Indian Journal of Animal Sciences **87** (1): 80–86, January 2017/Article

Biodiversity among sheep and goat reared under different agroclimatic regions of West Bengal, India

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Received: 8 December 2015; Accepted: 13 June 2016

ABSTRACT

Biodiversity in West Bengal, India is enormous in terms of climate and living creatures, particularly animals. Livestock species as sheep and goat forms the main backbone for rural economy and reared basically for meat production. The objective of the present study was to characterize them and study the growth, biomorphometric traits, reproduction, wool characteristics, blood biochemical profile, and disease resistance traits and aimed to reveal the biodiversity existing within the goat and sheep breeds reared in different agroclimatic zones of the state. Phylogenetic tree constructed by multivariate cluster analysis with identified traits of eight different sheep breeds of eastern India revealed the emergence of Birbhum sheep as a genetically distinct sheep breed of dry arid region of India with greater percentage of rudimentary ear as its unique physical characteristic. Molecular characterization with mitochondrial gene as Cytochrome B gene confirmed genetic uniqueness of Birbhum sheep. Black Bengal (BB) goat was found to be the only goat breed distributed throughout the West Bengal.

Key words: Biodiversity, Biomorphometric traits, Cytochrome B, Goat, Molecular characterization, Phylogenetic analysis, Sheep

India has vast genetic resource, ranked 2nd in goat and 3rd in sheep population, whereas West Bengal shares 10.72% for goat and 2.3% for sheep of their total Indian counterparts (19th Livestock Census 2014). Sheep and goat, meat purpose animals, form the main backbone for the rural landless and marginal farmers in West Bengal particularly for their high prolificacy and uniqueness and are very popular due to small land holding pattern and keen interest of female enterprises. Biodiversity in this state is enormous in terms of climate and living creatures. Farmers often get attracted to bred their livestock with higher body sized breeds, leading to breed dilution and loss of genetic merits. Hence, there is an urgent need to characterize the existing populations in different regions of the state and biodiversity analysis through construction of phylogenetic tree for goat and sheep. Keeping the above facts in view, the present study was aimed at phenotypic and molecular characterization of the sheep and goat population of West Bengal and biodiversity study through construction of phylogenetic tree for goat and sheep reared under different agroclimatic zones of West Bengal with respect to other breeds of Eastern India.

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MATERIALS AND METHODS

Phenotypic characterization based on economically important traits

Animals and area of study: The present study covered randomly chosen 1,076 sheep and 920 goats maintained by marginal farmers as well as government farms in different agroclimatic zones of West Bengal (Table 1). The state is situated between 21°252 243 and 27°132 153 N latitudes and 85°482 203 and 89°532 043 E longitudes and shares its international borders with Bangladesh, Bhutan and Nepal as well as four other Indian states- Odisha, Jharkhand, Asom and Sikkim.

Growth and reproductive traits: Growth parameters recorded includes body weight (BW) and other biomorphometric traits like heart girth (HG), body length (BL), body height (BH), pelvic width (PW), tail length (TL), head length (HL), ear length (EL), ear width (EW) and distance between two eyes. BW was measured with spring balance whereas other biomorphometric characters were measured with measuring tape. Reproductive traits considered in the present study include recording of litter

Table 1. Agroclimatic variables and study area

Agroclimatic region	Area covered in study		Latitude (N)	Longitude (E)	Avg. temp. (°C)
Terai	Jalpaiguri	Ramsai Farm Rajganj	26°15′47″ to 25°55′34″	88°23′2″ to 89°7′30″	20.4 to 31.4
Old-Alluvial	Dinajpur (S)	Balurghat	26°35′15″ to 25°10′55″	85°00′30″ to 87°48′37″	20.4 to 31.4
New-Alluvial	Nadia	Haringhata State Farm, Kalyani	22°52′30″ to 24°05′40″	22°08′10″ to 88°48′15″	20.6 to 32.4
Red-Laterite	Birbhum	Dubrajpur, Khoyrasole, Rajnagar	23°32′30″ to 24°35′0″	88°1′40″ to 87°5′25″	15 to 40
	Bankura	Indpur (Chota nagpur Plateu)	22°38′ to 23°38′	86° 36′ to 87°46′	27 to 45
Coastal-Saline	24-Pgs (S)	Sagar, Gosaba	21°25′30″ to 23°16′50″	88°01′10″ to 89°06′15″	16.2 to 43.4

size, age at first kidding/lambing, age at first service, gestation period and post partum estrus.

