming were collected, compiled, and analysed using Microsoft excel 2007.

Population trend of pigs in Uttarakhand

Various livestock species are found in all the four physiographic zones of Uttarakhand (SAP 2017); the species-wise contribution of the livestock population in Uttarakhand and its population trend during various livestock censuses of India is shown in (Fig 2). The population trend showed that the piggery sector contributes much less than different livestock species of Uttarakhand and its population is continuously declining in each progressive census year (Fig. 3).

The current pig population of Uttarakhand consists of indigenous/non-descript types and exotic/crossbred pigs (Fig. 4). A rapid decline in the indigenous breed population since the 17th livestock census and an increase in the population of the exotic breed has been observed from the census data. The introduction of the exotic breeds of pig from western countries in India had started before independence. Since then, the government breed improvement program has used these exotic animals to upgrade the native stock for superior performance in the economic traits such as high growth rate, high feed conversion efficiency, good litter size and carcass quality (Deka 2015). The exotic pig and its crossbreed are gaining preference among the pig breeders due to changes in the taste preference of the consumers. The exotic breed such as Landrace, Yorkshire and their crosses are raised in Uttarakhand (Breed survey 2013).

The overall pig population recorded in 2003 (17th Indian livestock census) in Uttarakhand was 31000; this reduced to 17659 (20th Livestock Census), which was a two-fold decline in the pig population since the 17th livestock census (after the formation of the new State). On contrary, the estimated number of the animal slaughtered each year in Uttarakhand surpass the count of pigs present in Uttarakhand (Fig. 5).

It is clear that the demand for pork products has shown a continuous rise over the last decade, but the supply of live animals cannot be fulfilled by the number of pigs raised in the State. This contrary data revolves around multiple reasons. The probable reasons for the rise in the demand for pork meat and its products can be linked to the increased inflow of tourists of preferential pork taste from other states of India and abroad (Table 1). The pork dishes are now included in the meal plans of various hotels, restaurants and institutions in Uttarakhand. Hence to meet the consumers' demand, more animals are slaughtered (Banik et al. 2019). Other possible reasons for this huge decrease in population are the increased influence of rapid urbanization and scarcity of open and empty land areas due to rapid construction. In Uttarakhand, the pig farmers rear pigs in semi-intensive management and pig are seen roaming and foraging on the garbage pile. Due to the government provision of making these urban districts clean, the municipal corporation confiscates roaming pigs on the street. These scenarios compelled pig rearers to farm pigs in small herds. Another probable reason may also be the availability of an alternate source of employment due to job opportunities and the growing social stigma against pig farming.

During our survey, one respondent said that 'if they get employment of 15000 INR (197 USD) per month, they will leave pig rearing practices as they were tired of the administration and social stigma associated with pig rearing. Another said that 'their children do not prefer to take the practice further as they are going to school and according to them rearing pig is one of the filthy practice among their friends.'

Indigenous pigs are found in all the districts of Uttarakhand and have a close association with the wild pigs. Moreover, they have better adaptability to both the plain and the hill districts of Uttarakhand. The sharp decline in the population of the indigenous pig needs to be immediately scrutinized by the government as the scientific community has been trying to investigate the origin and relationship between indigenous and wild pigs in the Hindukush Himalayan region for the last two decades (Larson et al. 2005; Nidup 2006; Tanaka et al. 2008).

Breed characterization and cataloging of the distinct indigenous breed populations have gained momentum in India during the last few years. Till date, ten native pigs (Agonda Goan, Ghoongroo, Gurrah, Mali, Niang Megha, Tenyi Vo, Nicobari, Purnea, Doom and Zowak) have been characterized and accorded the status of indigenous registered breeds (ICAR- NBAGR 2018), which are mostly from the eastern states of the country. We can initiate the process of breed study in Uttarakhand and characterize with appropriate scientific tools to study morphometric traits and production parameters.

Map

GPS (global positioning system) coordinates were plotted in the map using Google Map and further preparation of the map was done in arc GIS software (Fig. 6). According to our survey findings, 60.7% of households raised pigs near seasonal streams or rivers. These household locations coincide with slums (poverty pockets) areas of the surveyed urban districts (PCA slums 2011). 21.4% of households interviewed raise pigs near forest areas. 10.7% of households raise pigs in the residential areas with small or no open space and no water bodies for the pig to roam and make sheds within the house premises.

Pig rearer households were distributed in random patches throughout Dehradun, Haridwar and Nainital districts. Urban livestock plays a major role in fulfilling the growing demand for animal-related products in urban areas. However, livestock keeping and their maintenance in the urban landscape possesses an important risk of disease transmission (zoonotic and non-zoonotic), which has been widely studied (Alirol et al. 2011; Alarcon et al. 2017; Ahmed et al. 2019). Hence, two more aspects were considered to give a holistic picture of the impact of raising pigs in Uttarakhand. These are (1) the present pace of rapid urbanisation of Uttarakhand and (2) contact exposure of wild boars to humans and domestic animals.

There has been a rapid increase in infrastructural growth, a rise in migratory population, continuous flow of tourism and other commercial activities, particularly in the planar districts like Dehradun, Haridwar, Nainital and Udham Singh Nagar. To accommodate all the above activities, unplanned urbanization has taken place in these planar districts. The existing natural resources and ecology of these districts support all these activities and the increased number of livestock populations beyond their carrying capacity. The unplanned urbanisation has boosted the growth of slums in Dehradun along the bank of the perennial streams like Bindal and Rispana Rao. These streams run within the district and are degraded from freshwater streams to the city's drainage (Dehradun City Development Plan 2007). A similar pattern was observed in Haridwar and Nainital districts. The surveyed household was in Haridwar's most congested areas of Jwalapur close to the Ganga canal (Haridwar City Development Plan 2007) and slums settlements areas near Ramnagar close to river Kosi in Nainital (District census handbook Nainital 2011).

In all these districts human population is staying near pigs and other domestic animals. Our findings indicated that 60.7% of households raise pigs near slum settlements with inadequate sanitation, making these areas prone to zoonotic and non-zoonotic diseases. Moreover, close contact with the pigs living in these unhygienic environments poses a risk to animal health and human health (Alirol et al., 2011; Ahmed et al., 2019).

The other aspect unique to Uttarakhand is the wildlife–livestock–human interface, which poses another risk to human health. Uttarakhand has 45.44 % of the State's geographical area covered by forest and Dehradun, Udham Singh Nagar, Nainital and Haridwar contribute 23.32% of the total forest cover (State of Forest Report, 2019).

Due to the increased use of land for construction activities, there has been rapid deforestation in the urban districts, increasing the chances of contact exposure of wild boars to humans and domestic animals. The wild pig is a protected species under Schedule-III of the Indian Wild Life (Protection) Act, 1972. Uttarakhand government declared wild boar as "vermin" during 2016-2019, which authorised the local people to cull the animals (as per the guideline issued by the forest department) if it is found on agricultural land raiding crops (Azad 2020). 100% of households were hesitant to talk about wild boar, but some local people stated that hunting wild boars and their meat consumption is prevalent in Uttarakhand. Except for Brahmins, Pahari Rajput and other castes relish the taste because it is a clean feeder, and its meat has low-fat content (Sethi et al., 2010).

Wild boars harbor several viruses, bacteria, and parasites diseases that can transmit to humans through the consumption of meat and other domestic animals when they come in close contact with them (Meng et al., 2009). Our survey data clearly state that 21.4% of households interviewed raise pigs near forest areas. The domesticated pig remains forest during the day and returns to its shed at night. Hence this increases the chance of contact of wild boars with domestic pigs and humans. Thus, the chances of transmission of pathogens between wild boars and humans are high in Uttarakhand's highly populated districts. Very few cases of disease transmission to humans have been reported and documented in Uttarakhand. A report of multiple outbreaks of 'Human Trichinosis' caused by eating undercooked wild boar meat pork infected with the larvae of a nematode parasite of *Trichinella sp.* has been reported in Tehri and Pauri Garwal districts of Uttarakhand during 2009-2011 (Sethi et al., 2010). Swine flu which belongs to the influenza virus group endemic in pigs, continues to report every year. The number of patients was more from urban areas of Uttarakhand due to congested infrastructure and inadequate hygiene (Pandita et al., 2021).

Due to the location of the pig rearer, chances of an outbreak of disease either through the animal itself or through contamination of water bodies in urban sites become more prevalent. There is an immediate need to increase the awareness of disease emergence among the policymaker, pig rearers and veterinary practitioners in the urban settings. Improving the surveillance strategies for pathogens shared between wildlife and domestic animals will avoid any risk of pathogen spillover from the wild (Wu et al., 2012; Hassell et al., 2017).

