Conservation status of Asiatic Wild Buffalo *(Bubalus arnee)* in Chhattisgarh revealed through genetic study

A Technical Report

Prepared by

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Foreword

The wild buffalo, *Bubalus arnee* is an endangered species and it requires conservation interventions to sustain viable populations. It has rightfully been recognized as a protected species in Indian Wildlife (Protection) Act, 1972. The State of Chhattisgarh has taken up laudable efforts to bring back the population in Udanti Sitanadi Tiger Reserve. Overall, the efforts taken indicate the importance this species has been given at the state and national levels.

The wild buffalo was widely distributed in the riverine forests and in the flood plains of Brahmaputra and Ganga, and in the South, up to the Godavari River. The rapid decline of their distribution range is exemplified by reports of the species from Odisha as recent at 1966. In 1997, the last population in the State of Maharashtra went extinct. Finally, today wild buffalo survives in Central India in five small populations with less than 100 animals.

This situation should be reversed so that wild buffalo makes a comeback in the Central India. The State of Chhattisgarh has already come to grips with the situation. They have established a captive population. They have also decided to use Assisted Reproduction Technologies to enhance the populations in captivity. This report is an outcome of the efforts taken by Wildlife Trust of India and CCMB's Laboratory for the Conservation of Endangered Species (CCMB-LaCONES) to highlight the conservation status of wild buffalo after performing DNA based analyses of wild buffalo populations. This report will change the narrative of conservation options for wild buffalo in Central India. It opens up the possibility of bringing animals from the plains of Assam to Central India. I congratulate the efforts made by the biologists to bring out these findings that will help the future of wild buffalo conservation in Central India.

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Foreword

The Asian Wild Buffalo (*Bubalus arnee* kerr. 1972) is an endangered species as per criteria of IUCN. It is enlisted in Appendix III of CITES and in Schedule I of Indian Wildlife (Protection) Act, 1972. Wild buffalo is the third largest land mammal in India, after the Elephant and Rhino it carries the largest horns among the surviving animals of the world today. Wild buffalo was declared as State Animal of Chhattisgarh in 2001.

This technical report is an out come of the efforts taken by Wildlife Trust of India, CCMB Laboratory for the Conservation of Endangered Species (CCMB-LaCONES) and Chhattisgarh Forest Department. The reports highlight the status of wild buffalo of Chhattisgarh on basis of DNA analysis.

I Congratulate the efforts made by the biologists to bring out these findings that will help the future of wild buffalo conservation in Central India.

(S. K. Singh)

Date : 24-01-2020

Introduction

The wild buffalo (*Bubalus arnee* L.) is listed as “Endangered” in the Red Data book of IUCN. It has been categorized in appendix III of Convention of International Trade for Endangered Species of Wild flora and fauna (CITES). It is also classified in schedule I of Wildlife (Protection) Act, 1972, which indicates that it has been given top priority for conservation. It is the third largest land mammal in India, after the Elephant and Rhino. The wild buffalo has been declared as the state animal of the Chhattisgarh State in July 2001.

The Asian wild buffalo (*Bubalus arnee)* is endangered wild cattle restricted in its distribution to southern Nepal, eastern and central India, southern Bhutan and western Thailand. The wild buffalo is included in the species lists of Protected Areas in Cambodia and Vietnam. The Myanmar also consider as part of the range of the speciesbut some scientist considers it as having become extinct in the country by the 1930s and feels that the present population is feral. The species is reported to be extinct in Bangladesh, peninsular Malaysia, Sumatra, Java and Borneo. Sri Lanka is included in the historic range of the species although there is some disagreement on the origin of the population.

Distribution

(a) Past distribution

In India, the buffalo was once numerous and widely distributed in the riverine forests and plains of the north east, extending west from the plains of the Ganges and Brahmaputra to Uttar Pradesh, and southwards through eastern peninsular India to the Godavari river. Daniel & Grubh (1966) reported about 100 animals, based on reliable sources in Kondakamberu valley and Maheswarpur near Balimela in Odisha. They were believed to be extinct in Odishatill 1980 when a small number was reported from along the borders of Andhra Pradesh and Chhattisgarh (Thornback, 1983; Ranjitsinh *et al.*2002). The population has disappeared from Chandrapur district of Maharashtra, where it occurred till the 1970s (Divekar *et al.*1979). However, the sanctuary of Bhamragarh was notified in 1997 by the state to protect what was believed to be the last herds in the state. They are reported to migrate between