Wool traits: Different wool characteristics like fiber length, fiber diameter and medullation percentage were assessed following standard methods (Wang *et al.* 2007). Weight of wool per shearing were recorded as per standard animal husbandry practices and compared with normal sheep standards.

Blood biochemical traits: Haemoglobin content was estimated from the whole blood of individual animal by cyano-methaemoglobin method. Total erythrocytic count (TEC), total leucocytic count (TLC) and differential leucocytic count (DLC) were enumerated by haemocytometer (Schalm *et al.* 1975).

Disease resistance traits (parasitic): It includes recording of incidences for parasitic infestations. Nematode infestation has been measured as total faecal egg count observed under microscope with the help of a McMaster slide (McMaster 2014).

Statistical analysis: Analysis of variance was employed for studying the economic traits at different agroclimatic zones. Multivariate cluster analysis was employed (SYSTAT 13) based on Euclidean distance and complete linkage method for estimation of genetic distance and construction of phylogenetic tree for goat and sheep population at different agroclimatic regions of West Bengal with reference to the native population of eastern India.

Molecular characterization with Cytochrome B gene

Mitochondrial DNA was isolated from representative samples of the sheep breeds as Garole, Bonpala, Chotanagpuri and Birbhum reared in different agroclimatic regions of West Bengal (Sambrook and Russel 2001). Cytochrome B gene was amplified with the undernoted forward and reverse primers Cyt B1. 5'CATTGATCTC CCAGCTCCA3' and Cyt B2. 5'GATGTAGGGGT GTTCAACTGG3'. PCR was carried out in a final volume of 25 µl of reaction mixture containing 80–100 ng DNA, PCR buffer 1.2×, $MgCl_2$ 1.5 mM, dNTP 0.5 mM, forward primer and reverse primer 60 ng each , Taq DNA polymerase (1 unit) at annealing at 61°C. Amplified product was subjected to Sanger sequencing. Further bioinformatics analyses were carried out with suitable softwares. Phylogenetic tree was constructed based on the CytB gene sequences (Clustal W method, DNASTAR).

RESULTS AND DISCUSSION

Biodiversity analysis of sheep reared under different agroclimatic regions of West Bengal

Phylogenetic study based on phenotypic traits: Biodiversity study for sheep has been accessed through phylogenetic analysis of different sheep breeds of West Bengal along with their counterparts existing in eastern India (Fig. 1) namely Garole, Chotanagpuri, Bonpala and Birbhum sheep of West Bengal; and Ganjam, Balangir, Sahabadi and Tibetan from other parts of the region. It is evident from the constructed phylogenetic tree that the sheep of Hilly region (i.e. Tibetan and Bonpala) remained genetically most distant from other breeds of eastern India. Thus, Bonpala is genetically most distant to other natives

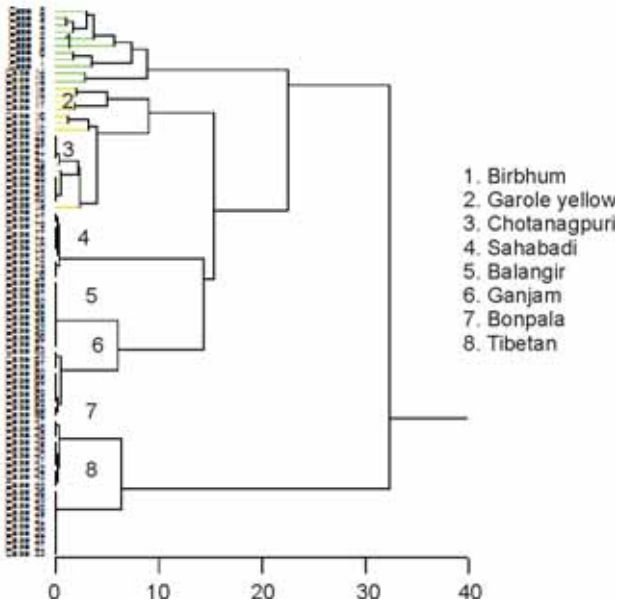

Fig. 1. Phylogenetic tree for different sheep breeds of Eastern India.