Pig husbandry practices in Uttarakhand

Household characteristics:

The households were predominantly headed by males (100%) and only male members were the main respondent in the urban areas of Dehradun. The female participation in the survey response was in places where the pig was raised in the backyard i.e., not far away from the house. Regarding the age of household head, the highest proportion (85.7%) were in the middle age group (30-55 years), only (4.3%) were in the young age group (20-29 years). 100% of households surveyed in this study belonged to the Scheduled Caste (SC) community of a specific caste. In Uttarakhand, among SC community, this particular caste has a 70.64 % urban population (PCA SC 2011). Haridwar, Dehradun, Udham Singh Nagar, and Nainital districts together accommodate 86.7% of the total population of this specific community in the State (PCA SC 2011). These four urban districts also have a considerable pig population in the State (Fig. 1), indicating that a specific caste is associated with pig farming in Uttarakhand. Caste-based spatial segregation has been observed in these districts (Sidhwani 2015). Due to the urbanized nature of this community, female participation is significantly less in piggery enterprises. The work participation rate is also the lowest in this community compared to other scheduled castes, only 33.49% (Census 2011).

About 50% of household heads had primary as the highest education level attained, 46.4% by those with secondary education, and 3.6% had no formal education. As for household size, 89.29% of the household have a nuclear family (3-6 members), and only (10.71%) have a joint family (>6 members) in the home. The data suggest that though the community is highly urbanised, the economic status is dismal; hence they prefer nuclear families over joint families to avoid the financial burden of any extra members (Saggurti et al., 2005).

The house construction type of the pig rearer in all the surveyed households was 100% solid construction with bricks and cement. They have 100% water supply either from hand pump or other sources and electricity supply. Every (100%) household has Television as a source of entertainment. Only (21.4 %) of the family had taken pig rearing as a primary activity. They were enthusiastic about giving the traditional information about their rearing practices; otherwise, 78.6% had other odd jobs and rearing pigs in a small herd. In Uttarakhand, the pig rearer community has been continued to be known by their caste occupation and as observed that 64.3% work as sweepers in the various institute and municipal corporations (Ganguly 2019)

Due to urbanisation and improvement in community literacy, our survey data suggest a diversified occupation, with 10.71% in the army and 3.6 % in other jobs. Other activities for their livelihood are ceremonial music and poultry and some are also involved in game animal sports like cockfighting and pigeon flying. None of the households owns agricultural land to cultivate in other fields.

Livestock inventory

39.29% of the participating households raise chicken for commercial purposes. 25% kept cattle. Few families kept pigeons and wild cock for gambling and betting purposes. Households kept 2-4 adult sows and 1-3 hybrid or boars (need confirmation) with their piglets in their herd (Fig. 5a, 5b, 5c). They identified the breeds in their herd as indigenous and mixed parentage of indigenous crossed with the exotic breed and putative crossed with a wild pig. 28.6% of pig rearers maintained probable breeding boar of feral origin in secrecy due to its easy availability and high sale value (Fig. 5d). Detection of wild boar-like piglets in the head could be due to the possible hybridisation of the domestic pig with the wild boar in the proximity near the fringes area of the forest. However, these need to be examined by molecular genetic data to confirm the intentional or by mistake rearing of wild boar-like piglets for commercial use.

Production and management of pig

The combination of farrow to finish (piglets born in the herd kept by the household are raised in the same herd till slaughter) and piglet production (more sows are kept in a herd for piglet production to sell the piglets) systems is a type of production system observed in 85.7% of the households surveyed. Only 14.3 % of the households practiced fattener production (piglets were purchased from the households and raised for slaughter). The major rearing pattern observed in the study area was a semi-intensive system (96.94%) (Fig. 5e) followed by an extensive (3.57%) (Fig. 5f) system. Though these systems need less capital investment, disease incidence and parasite infestations are high. The girth tethering of the pig is not followed in any area. Almost all the pig farmers feed swill (from restaurant and kitchen) to their herd, which costs them INR 500 (6.57 USD) per month. Feeding and watering are given twice a day to the household's herd that follows a combination production system. During winter, warm water is mixed with food to keep them away from the cold. The pig rearer did not measure the quantity of the feed provided to the pigs.

Animal health services are available at INR15 (0.20 USD) as a consultation charge, but no veterinary doctors visit the sick pig. 100 % of the households said that there is no service for vaccination and treatment of ill animals. Decreased appetite, classical swine fever, nails cracking, diarrhea, worm's infection, and foot and mouth disease are common among pigs.

Reproduction, breeding and breed preference

The natural and controlled breeding method is the only available method for reproduction present in the area. The breeding males are from their farms or nearby farms and sometimes wild relatives. The castration of the old boar was done by 100% of the respondents. They castrate their male pigs by traditional indigenous methods and these pigs get a good amount if given for religious sacrifices in festivals and ceremonies. 100% of the respondents had recorded the farrowing of indigenous pigs twice a year, with a litter size of 6-8 piglets on average. 100% of the respondent preferred the traits like mothering ability, reproductive performance, and disease tolerance over general appearance and scavenging ability as a quality of the females that were to be kept by the owner in the herd for future production.

Marketing and Annual income

The majority (100%) of pig farmers have a family income ranging from INR 30000 - 1.0 lakh (394 USD- 1313 USD) per annum. The selling price of the live adult pig range INR 3500-4000 (45-USD -52 USD) and piglets INR 2500-3000 (32 USD- 39 USD). The animals are sold to the traders, butchers and the village animal fair. The butchering is done by other scheduled caste communities. They sell the meat INR 180-

200 (2 USD) per kg in the shop after inspection by veterinary doctors in a registered slaughterhouse. If sold directly to the consumer, then assessment is not done.

Limitations to pig-keeping

Pig husbandry practices are an unorganised sector in Uttarakhand; hence 100 % of the households considered rapid infrastructure growth and administration pressure a significant constraint to pig keeping. Few families also mentioned that pig raising forms major conflict issues with the neighbor who follow Islam as a religion. No household mentioned the diseased condition of the pig as a constraint because the majority kept mixed parentage herd of wild and indigenous breeds hence better adapted to the climatic condition and low management inputs.

Conclusion And Recommendation

The findings from the small representation of the pig rearer in Uttarakhand give us an understanding of the socio-economic status of pig rearers with their locations. The research analysis suggests that the pig is predominantly reared by the scheduled castes community in Uttarakhand. Due to religious and cultural customs, pig farming is a highly unacceptable occupation among the higher caste. Caste-based spatial segregation of the pig rearer group has been observed in Uttarakhand (Ganguly 2019). These communities have their accommodation concentrated in the squatters and slums, which indicates their socio-economic conditions. As observed, the caste occupation is still attached to them and most of them work as a sweeper in Municipal Corporation. The demand for pork and its product is continuously rising in the State. But the policy made on pig farming is not adequate for developing the piggery sector in Uttarakhand. Presently there is two government pig farm in Uttarakhand, but none has contributed significantly to the promotion of pig farming and these have been declared defunct for many years (SAP 2017).

This study also suggests that the wild or hybrid pigs are included in the herd because of their easy availability, disease resistance, meat preference, and medicinal values; hence, they come in close contact with humans and other livestock species (Namusisi et al. 2021). These interactions create a potential threat for disease spillover from wild pigs to humans and other livestock species. The intensity of the risk of transmission becomes more profound with an increase in urbanisation and anthropogenic influence beyond the carrying capacity of the area's ecological system. People in Uttarakhand are unaware of the zoonosis diseases of wildlife origin and the risk associated with their close contact. Hence sensitization and awareness campaigns should be promoted to address the zoonotic disease transmitted by the consumption of wild pigs and other wildlife origin animals. The government often neglects public health policies while planning for urban development. This neglect poses a higher risk of zoonotic and non-zoonotic diseases to the population living near slums and informal settlements. Hence these settlements can become an epicenter for the outbreak of infectious disease and due to pigs roaming in these areas, the chances of zoonotic disease will also increase. The location of the herd and its foraging should be strictly monitored. Understanding the dynamics between wildlife, livestock and humans and its detailed surveillance should be focused on by the government of Uttarakhand to prevent the emergence and spread of disease.