Chhattisgarh (Bastar) and Maharashtra (Bhamragarh region). By 1988, wild buffalo were reported to be restricted to five populations in Central India with a total number of about 125 animals. These were in Indravati National Sanctuary, Bhairamgarh, Pamed, Sitanadi and Udanti wildlife sanctuaries in Bastar, and Raipur districts of Chhattisgarh. In northeast India, Daniel & Grubh (1966) considered Manas, Kaziranga, Pabha, Laokhowa, the Sankos-Manas riverine tract and Lakhimpur as the range of this animal in Assam. There have been reports of buffalo in Meghalaya (Rodgers & Panwar, 1988; Choudhury, 1994) and Arunachal Pradesh (Ranjitsinh, 1997, 1982; Choudhury, 1994).

(b) Present distribution

In presently, there are about 4000 wild buffalo found in India and about 50 wild buffalo are occurring in hard ground habitat in Central India. Today, the last bastion of the wild population in India lies in Assam in North-east India and in Chhattisgarh & Maharashtra in the Central India. The Udanti- Sitanadi and Indravati tiger reserves are main habitat in Chhattisgarh while some animals moving in Maharashtra area adjoining to Indravati TR.

Udanti Wildlife Sanctuary

UdantiWildlife Sanctuary (now tiger reserve) is famous as natural home of wild buffalo in Central India. It is situated in Gariyaband and Dhamtari districts of Chhattisgarh State. This sanctuary supports best potential habitat for Indian wild buffalo. It was declared as a wildlife sanctuary in 1985, under the Wildlife (Protection) Act, 1972 by the Government of Madhya Pradesh vide notification number 15/4/83/10/2, Bhopal, Dated 09/03/84 (Table1; Figure1). Now it was declared as tiger reserve in the year of 2009.

Geographically the Udanti Wildlife Sanctuary is located between longitude $82^0$11’10” to 82^0 24’10” E and latitude 19^0 55’30” to 20^0 11’15” N. The tropic of cancer passes through the sanctuary. It falls in bio geographic zone 6 E (Rodgers and Panwar, 1988 Vol. 1&2). The total area of sanctuary is 237.27 square Km (Garbyal, 1995-96). The sanctuary is situated on the bank of Udanti River after, which it derives the name as

Udanti Wildlife Sanctuary. The extent of cross section area is 22.350 Km from east to west and 19.00 km from north to south of the sanctuary. The sanctuary is strategically situated on the interstate border of Chhattisgarh and Odisha. Across interstate border in Odishastate there lies the Sunabeda wildlife sanctuary which is about 20 Km away.Most of the peripheral area of the sanctuary is hilly while the middle is plain to gentleslopes. The elevation of the area is between 335 m and 674 m. The average annual rainfall is between 1300 mmand1500 mm.

Table 1. Characteristic features of Udanti Wildlife Sanctuary

Topography	Hilly, Undulating terrain, moderate slope with flat plateau and valleys
Altitude	Between 320 to 370 m. above MSL
Drainage	Udanti and Indravan river basin beside seasonal nullahs
Geology	Archaean and Vindhyan sand stone
Soil	Laterite and sandy soil
Slope	Moderate, plateau steep and plain
Climate	Monsoonic viz. Rainy, Winter and Summer
Rainfall	1200 – 1500 mm/year
Temperature	Maximum 41^0 C and Minimum 7^0 C
Humidity	70 to 90 % during rainy season and 20 to 30 % during summer season.
Wind velocity	1.7 to 10 km /hr.