of West Bengal (i.e. Garole, Birbhum and Chotanagpuri). There is possibility of slight genetic admixture of Garole and Chotanagpuri breeds which emphasizes the need for adoption of immediate conservation strategies for these two breeds to prevent the dilution of genetic merits. Garole and Birhum sheep found to exhibit more genetical closeness. Hence further analysis has been done for these two breeds covering a large population (Fig. 2). They remained in two distinct clusters which might strengthen the hypothesis of genetic uniqueness of Garole and Birbhum sheep. Birbhum sheep existing in the dry-arid region as formed a separate cluster might form the basis for emerging as a new sheep breed. Ganjam and Balangir (of adjoining Orissa state) depicted their genetic closeness (Fig. 1) with genetic closeness with Sahabadi. Similar studies were observed by multivariate analysis of morphological characteristics in Nigerian native sheep have been studied in recent past (Yakubu *et al.* 2011) where different clusters were identified for breeds of sheep.

Molecular phylogeny of sheep based on Cytochrome B gene: The dendogram reveals genetic distinctness of Birbhum sheep (KU246233.) compared to other sheep breeds of WB as Garole (KU246232), Bonpala (Gene sequence submitted) and Chotanagpuri (KU246232) (Fig. 3). This confirms the findings as revealed by phenotypic characterization. Mitochondrial gene as Cytochrome B, the only gene coding polypeptide is very effective for carrying out evolutionary study.

Similar studies were conducted as Sheep biodiversity from Southern peninsular and parts of Eastern regions of India had been tried by microsatellite DNA marker (Arora *et al.* 2011) and mitochondrial DNA marker (Othman *et al.* 2015).

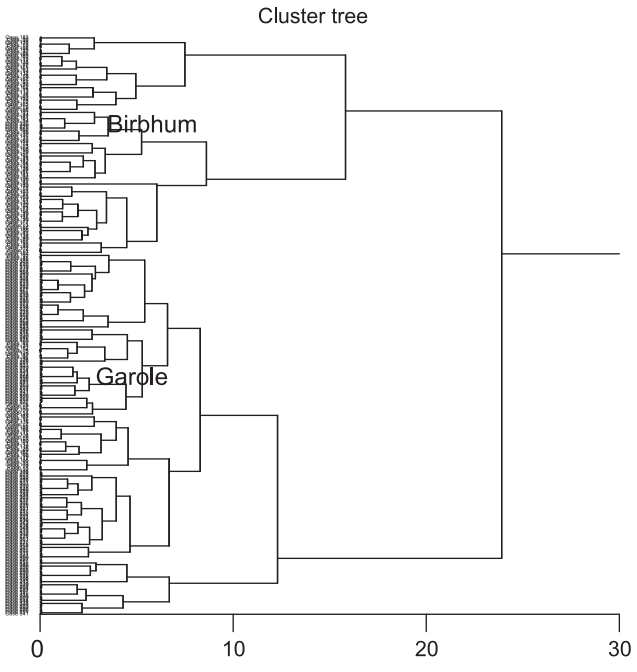

Fig. 2. Phylogenetic tree for Garole and Birbhum sheep. The upper cluster reveals sheep from dry arid region of Birbhum district, whereas lower cluster reveals Garole sheep.

Table 2. Growth parameters for different sheep breeds of West Bengal

Particular	Garole	Bonpala	Chotanag-puri	Birbhum
Body weight (kg)	10.4± 0.33[a]	41.68± 0.28[c]	19.47± 0.13[d]	12.98± 0.28[b]
Body length (cm)	45.31± 0.33[a]	74.79± 0.53[b]	52.73± 0.23[c]	47.53± 0.21[a]
Heart girth (cm)	62.905± 0.35 [a]	80.39± 0.67[c]	71.14± 0.56[d]	60.23± 0.31[b]
Paunch girth (cm)	63.1± 0.33[a]	81.87± 0.63[c]	72.54± 0.45[d]	60.43± 0.42[b]
Body height (cm)	47.625± 0.33[a]	73.34± 0.46[c]	54.58± 0.45[d]	51.43± 0.32[b]
Head length (cm)	15.56± 0.33	18.97± 0.39	17.97± 0.38	17.54± 0.35
Tail length (cm)	9.98± 0.34	15.38± 0.34	13.15± 0.18	11.75± 0.33
Ear length (cm)	6.455± 0.34	7.89± 0.18	9.38± 0.17	4.84± 0.42
Ear width (cm)	4.655± 0.34	3.56± 0.32	4.2± 0.37	3.87± 0.28
Distance between two eyes (cm)	11.025± 0.34	13.67± 0.67	12.45± 0.45	9.12± 0.37
Presence of horns	Present in ram but absent in ewe	Both sexes are polled	Both sexes are polled	Present in ram but in absent ewe

*Mean values bearing different superscripts (a,b,c,d) in a row differ significantly (P<0.01).