Before planning for piggery development by the government in Uttarakhand, multiple factors should be considered. The survey gave us an idea that the socio-economic factor such as participation of the young age group in the piggery sector is only 4.3% moreover, the work participation rate among the pig rearer community in Uttarakhand is lowest (census) hence before planning for the pig improvement program in Uttarakhand, motivation and proper training should be given to the younger generation of this particular community to carry the pig rearing activity. Lastly, the government policy of piggery development in Uttarakhand should also consider the indigenous breed due to their adaption to the plain and mountainous districts. Therefore, documentation and phenotypic characterisation of indigenous pigs and their production system is crucial for making the strategic decision for its promotion and conservation.

Declarations

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Conflict of interest: The authors declare no competing interests.

Ethics approval: Not applicable

Consent to participate Informed consent was obtained from the households before the interviews and to publish the data anonymously.

Consent for publication: Not required

Authors' contributions: Conceptualization: KS, SKG; Methodology, formal analysis and drafting: KS; and writing review and editing: SKG

Data availability statement: We have no data to show; however, samples questioner form is provided in the supplementary materials

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Tables

Table 1 is not available with this version.

Figures

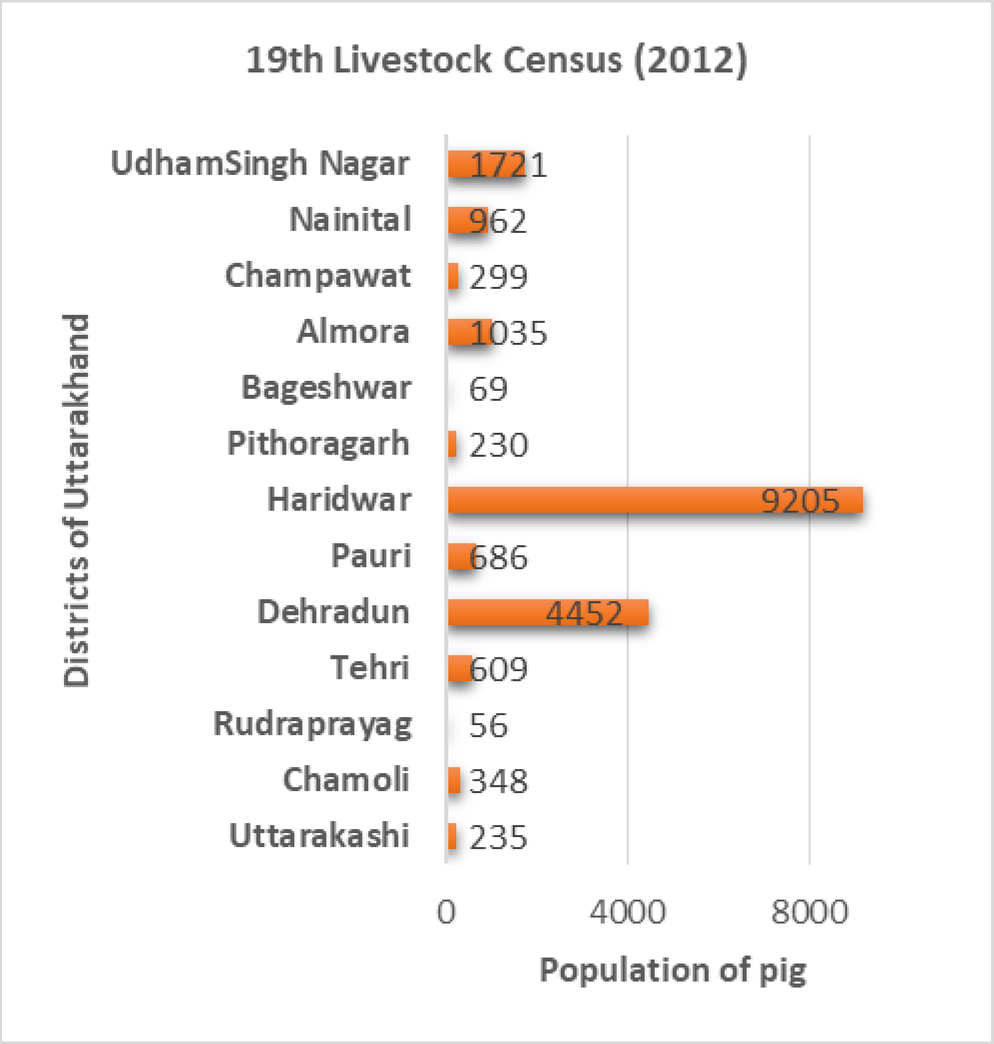

Figure 1

District wise population of pigs in Uttarakhand (Redrawn from 19th Livestock census 2012)

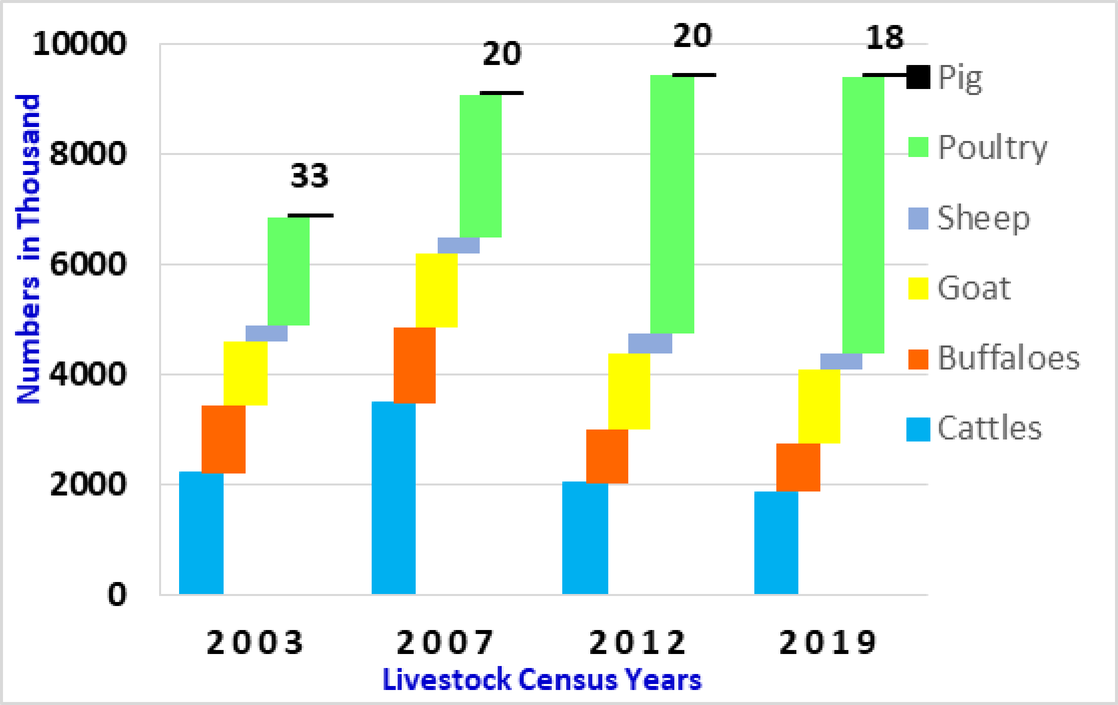

Figure 2

Comparative population trend of various livestock species in Uttarakhand (Redrawn from 17th to 20th Livestock Census, GOI)

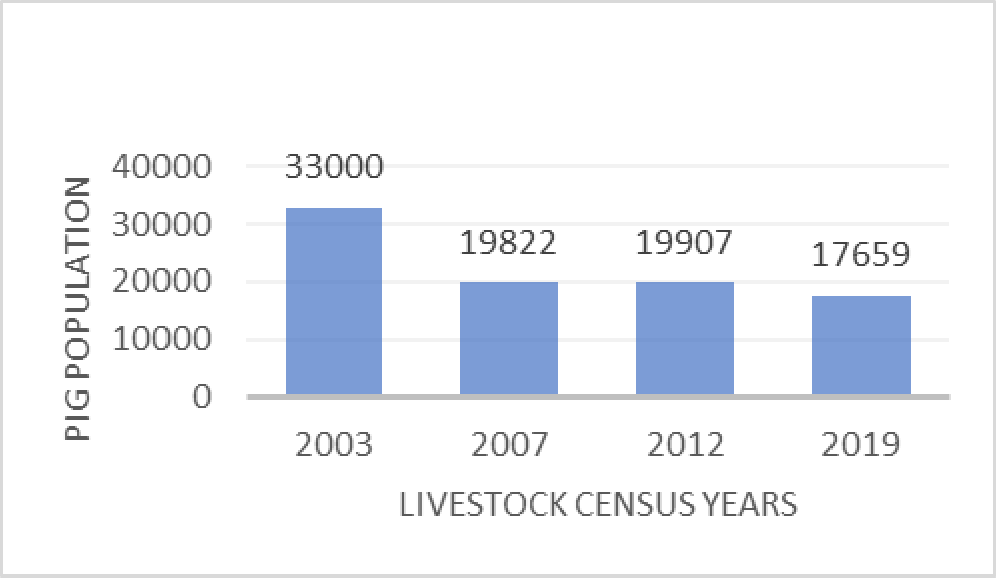

Figure 3

Population trend of pigs in Uttarakhand after the formation of State (Redrawn from 17th- 20th Livestock census of India).