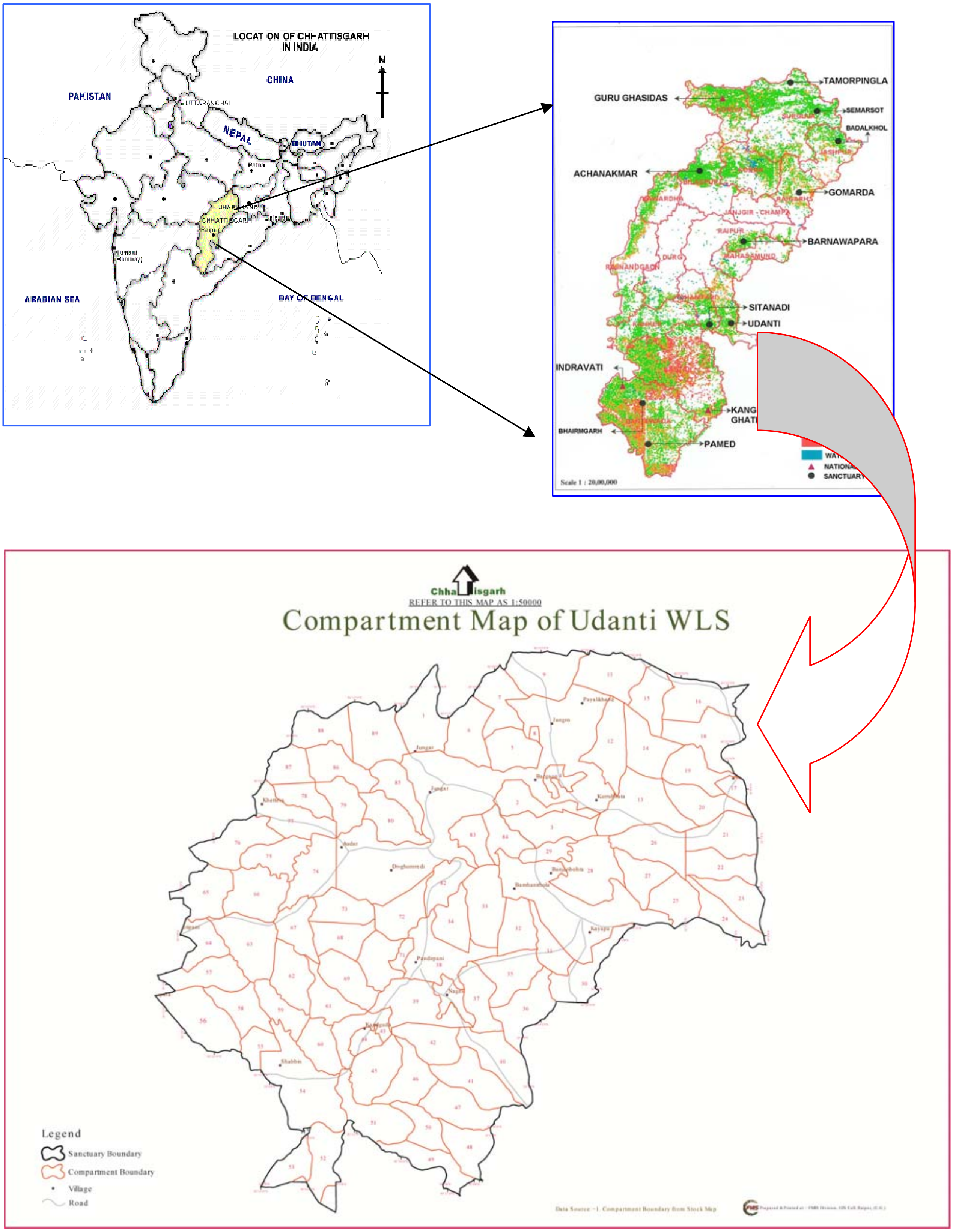

Figure 1. Location of Udanti Wildlife Sanctuary

Flora and Fauna

A. Flora

According to Champion and Seth (1968), the forests of Udanti belong to following types

(i) 5A/C$_{I\ b}$ Southern Tropical Dry Deciduous Dry Teak forest.

(ii) 3C/C$_{2\ e\ (i)}$ North Indian Tropical Moist Deciduous Moist peninsular high level Sal.

(iii) 3C/C$_{2e\ (ii)}$ North Indian Tropical Moist Deciduous -Moist Peninsular low level Sal

(iv) 5B/C$_{I\ c}$ Northern Tropical Dry Deciduous – Dry peninsular Sal.

(v) 5A/C3 Southern Tropical Dry Deciduous – Southern dry mixed deciduous forest.

The dense forests occur in most of the sanctuary particularly plain area. The deciduous forest contains a large number of trees, producing timber and a variety of non-timber forest products. Some of the common tree species are *Shorea robusta, Terminalia tomentosa, Diospyros melanoxylon, Anogeissus latifolia, Bombax ceiba, Terminalia chebula, Tectona grandis, Boswellia serrata, Pterocarpus marsupium,* etc. In the middle storey species are *Emblica officinalis, Buchanania lanzan,Cleistanthus collinus, Grewia tiliafolia, Cassia fistula, Soymida febrifuga, Ogenia oojensis* etc. The *Soymida febrifuga* and *Acacia catechu* depicts a spatial distribution pattern being more common at certain places like Nagesh, Koeba andJungad forest beats.In under growth species like *Indigofera cassioides,Chloroxylon sweitenia, Dellenia aurea, Randia dumetorum, Ixora arborea, Woodfordia fruticosa, Embelia ribesHelicteris isora*etc are found.The common herbaceous species are *Chlorophytum arundinaceum, Grewia hirsuta, Curcuma angustifolia,Andrographis paniculata, Urena lobeta, Side acuta*etc. The important grasses are *Heteropogon contortus, Saccharum spp., Bothriochloa spp, Eragrostis tenella, Chrysopogon fulvus, Vetiveria zizanoides, Panicum sp. Apluda mutica, Themeda quadrivalvis, Themeda triandra,* etc. Many species of orchids and tree parasites are also found in sanctuary.