Table 3. Growth performances of different sexes of Garole and Birbhum sheep

Particular	Garole		Birbhum	
	Male	Female	Male	Female
Body weight (kg)	10.3± 0.31	9.72± 0.28	12.98± 0.28	11.42± 0.30
Body length (cm)	44.76± 0.33	40.5± 0.31	47.53± 0.21	43.68± 0.23
Heart girth (cm)	62.85± 0.35	56.6± 0.33	60.23± 0.31	55.84± 0.34
Paunch girth (cm)	62.7± 0.33	59.5± 0.33	60.43± 0.42	56.68± 0.43
Body height (cm)	47.32± 0.33	43.03± 0.34	51.43± 0.32	47.6± 0.34
Head length (cm)	15.16± 0.33	14.00± 0.34	17.54± 0.35	16.2± 0.36
Tail length (cm)	09.86± 0.34	9.09± 0.33	11.75± 0.33	10.88± 0.34
Ear length (cm)	6.05± 0.34	7.07± 0.34	4.84± 0.42	5.85± 0.43
Ear width (cm)	4.35± 0.34	6.09± 0.34	3.87± 0.28	4.17± 0.29
Distance between two eyes (cm)	10.95± 0.34	9.43± 0.34	9.12± 0.37	7.98± 0.38

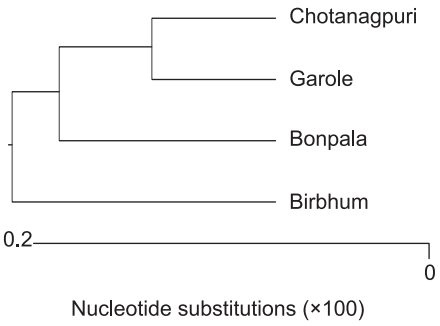

Fig. 3. Molecular phylogeny for sheep breeds of West Bengal based on cytochrome B gene.

Characterization of sheep at different agroclimatic regions

Growth traits: Significant difference among the growth traits for different sheep breeds of West Bengal has been observed and characterized accordingly (Table 2). Birbhum sheep exhibited significantly better (P<0.05) BW and BH compared to the Garole, but HG and PG remained superior for the Garole. It might be due to more compactness of body structure of Garole. Similar trend was observed for either sex within the breed under study (Table 3). About 30.6% of the Birbhum sheep found to have rudimentary ear, which seems to be a unique characteristics for this particular breed. Body colour varied from black, brown and white for the Birbhum sheep. Horn is present in ram but absent in ewe in both the breed. Average ear length and ear width was exceptionally less for Birbhum as compared to Garole, might be due to higher percentage of rudimentary ear. Hence, Garole and Birbhum sheep (Fig. 4) have distinctly separate morphological characteristics.

Fig. 4. A flock of Birbhum sheep in its natural habitat (left: at farmer's house; right: grazing) reared at dry arid region of Birbhum District of West Bengal, India.

Table 4. Reproductive performances of sheep at different agroclimatic regions of West Bengal

Particular	Garole	Birbhum
Age at first lambing (days)	419.56±11.46[a]	390.40±10.43[b]
Litter size	1.50±0.14	1.71±0.16
Age at first service (days)	250.45±11.78	240±10.45
Gestation period (days)	151±11.45	150.40±11.58
Post-partum estrus (days)	91.12±10.34	90.56±9.80

*Mean values bearing different superscripts (a,b) in a row differ significantly (P<0.01)

Reproductive traits: Age at first lambing was significantly better in Birbhum sheep compared to that of Garole. A tendency has been observed for better age at first service and post-partum estrus in Birbhum sheep (Table 4).