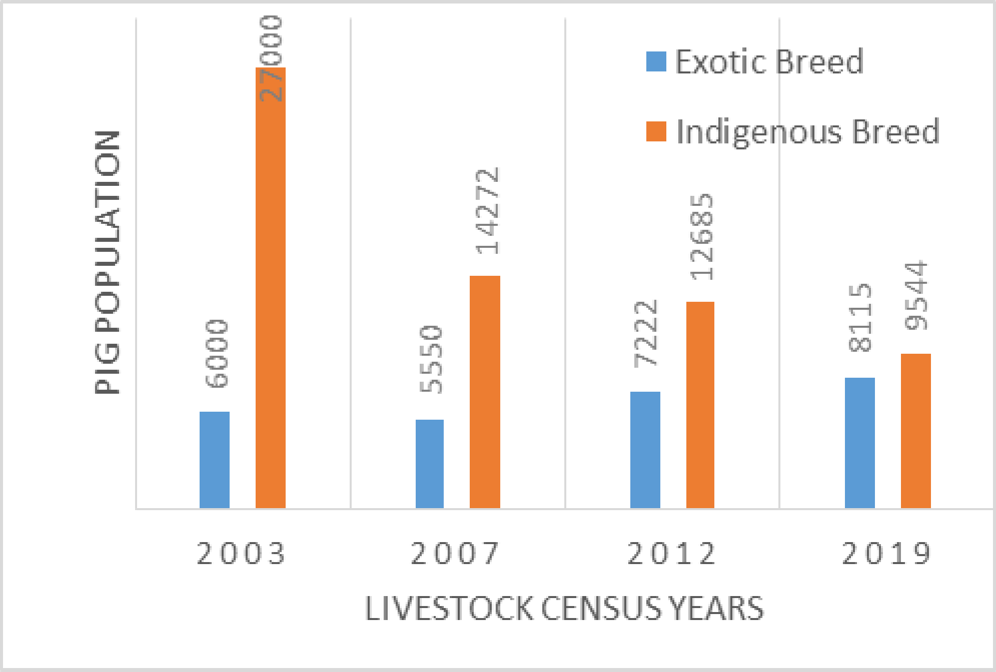

Figure 4

Population trend of Indigenous and Exotic breed in Uttarakhand (Redrawn from 17th-20th Livestock census of India)

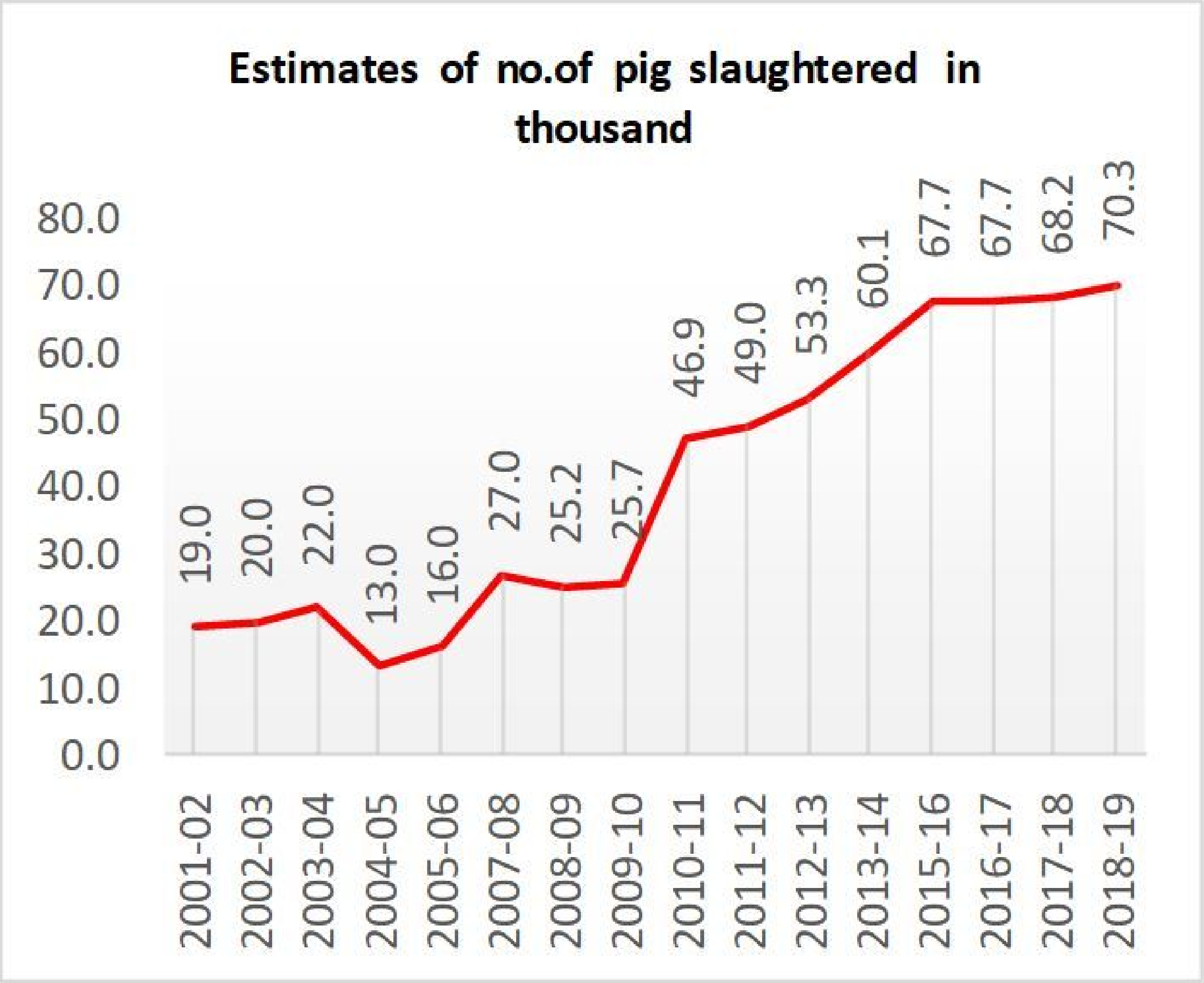

Figure 5

Year-wise estimates of the number of pigs slaughtered in Uttarakhand (Redrawn from Basic Animal Husbandry and Fisheries Statistics)