B. Fauna

Udanti wildlife sanctuary is famous for hardground wild buffalo. There is a large verity of animal populations comprising of prey and predators. The most of the wild animals found in Central India are found in Udanti. Important large prey animals comprise of three species of deer (*Axis axis, Cervus unicolor, Muntiacus muntjak)* two species of antelopes (*Tetracerus quadricornis& Boselaphus tragocamelus)*, *Bos gaurus*, *Sus scrofa*, *Presbytis entellus, Lepus nigricollis* etc. The main predators are *Panthera tigris, Panthera pardus, Cuon alpinus. Viverricula indica* and *Hyaena hyaena*are scavengers. Omnivorous *Melursus ursinus* also occurs. Apart from these, other animals such as reptiles (Snakes, lizards), birds (more than 180 species), insects and butterflies are also found.

The People

There are 17 small revenue villages inside the sanctuary. About 5000 people live within the sanctuary along with over 4,000 livestock. About 8 villages also situated periphery of the sanctuary and villagers mostly dependent on the sanctuary along with 3000 livestock. *Gond, Bhunjia and Kamar* are the major tribes in the sanctuary.

Population of wild buffalo

Every compartmentwas visited and tried to find out the number of wild buffalos with their age-sex composition. Also surveyed territorial forests adjoining to the sanctuary but could not found evidences of Wild buffaloes. The observation survey found only seven numbers of individuals in Udanti Wildlife Sanctuary. Subsequently, the Chhattisgarh Forest Department conducted survey between31st May and 2ndJune, 2007. All the forest staff including senior forest officials of Udanti Forest Division was engaged in this survey. The each and every compartments of the sanctuary were covered during the survey. The forest department also found the same number of wild buffalo in Udanti Wildlife Sanctuary. Among these, five individuals are adult male and one each adult female and male calf (Figure 2). So, the sanctuary not only has very few numbers of wild buffalo but it has a skewed age-sex ratio.

Figure 2. Wild buffaloes at Udanti Wildlife Sanctuary, Chhattisgarh

New action taken

See the poor population of wild buffalo in Udanti, CEC directed to the state to constitute a wild buffalo task force in the year of 2007. The first meeting of the task force was organized in Udanti between 5thand 6th July 2007. The task force has suggested for following five points to conservation of wild buffalo in Udanti WLS.

a. Action Plan as per IUCN guidelines
b. Genetic studies
c. Patrolling and monitoring
d. Replace domestic buffaloes around Udanti for suitable replacement
e. Explore possibilities of conservation breeding

The all above four points has been implemented by the Chhattisgarh Forest Department with technical support by Wildlife Trust of India. To know the genetic status of wild buffalo of Chhattisgarh and northeast WTI & Chhattisgarh Forest Department requested to take up the study with CCMB, Hyderabad. The present technical report highlights the findings and recommendations of genetic studies conducted in wild buffaloes.

Genetic Studies

Genetic variation is important for the viability of populations.The existing populations of wild buffalo in India are highly endangered because of habitat loss and degradation due to anthropogenic interference. Furthermore, the loss of genetic purity because of cross breeding with the domestic and feral animals is the other major threat. The wild buffalo can occupy and disperse through a wide range of habitat types and is physically capable of moving over large distances. Wild buffalo males are reported to kill domestic buffalo males to mate with domestic females. Since it is difficult to distinguish between wild buffalo, feral, domesticated and their hybrids, the actual conservation status is uncertain and the possibility of extinction of wild populations is quite real. Therefore, there is an urgent need to review the genetic status of this endangered species for ascertaining its genetic uniquenessand assessing the level of genetic diversity in the existing populations, using tools of molecular genetics.

The availability of polymorphic genetic markers, microsatellite markers in particular, has made it possible to test the effect of inbreeding on fitness in the field and in the absence of pedigree information. These can provide information that can help define the distinctiveness of species, their ranking according to the number of close relatives and phylogenetic position, how populations of a given species are distributed, how the different populations are genetically distinct, how much genetic variation is present within and among population and whether there is a gene flow or migration between them. The acquired information holds promise to predict future population dynamics and extinction risks, resulting from demographic or genetic processes.

Sample details:

Total 69 samples were collected from Udanti Wildlife Sanctuary, Chhattisgarh (n=10) and other locations viz. CWRC Rescue Centre and DibruSaikhowa, Assam (n=3), Kaziranga National Park, Assam (n=45); Sironcha Forest Division, Maharashtra (n=1), Skin sample (n=1) from Ambikapur Palace, Chhattisgarh; skin sample (n=1) from Dharamjaygarh Palace, Chhattisgarh; skin sample (n=1) from Udanti WLS, Chhattisgarh and control samples in the form of tissue biopsy and blood samples of known wild and domestic buffalo (n=6) collected from Udanti Wildlife Sanctuary, Chhattisgarh were received for genetic analysis (Figure 3).

Figure 3. Collection of a) Dung Sample; b) Tissue Biopsy and c) Blood sample

The first part of sample analysis involved the extraction of high molecular weight DNA. A modified phenol-chloroform (Sambrook *et al* 1989) method for DNA isolation from non-invasively collected dung samples was successfully standardized (Figure 4). The DNA was quantified both by spectroscopy and in-gel analysis and diluted accordingly for the PCR amplification. The three skin samples received were highly degraded and failed to give any DNA.

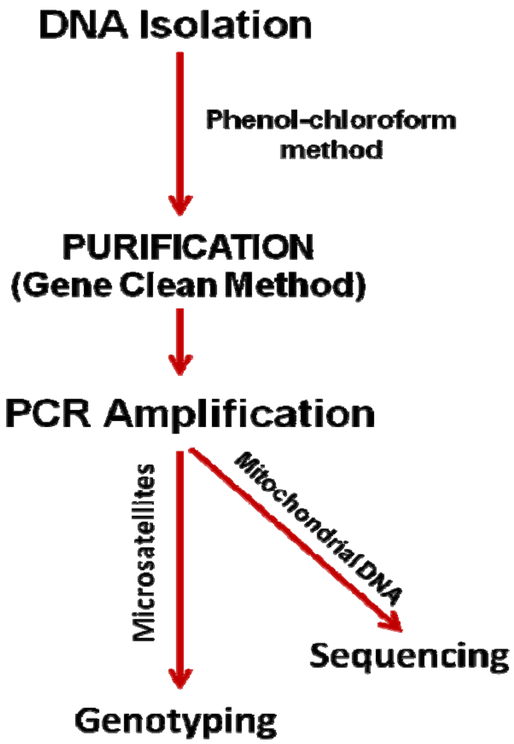

Figure 4. Flow diagram protocol employed for DNA analysis of wild buffalo samples

Mitochondrial DNA sequencing:

Primers for PCR amplification of the four mitochondrial DNA (mt DNA)regionswere designed (Table 2). Amplification conditions for each marker was optimized by variation of annealing temperature, Magnesium concentration, number of cycles, concentration of primers, and DNA in the reaction mixture.

Table 2. Details of mitochondrial DNA markers used in this study

S. No.	**Region of Mt DNA**	**Size of Amplicon (bp)**	**Reference**
1.	Cytochrome b	489 bp	Kumar *et al* 2007
2.	Hyper Variable Region I (HVR I)	468 bp	Vila et al. 1999
3.	Hyper Variable Region II (HVR II)	380 bp	Kumar et al. 2007
4.	16 S rRNA	581 bp	Palumbi et al. 1991

Mt DNA sequences were initially edited and aligned using AUTOASSEMBLER software (Applied Biosystems). Initial sequence comparison, measures of variability and phylogenetic relationships among the haplotypes from different groups were examined using Neighbor-Joining analysis based on Kimura two-parameter (Kimura 1980) distance with gamma correction, as implemented in MEGA version X (Kumar et al. 2018). *Bos taurus* was used as the outgroup. The robustness of the neighbor-joining tree was assessed by 500 bootstrap replications (Felenstein 1985).