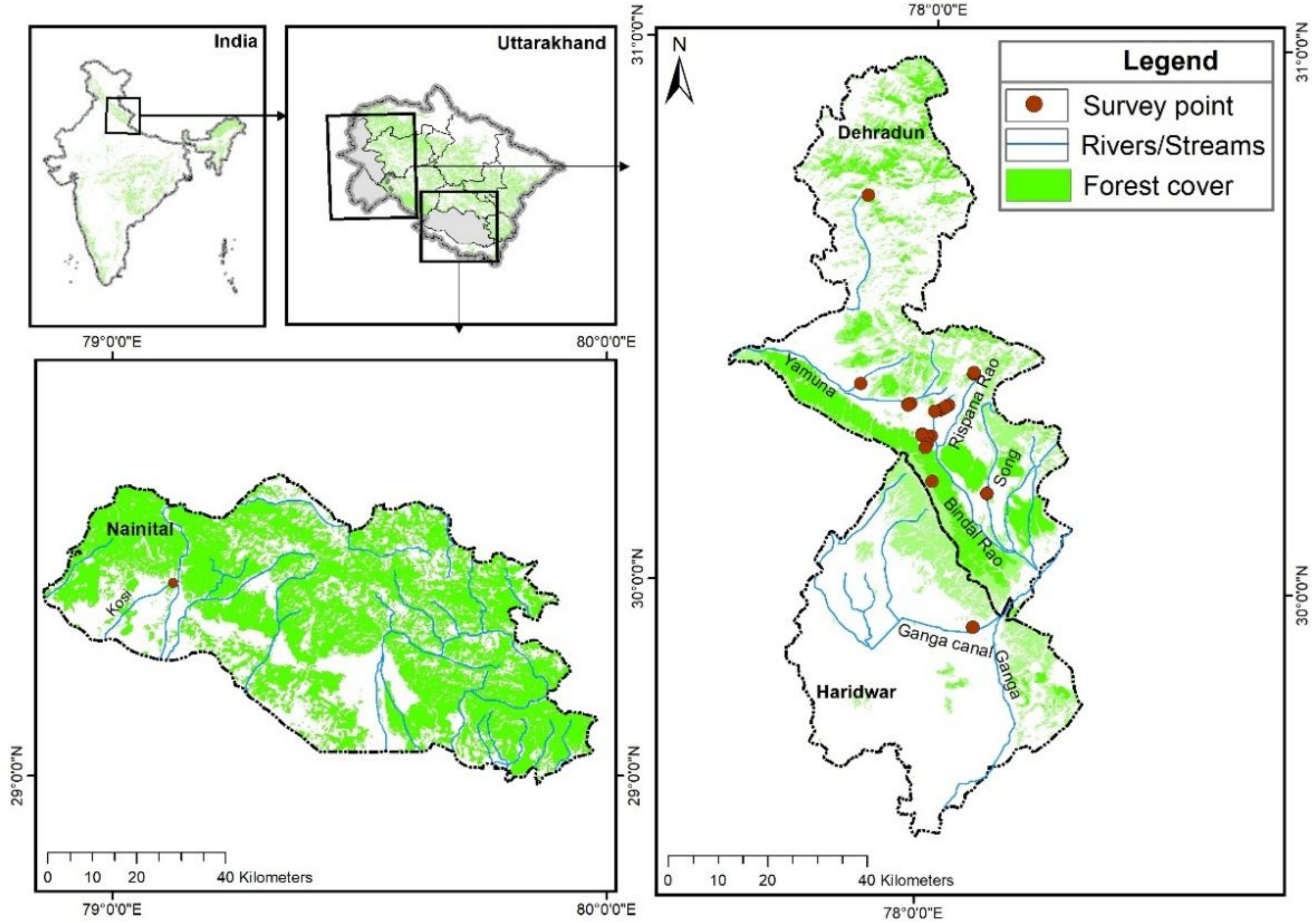

Figure 6

Map showing the location of surveyed household in the study area of Uttarakhand

Figure 7

(a) Pig herd in Ramnagar (Nainital district of Uttarakhand) (b) Pig herd of mixed parental origin observed in Chandrabani (Dehradun district of Uttarakhand) (c) Pig herd in Jwalapur (Haridwar district of Uttarakhand) (d) Possible hybridised feral origin or boar piglet for sale or mating purpose (e) Semi-intensive system of pig management with housing facility (all the pen is made of brick with tin as a roof within the house premises) (f) Extensive system of pig management with no housing facility

Supplementary Files

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- Supplementarymaterial.docx

Tracing the maternal lineage of Sus scrofa in Northern India and its correlation with ancient human migration patterns

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Research Article

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Abstract

We aimed to explore the distribution and variation of porcine mitochondrial DNA (mtDNA) lineages in northern India. The mtDNA control region (417 bp) was sequenced from 83 domestic pigs from three North Indian states. The 18 haplotypes that were obtained were compared with 1518 published mtDNA control region sequences of *Sus scrofa* from various regions across the world. The analysis revealed complex clustering patterns of porcine haplotypes with distinct phylogeographic signals. The study unveiled diverse maternal lineages among the domestic pig populations in northern Indian states situated in the foothills of the majestic Himalayas. Additionally, the study identified the presence of two distinct subspecies of wild boar in India (W1 and W2), demonstrating their migration routes and offering a strong phylogeographic signal. Evidence for a separate domestication center in the Himalayan foothills and Indian sub-continent was found within Mixed Clade 5 (MC5). A novel clade (MC4) with a localized distribution in the Chotanagpur plateau was detected, suggesting a unique population of wild boar in this region. Diverse maternal lineages among domestic pigs were observed, which may reflect human-mediated dispersal of pigs within and into the region; hence, a hypothesis linking ancient human migration with the presence of these maternal lineage clades has been proposed. Overall, the porcine haplotypes discovered in the Indian subcontinent highlight its unique and significant contribution to genetic resources.

Introduction

The advent of the Neolithic period witnessed humans establishing themselves across diverse landscapes without relying on domestic animals. However, subsequent domestication of animals facilitated significant migrations as farming societies travelled with their livestock, hence the preserved archaeological remnants and genetic imprints of both historical and contemporary domesticated animals offer a direct avenue to decipher the timelines and pathways of ancient migrations (Bellwood 2009, Stoneking et al. 2023). This concept has been validated in the case of various domestic species such as sheep (Rocha et al., 2011), goats (Peng et al., 2022), cows (Edwards et al., 2007), and notably, pigs (Larson et al., 2005; 2007; 2010)

By focusing on mitochondrial DNA markers, genetic studies have achieved a more precise understanding of numerous geographically distinct populations of wild boar characterized by unique genetic signatures (Giuffra et al., 2000; Larson et al., 2005; 2010). The work conducted by Larson et al. (2005) has laid a substantial groundwork for advancing the study of origins, migration, domestication, and the dispersal of *Sus scrofa* through analysis of mtDNA control region (D-loop) sequences obtained from global wild boar, domestic pig, and ancient samples. The study established a cohesive framework outlining the origin and global path of wild boars, including their passage through the Indian subcontinent and subsequent radiation to other continents. The work highlights human influence on pig genetics and distribution, revealing six distinct mtDNA lineages (D1-D6) among wild pigs worldwide.

The northern Indian population of wild boars is descended from *Sus scrofa* that migrated from ISEA to Asia, with a unique lineage (D3) indicating an independent domestication event in India. The subspecies *S. s. cristatus* thrives in the Himalayas and parts of northern India, like Jammu and Kashmir, Chhattisgarh, and West Bengal (Larson et al., 2005; Tanaka et al., 2008). Two more wild boar lineages have been recognized in the Indian subcontinent detailing their migration route, but with limited influence on current domestic pig populations. Additionally, two distinct wild boar populations and their migratory routes, associated with unique mitochondrial traits, possibly corresponding to subspecies *S. s. davidi* and *S. s. affinis*, are also identified in the Indian subcontinent, though their influence on present-day domestic pigs is limited (Nidup 2011; Choi et al., 2020). Despite the exploration of mtDNA lineages, there is still an inadequate understanding of the origin, distribution, and domestication of pigs in the Indian subcontinent. This study aims to examine the distribution of porcine mtDNA lineages in Northern India, as well as to assess the potential of using pigs as a proxy to trace human migration, especially concerning the D3 haplotype and other distinct haplotypes found within the Indian subcontinent.

Materials and Methods

1.1. Sample Collection, DNA Extraction, Amplification and Sequencing

Non-invasive biological samples (hair) were collected from 83 unrelated pigs from the surveyed households in Uttarakhand, Jharkhand, and Bihar. Genomic DNA was extracted using DNeasy Blood & Tissue kit (Qiagen, Hilden, Germany) in a final elution volume of 40–80 µL. DNA extraction products were electrophoresed on 0.8% agarose gel and visualized under UV light. The amplification of the mtDNA hypervariable control region was recorded using a control region-specific primer pair (Balakrishnan et al., 2003). The reactions were performed in 20µl volumes containing 10–20 ng of extracted genomic DNA. PCR master mix contained: 1× PCR buffer (Applied Biosystems), 2.0 mM MgCl2, 0.2 mM of each dNTP, 2 pmol of each primer, and 5U of Taq DNA polymerase. The PCR conditions for both the primers were as follows: an initial denaturation for 5 minutes at 95°C, followed by 35 cycles at 95°C for 45 seconds, 55°C for 45 seconds and 72°C for 1 minute 30 seconds, with a final extension of 72°C for 10 minutes. The PCR products were electrophoresed on 2% agarose gel and visualized under UV light. Positive amplicons were treated with Exonuclease-I and Shrimp alkaline phosphatase (USB, Cleveland, OH) for 15 min each at 37ºC and 80ºC, respectively to degrade any PCR reaction’s residues. The purified fragments were sequenced directly in Genetic Analyzer 3500XL from forward primers set using a BigDye v3.1 Kit (Applied Biosystems).