Microsatellite genotyping:

Twelve microsatellite loci, moderately to highly polymorphic, distributed across 17 cattle chromosomes were used for the genotyping of the fecal samples. All these markers have been selected from BOVMAP database (available at www.marc.usda.gov). One of the primers for a given locus was labelled with fluorescent dye (FAM or HEX). Fluorescent tagged amplification products were size fractionated and visualized on ABI 377 DNA sequencer (Applied Biosystems Inc, USA), and allele size was determined using HD400 [ROX-0350] or [Tamara-350] size standard and the GENESCAN 3.1 software [Applied Biosystems Inc, USA].

Results and discussion

Median Joining Network of Wild and domestic buffalo haplotypes based on variation in Cytochrome B sequences showed that the wild buffalo from Central India and North East had a common genetic ancestor (Figure5). Most of the domestic samples from Kaziranga showed distinct variations as compared to their wild counterparts. Still some of the domestic samples were found to be sharing haplotypes with wild samples. The samples from Udanti Wildlife Sanctuary also show clear cut distinction between wild and domestic samples. The samples from Udanti wildlife sanctuary were found to be more close to the wl-ag/bg_kaz series of wild samples from Kaziranga. But these samples showed overall distinct variations from Kaziranga samples, may be because of the geographical distance. There were three distinct haplotypes among the wild animals of Udanti based on 18 polymorphic and variable sites with a nucleotide diversity of 0.667. Asha, Raja and recently born Prince shared same haplotype, but are clearly different from other animals. The partial sequences of cytochrome b (489bp), Hypervariable region I of d-loop (468bp), 16S (581 bp) and hypervariable region II of d-loop (380 bp) were generated from the DNA obtained from the faecal samples. The neighbor-joining (Figure6) and UPGMA phylogenetic trees constructed based on 1917 bp of combined sequences of the 4 markers.

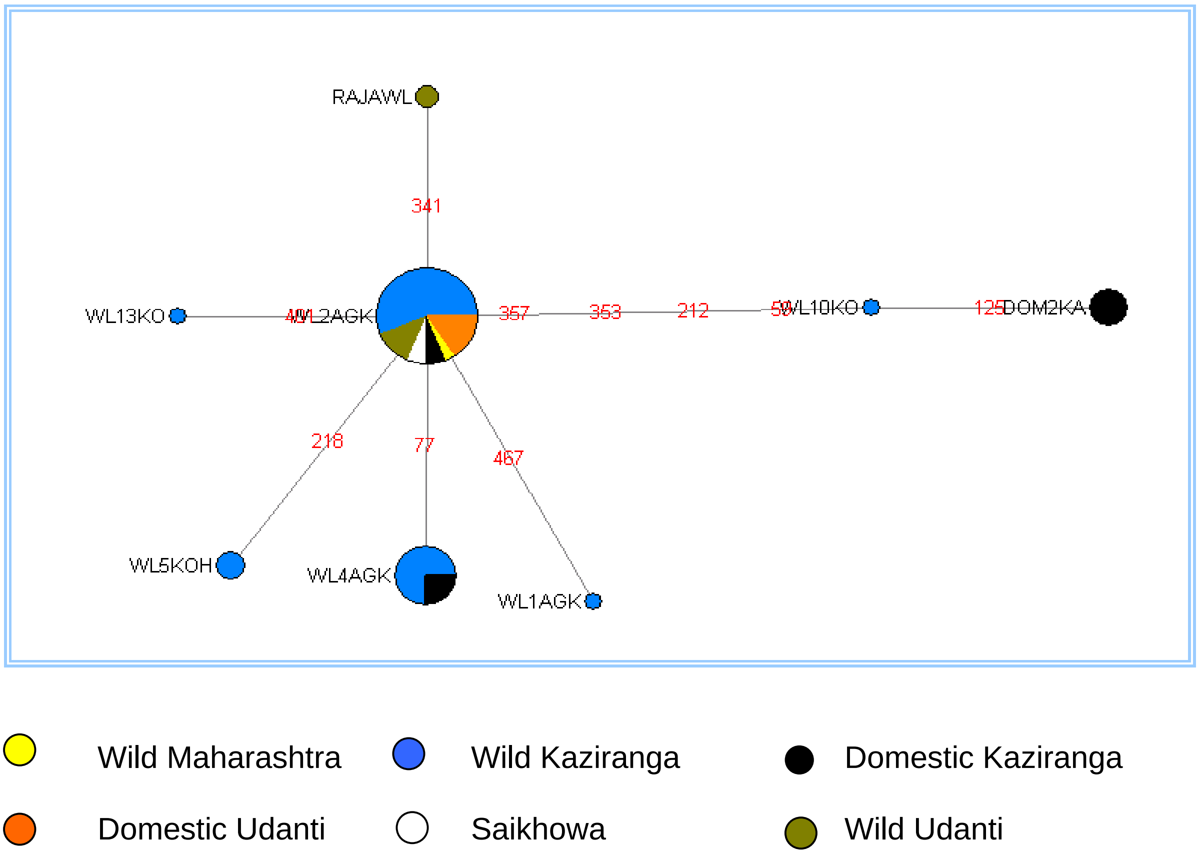

Figure 5. Median Joining Network of Wild and domestic buffalo haplotypes based on variation in Cytochrome b Sequences

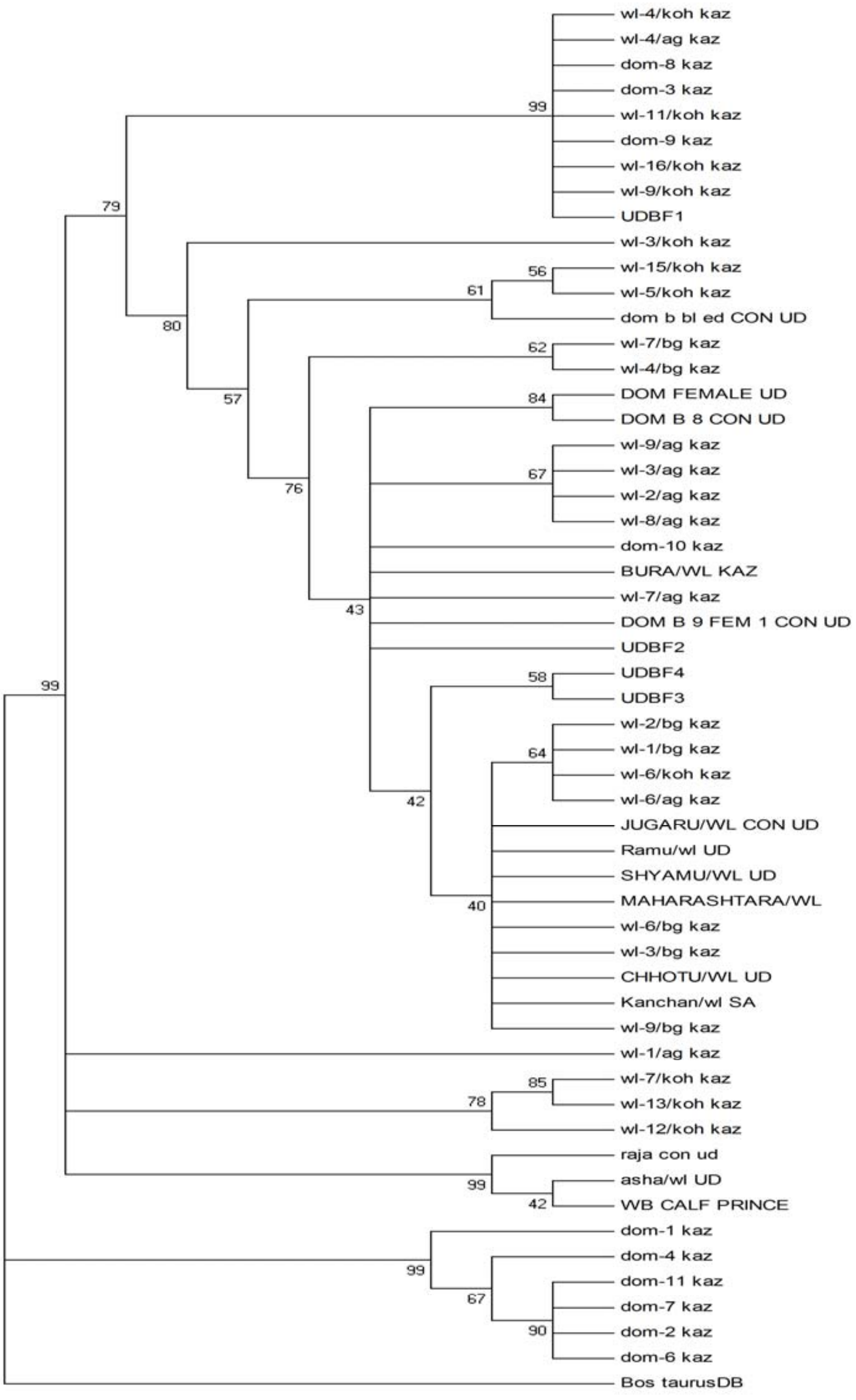

Figure 6. Neighbor-joining tree of combined mitochondrial markers sequence (1917 bp). UD: Udanti Wildlife Sanctuary; KAZ: Kaziranga National Park; SA: Dibru, Saikhowa; WL: Wild; DOM: Domestic