1.2. Sequence data analysis

417 bp of mtDNA control region were obtained, which were confirmed using the BLAST tool on NCBI (http://ncbi.nlm.nih.gov/). The generated sequences obtained were clean and edited with SEQUENCHER® version 4.9 (Gene Codes Corporation, Ann Arbor, MI, USA)

1.3. Phylogenetic and population structure analysis

The 83 mitochondrial control region sequences (68 Uttarakhand, 1 Bihar, and 14 Jharkhand) were combined with 1518 published *Sus scrofa* mitochondrial D-loop sequences (NCBI). These were aligned using the CLUSTAL W algorithm (Thompson et al., 1994) in program MEGA X (Kumar et al., 2018) and alignment was checked by visual inspection and was submitted to National Centre for Biotechnology Information (NCBI) GenBank (OP382063-82).

The spatial relationship among the haplotypes was reconstructed using the median-joining network in PopART v.1.7 (Leigh et al., 2015). Phylogenetic relationships among the sequences were reconstructed using the partial fragment of the control region based on the Bayesian approach as implemented in program MrBayes v.3.2 (Ronquist et al., 2003). The most appropriate model (GTR + I + G) for nucleotide substitution was selected based on the Bayesian Information Criterion (BIC) values using program jModelTest v.2.1.3 (Posada 2008). Two MCMC chains of 10 million simulations sampling at every 5,000 generations were performed.

The output tree topologies were edited using FigTree v.1.4.2 (http://tree.bio.ed.ac.uk/sofware/fgtree/). Warthog (*Phacochoerus africanus*; NC_008830) was used as an outgroup. The nomenclatures described by Larson et al., 2005 with six clades (D1 to D6) were used as the reference for clade/ haplogroup notation in order to accommodate the major porcine haplotypes (Table 1).

Table 1
DNA samples used and haplotype generated in this study.

Haplotype	Sample ID	no. of samples	Haplogroup (Larson et.al 2005)	Haplogroup Notation in this study	Location
H2, H7, H11	UK63DP, UK30DP, JH1DP, UK62DP	4	-	W1	Uttarakhand, Jharkhand
H5, H6, H16	UK12DP, UK67DP, UK2DP, UK3DP, UK42DP, UK48DP, UK49DP, UK5DP, UK61DP, UK6DP,UK40DP	11	D6	MC1	Uttarakhand
H9	UK47DP, UK55DP	2	-	-	Uttarakhand
H14	UK7DP	1	D2	MC3	Uttarakhand
H17	UK53DP	1	-	-	Uttarakhand
H18	BH1DP JH10DP JH11DP JH12DP, JH13DP, JH14DP, JH2DP JH3DP, JH4DP, JH5DP, JH6DP JH7DP, JH8DP, JH9DP	14	-	MC4	Bihar Jharkhand
H1, H3, H8, H10, H13	UK10DP, UK11DP, UK13DP, UK14DP, UK15DP, UK16DP, UK17DP, UK18DP, UK19DP, UK1DP, UK20DP, UK21DP, UK22DP, UK23DP, UK25DP, UK41DP, UK43DP, UK44DP, UK45DP,UK46DP, UK4DP, UK50DP, UK51DP, UK52DP, UK54DP, UK57DP, UK58DP, UK59DP, UK60DP, UK65DP, UK66DP, UK68DP, UK8DP, UK26DP, UK27DP, UK28DP, UK29DP, UK31DP, UK32DP, UK33DP, UK34DP, UK35DP, UK36DP, UK39DP, UK24DP, UK38DP, UK37DP	47	D3	MC5	Uttarakhand
H4	UK56DP	1	-	-	Uttarakhand
H12,H15	UK9DP UK64DP	2	D1	MC2	Uttarakhand

Results

2.1. Clustering of haplotypes in Phylogenetic tree

177 haplotypes were obtained from 1601 aligned sequences of which the 83 samples of this study form 18 haplotypes (Table 1). Based on Bayesian phylogenetics, these were classified into six major clades, each further divided into sub-clades (Fig. 1).

It indicated two distinct sub-clades (W1 and W2) within the initial clade (W), consistent with previous research (Larson et al., 2005; Nidup 2011). W1 includes domestic samples from Uttarakhand and Jharkhand, sharing haplotypes with northern/central India, Nepal, and Pakistan wild samples. W2 consists of wild haplotypes from northern/central/southern India, and Sri Lanka.

The consensus Bayesian tree differed from earlier research mainly in the placement of the wild clade (W) which lies within the continental clade. The position of the remaining clades in the tree showed similarity to previous findings (Larson et al., 2005; 2010; Tanaka et al., 2008).

Clade MC1 includes domestic pig haplotypes from northern India, north-eastern India, and the Philippines, as well as a wild pig haplotype from Indonesia. This clade contained 11 Uttarakhand samples and incorporated the reference sequence of lineage D6.

Clade MC2 includes two Uttarakhand samples and European domestic pig breed haplotypes. It shared sister clades with Indian wild and domestic Andaman pigs and domestic Nicobari pigs. The reference sequences, of the D1 lineage were present within this clade.

Clade MC3 comprises one sequence from Uttarakhand and includes domestic pig sequences from Asian countries such as the Philippines, Indonesia, Vietnam, and northern India. This clade falls within the D2 lineage.

It revealed a unique clade, MC4, comprising one sample from Bihar, 13 out of 14 domestic pig samples from Jharkhand, wild pigs from Bhutan, eastern India, and domestic pigs from central India. This novel clade does not align with any proposed reference sequences. The wild pig sequence from Bhutan, found within this clade, was previously identified as a new haplotype (W12) (Nidup 2011).

The general Asian clade corresponds to the basal Island of South East Asia (ISEA) clade (Larson et al., 2005). The clade includes ancient haplotypes and exhibits a large polytomy of clades and individual branches representing samples from various Asian countries (Indonesia, Myanmar, the Philippines, Japan, Malaysia, Thailand, Cambodia, and Laos).

Three haplotypes from the studied samples formed the basal branch of the tree H4, H9 and H17. H4 and H17 were widely distributed and associated with diverse populations, supporting their broad geographic presence. H4 served as a common ancestor connecting Clade W and Clade MC1. H9 was a unique haplotype found only in Uttarakhand, bridging Clade MC1 and MC2. H17 connected Clade MC3 and the distinct Clade MC4.

A significant proportion (47 out of 68) of the samples from Uttarakhand belonged to the MC5 clade, previously known as D3. This clade includes domestic pig sequences from Bhutan, Nepal, and indigenous registered breed sequences that are native to Assam, West Bengal, and Meghalaya. Additionally, museum specimens of wild sequences from India and Bhutan were also included in this clade. Most clades had high posterior probability support, with over 90% for the W and D3 clades and 71% for the new MC4 clade. It reinforces the reliability of these clades.

2.2. Median Joining Network Analysis

The MJN analysis exhibited a similar clustering pattern to the Bayesian tree (Fig. 2). It confirmed the distinct separation of the W (including W1 and W2), MC1, MC2, MC3, and MC5 clusters, consistent with the phylogenetic tree. Notably, the newly discovered MC4 clade was observed emerging from the general Asian cluster.

Discussion

The presence of different haplogroup of porcine mitochondrial DNA in Uttarakhand is a result of human-mediated migration. This is facilitated by the historical role of north-eastern India, such as Assam, Manipur, and Mizoram, (LPAI, 2022) as a migration corridor between Southeast Asia and other regions that have served as connecting points, allowing for the exchange of cultures, languages, genes, and ideas throughout history (Reddy et al., 2007).

The MC5 clade, a significant maternal lineage, has a widespread distribution across various states in the Himalayan foothills of India, indicating its geographic expansion. The inclusion of a haplotype of domestic pig belonging to an ancient sample of Nepal dating back approximately 1500 years (Nidup, 2011) suggests that the genetic characteristics and lineage of the MC5 clade predate its estimated age, indicating a long-standing presence in domestic pigs. The presence of *Sus scrofa cristatus*, the wild boar species, in the Himalayas, including northern Indian states like Jammu and Kashmir, central states, and West Bengal (Larson et al., 2005; Tanaka et al., 2008) may likely influenced the domestication process of pigs in the area. The ancient sample of domestic pigs from Nepal also implies historical genetic exchanges and hybridization between wild boars and domestic pigs, highlighting their shared maternal lineages.

A potential hypothesis links the origin and evolution of the MC5 lineage to the ancient Harappan Civilization, which flourished about 5,300 to 3,300 years ago in present-day Pakistan and north-western India. Around 4,500 years ago, the decline of the civilization led to the migration of its people towards the eastern regions near the Himalayan foothills, where they established small agricultural communities (Malik, 2020). The consumption of wild pigs may have been a cultural practice during this time, as evidenced by the presence of pig bones and lipid residues at Harappan sites (Chase, 2014; Suryanarayan et al., 2021). The inclusion of museum samples from Jammu and Kashmir within the MC5 clade provides support for the hypothesis. Further genetic and archaeological evidence is required to confirm this speculation.

The confirmed presence of the *Sus scrofa davidi* subspecies in northwest India, Pakistan, and south-eastern Iran (Groves 2008, Choi et al., 2020) provides support for the existence of a distinct wild boar subspecies, potentially represented by the W1 clade. The W1 clade showed a correlation with the geographical distribution of the MC5 clade. Currently, there is no evidence suggesting any maternal genetic contribution from W1 wild boars to modern domestic pigs. However, the presence of specific haplotypes (H2, H7, H11) in domestic pig samples from Uttarakhand and Jharkhand raises the possibility of wild pig farming practices in these regions. The study highlighted the coexistence and potential interactions between the two subspecies, *Sus scrofa cristatus* and *Sus scrofa davidi*, in the foothills of the Himalayas and northern India.

The W2 clade confirmed the existence of a distinct mtDNA clade, likely corresponding to the subspecies *Sus scrofa affinis* (Nidup 2011). Additional samples from South Indian states would provide valuable insights into migration patterns and genetic diversity.

The presence and position of the H4 haplotype in the phylogenetic tree and the inclusion of samples of wild pigs from Malaysia and Vietnam offer valuable insights into the evolutionary history of W clade lineage. H4 acts as a common ancestor of both clades suggesting an ancient origin predating their divergence and indicating an early emergence of *S. scrofa* in island Southeast Asia (ISEA) (Fig. 3) (Choi et al., 2020, Larson et al., 2005). The H4 haplotype provides valuable insights into the evolutionary history and dispersal of W clade.

A new lineage of wild pigs, called MC4, has been discovered in northern India. Interestingly, the MC4 lineage also includes genetic variants of wild pigs from the southwestern region of Bhutan confirming that gene flow from East Indian wild boars, particularly in the southwestern region of Bhutan (Tanaka et al., 2008). Network analysis reveals the haplotype's position within the general Asian cluster, with a distinct circle of haplotypes emerging. Furthermore, the presence of missing haplotypes suggests the potential existence of another wild pig lineage in India, emphasizing the need for additional sampling in these areas. The presence of domestic pig samples in MC4 suggests the introgression of wild boar maternal lineage into local domestic pig populations due to crossbreeding driven by government initiatives in the piggery sector in Jharkhand, India. (Seth et. al.2022)

The MC4 lineage occupies a basal position in the phylogenetic tree and exhibits a star-like pattern in MJN within the Asian cluster in the network analysis. The proposed hypothesis for the presence of this clade in the region is suggested based on linguistic diversity. In the Indian subcontinent, four linguistic groups arrived at different times, with the Austro-Asiatic group being the oldest and most divergent (Gadgil et al., 1997). The Mundari branch of the Austro-Asiatic group, primarily residing in the Chotanagpur plateau region, overlaps with the region of the presence of the MC4 clade. This region includes parts of Odisha, Jharkhand, Madhya Pradesh, West Bengal, and north-eastern Maharashtra, characterized by similar habitats such as hills and forests (Kumar et al., 2003). The Mundari populations have a traditional hunting and gathering lifestyle, predominantly occupying areas unsuitable for agriculture, suggesting the continuation of their ancestral practices. Therefore, it is unlikely that they migrated during the Neolithic era when farming communities expanded (Thangaraj et al., 2005).

Analysis of mtDNA in present-day Mundari populations suggests their ancestors migrated to India during the Pleistocene era, which aligns with archaeological evidence indicating human presence in India since the early Paleolithic era (Kumar et al., 2007; Lal BB, 1956). This evidence supports that the haplotype H18 of the pig is concentrated in this region and as the community has maintained their ancestral ways of subsistence practices; hence, the migration is limited and so the haplotype of the pig raised by them is only restricted to eastern India and Bhutan. Further sampling is required in this region to better understand the unique population characteristics and establish its distinctiveness.

Conclusions

The study identified four distinct populations of wild pig lineages in Northern India, associated with different subspecies of wild boars. These populations have undergone genetic differentiation, indicating unique genetic variations likely shaped by local adaptation, historical isolation, and genetic drift. Further research is needed to explore additional porcine lineages of wild boar, especially in South India, for a comprehensive understanding of their genetic diversity and population dynamics in India. The study also highlights the complex history of domestication of a distinct pig haplotype and its population expansion in the Himalayan foothills and the Indian subcontinent. The discovery of another wild boar lineage in the Chotanagpur plateau region provides insights into regional dynamics, conservation efforts, and the historical connectivity of wild boar populations in India.

Declarations

Conflict of interest statement: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Ethics approval: Not applicable, as the samples were obtained non-invasively from the domestic pigs from the pig rearers, no permission was required to collect the samples.

Authors' contributions: Conceptualization: KS, SKG; Methodology, formal analysis and drafting: KS; and writing review and editing: SKG

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Figures

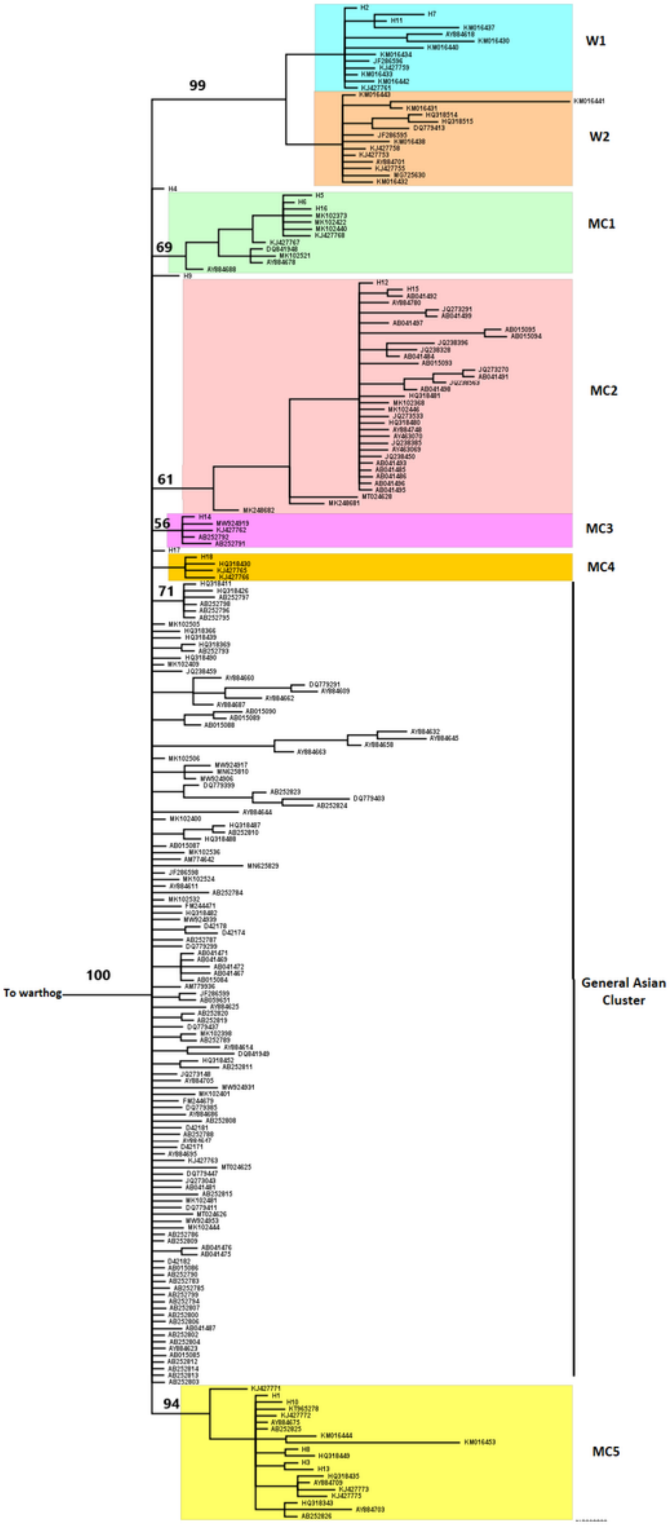

Figure 1

Phylogenetic relationships of North Indian (Uttarakhand, Bihar and Jharkhand) domestic pigs obtained from the Bayesian analysis of mitochondrial control region sequences (417 bp). Bayesian Posterior Probability (BPP) values are provided at respective nodes with *Phacochoerus aethiopicus* (NC_008830) used as outgroup and other reference haplotype sequences. The digits at the nodes are posterior probability (>50%; 10 million generations). MC4 is the Novel clade discovered in this study.