Proper amplification and genotypes could be obtained at 9 loci namely, BMS518, RM372, BMS2116, BMS2325, BMS1316, BMS1226, CA004, BMS2519 and ILSTS058. Allele frequency, number of alleles, observed and expected hyterozygosities were calculated using GENALX software. The comparative alleles for different genotypes of Udanti wild buffaloes at these nine loci is presented in Table 3. The observed heterozygosity among the wild animals was found to be about 45% (Table 4). The domestic animals showed a lower level of heterozygosity i.e. 24% only. Several alleles found in the wild animals only and not in the domestic ones and vice versa, which shows the genetic distinctiveness of both that was not observed clearly at mitochondrial markers (Uni-parental inheritance).

Table 3. Genotypes of the wild buffalo individuals of Udanti Wildlife Sanctuary at the nine selected microsatellite loci.

MARKER / WILD SAMPLES	**L1**	**L1**	**L2**	**L2**	**L3**	**L3**	**L4**	**L4**	**L5**	**L5**	**L6**	**L6**	**L7**	**L7**	**L8**	**L8**	**L9**	**L9**
ASHA	**151**	**151**	**118**	**120**	**106**	**106**	**111**	**113**	**115**	**115**	**157**	**163**	**153**	**153**	**139**	**139**	**105**	**105**
RAJA	**149**	**151**	**118**	**120**	**106**	**108**	**113**	**113**	**115**	**117**	**157**	**163**	**153**	**159**	**139**	**153**	**105**	**107**
PRINCE	**149**	**151**	**118**	**120**	**106**	**108**	**113**	**113**	**115**	**117**	**157**	**163**	**153**	**159**	**139**	**153**	**105**	**107**
SHYAMU	147	***149***	*118*	*120*	106	***108***	113	113	**117**	***117***	157	163	159	***159***	153	***153***	103	***107***
CHHOTU	151	153	126	126	106	106	105	107	115	117	163	163	171	171	119	121	105	109
RAMU	141	143	126	126	110	110	127	127	113	113	157	157	147	147	151	151	103	103
KALIA	149	151	114	116	106	106	115	115	115	115	157	163	147	149	151	151	105	105
JUGADU	151	153	122	124	106	106	111	111	115	119	157	163	139	153	151	151	105	109

Table 4. Comparative Observed (Ho) and Expected He) levels of heterozygosity in the wild and domestic buffalos from Kaziranga National Park (Pop 1 and Pop 2) and Udanti Wildlife Sanctuary (Pop 3 and Pop 4), respectively. N: Number of individuals; Na: Average number of alleles; Ne: Effective number of alleles

Mean and SE over Loci for each Population						
Population		**N**	**Na**	**Ne**	**Ho**	**He**
Pop 1	Mean	36	15.667	9.279	0.396	0.863
	SE		1.994	1.356	0.038	0.029
Pop 2		10	7.000	4.993	0.367	0.766
			0.782	0.596	0.076	0.037
Pop 3		08	5.556	3.971	0.456	0.691
			0.669	0.503	0.084	0.059
Pop 4		09	5.667	3.914	0.246	0.673
			0.799	0.692	0.068	0.055
Grand Mean and SE over Loci and Populations						
Total			**8.472**	**5.539**	**0.366**	**0.748**
			0.903	0.553	0.037	0.026

Conclusion

The genetic variation estimated with microsatellite size and mt DNA nucleotide variation, the significantly moderate level of heterozygosity as well as nucleotide diversity, is indicative of a genetically stable population in Udanti.However, due to their very small number, it was recommended to bring individuals from other parts since these have shown to share the haplotypes with wild buffaloes from Assam and even Maharashtra.The results of the present study would also facilitate identification of animals with unique genotype, which can be used in controlled breeding programs to ensure propagation and genetic variability.

Recommendations:

1. **Atan early point in time, the wild buffalo from Central India and North East had a shared ancestry.Therefore, it is advised to bring unrelated male and female individuals from Kaziranga and Manas National parks for conservation breeding with the already available animals in Udanti Wildlife Sanctuary.**
2. **Analysesof reference specimens both historical and recent in order to develop a benchmark to determine an acceptable level of genetic composition to be maintained in Wild Buffalo population is necessary.**

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