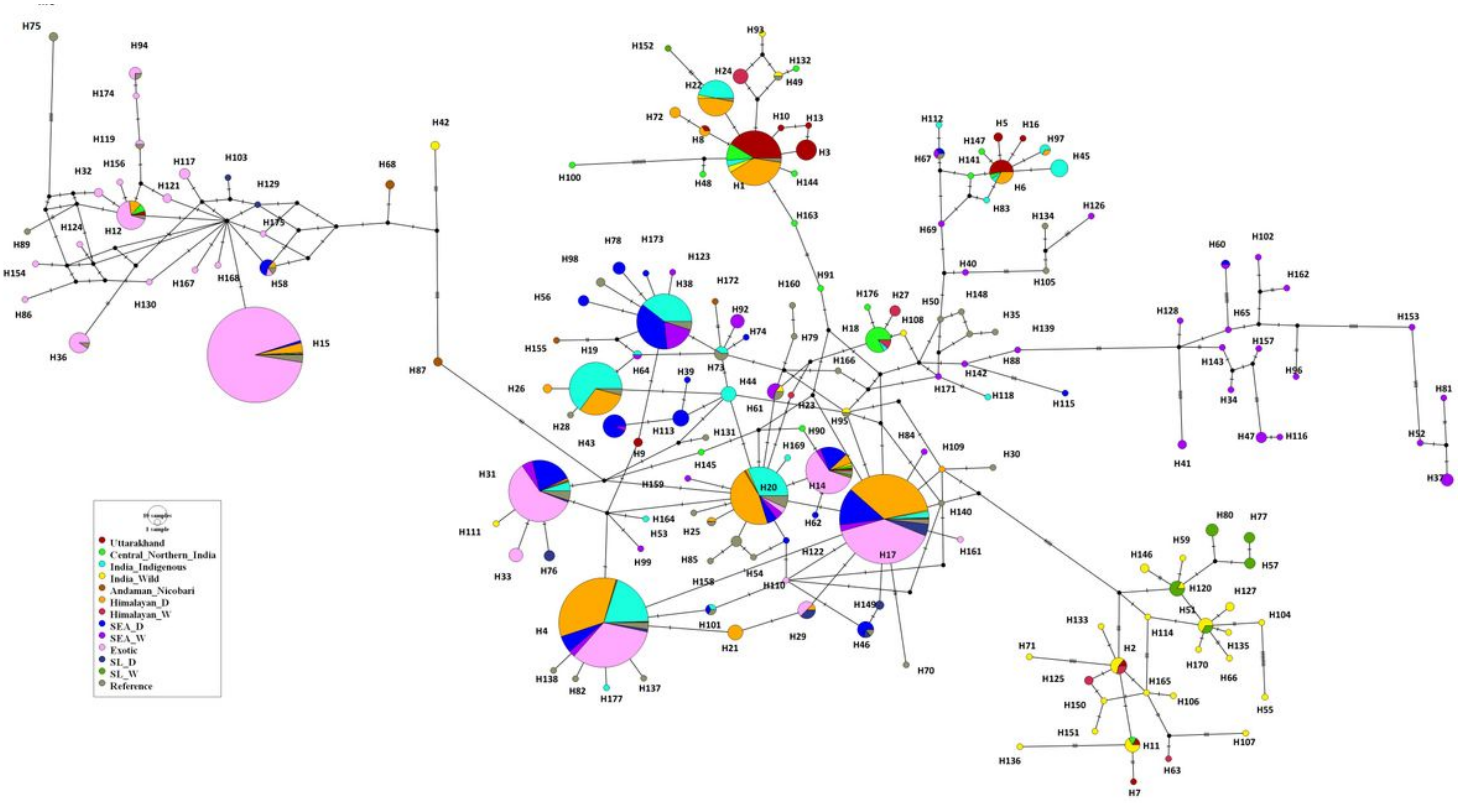

Figure 2

Relationship and clustering of 18 (H1-H18) Northern Indian (Uttarakhand, Bihar and Jharkhand) pig haplotypes with 158 global sequences haplotypes (H20-H176) of mtDNA control region (marked). Geographical locations of samples are given in colour and node size is proportional to the frequency of the corresponding haplotypes. Small black dots are median vector (mv) representing hypothetical sequences that were not detected in this study. Each bar on the branch indicates one nucleotide substitution (or an insertion/ deletion).

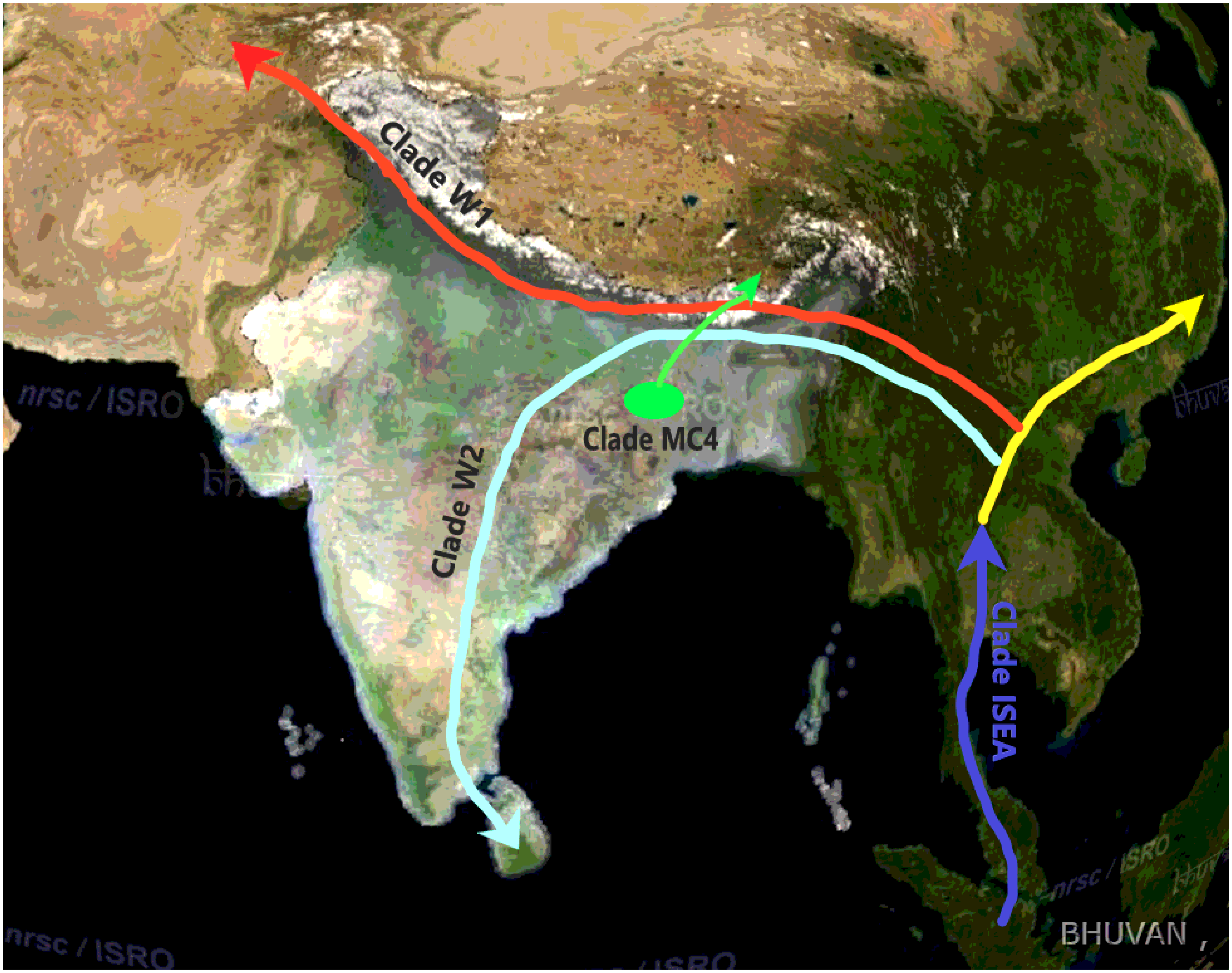

Figure 3

Suggested migration routes of wild boar in Indian Subcontinent

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