Wool quality: Wool or fibre parameters namely wool colour, staple length, fibre diameter, medullation percentage and weight of wool per shearing of the sheep breeds of

Table 5. Wool quality of different sheep breed of West Bengal

Particular	Garole	Bonpala	Chotanag-puri	Birbhum
Colour	White, grey, brown	Complete white to total black with a range of intermediary colour	Light grey to brown	White, grey, brown
Staple length (cm)	4.95± 0.23	9.63± 0.87	5.23± 0.12	7.46± 034
Medullation percentage	88.13± 0.56	95± 0.34	83.61± 0.23	78.34± 0.45
Fibre diameter (micron)	54.74± 0.34[a]	66± 0.56[a]	52.53± 0.34[a]	45.23± 0.23[b]
Weight of wool per shearing (g)	305.36± 10.03[a]	455.00± 8.34[b]	184.2± 2.45[c]	452.20± 9.89[b]

*Mean values bearing different superscripts (a,b) in a row differ significantly (P<0.01)

Table 6. Blood biochemical profile for different breeds of sheep of West Bengal

Particular	Garole	Bonpala	Chottanag-puri	Birbhum
Hb (mg/dl)	9.32± 0.22	10± 0.34	9.8± 0.32	11.7± 0.23
PCV	28.03± 0.33	28.9± 0.43	27.78± 0.45	30.45± 0.34
TEC (× 10^6)	3.27± 0.12	3.15± 0.24	3.18± 0.25	3.45± 0.12
Neutrophil (%)	42.59± 0.44	40.34± 0.54	40.24± 0.55	43.67± 0.45
Eosinophil (%)	3.95± 0.12	3.67± 0.14	3.34± 0.15	3.67± 0.13
Basophil (%)	0.00± 00	0.00± 00	0.00± 00	0.00± 00
Lymphocyte (%)	51.27± 0.44	53.54± 0.56	54.33± 0.57	50.19± 0.54
Monocyte (%)	2.18± 0.11	2.45± 0.12	2.18± 0.13	2.13± 0.11

West Bengal have been listed in Table 5. All the breeds usually produce mostly coarse carpet wool. A better wool yield per shearing was recorded for Bonpala and Birbhum sheep. However, wool of Birbhum sheep found to have a better demand might be due to its superiority over other breeds under study.

Disease resistance characteristics: The blood biochemical profile (as a marker for general health status) revealed no marked difference in haemoglobin, PCV, TLC, TEC and DC among different sheep breeds (Table 6) but Birbhum sheep were found to have numerically better polymorphonuclear leukocytes. This may attribute a better immune status to this particular breed.

Table 7. Disease resistance traits (Parasitic) for different breeds of sheep of West Bengal

Particular	Garole	Bonpala	Chottanag-puri	Birbhum
FEC for nematodes (number per gram of faeces)	155.38± 10.45[a]	109.67± 11.34[b]	100.45± 11.54[b]	80.45± 10.44[c]
Incidences for Eimeria (%)	77.38± 0.42	60.45± 0.59	42.78± 0.58	32.45± 0.43
Incidences for Strongyles (%)	55.95± 0.46	43.78± 0.56	32.56± 0.49	28.34± 0.48
Incidences for Trichuris (%)	4.76± 0.13	2.2± 0.24	1.13± 0.25	1.1± 0.20

*Mean values bearing different superscripts (a,b) in a row differ significantly (P<0.01).

Birbhum sheep also exhibited a better resistance against common parasitic infestations (Table 7) in the present study. Not only total faecal egg count (number per g of feaces) but also individual GI nematodes also remained lowered (P<0.01) in Birbhum sheep as compared to Garole, Chotanagpuri and Bonpala.

Phylogenetic analysis of goat reared under different agroclimatic regions of West Bengal

Genetic distance with multivariate cluster analysis revealed that the entire goat populations reared under different agroclimatic region formed a single cluster (Fig. 5). It reflects the genetic identity between the Black Bengal goat population reared under varying agro-climatic conditions. Since no separate cluster was formed within the goats in separate zone, it can be predicted that there may be the absence of inbreeding within a particular region. Hence it can be inferred that no distinct strain/line of BB goat has been developed in separate agroclimatic zones. No evidence of crossbreeding of BB goat population with other goat breeds was observed. It might be due to general preference of BB goat to rural farmers added with its lucrative market price.

Characterization of goat reared at different agroclimatic regions

Growth traits: The average of growth traits for BB goat reared in different agroclimatic regions has been depicted in Table 8. No significant differences were observed between the body weight and biomorphometric characters

Table 8. Growth performances of Black Bengal goat at different agroclimatic regions of West Bengal

Particular	Terai		New Alluvial		Old Alluvial		Dry Arid		Coastal Saline	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
BW (kg)	13.23± 0.56	13.56± 0.43	13.31± 0.34	13.67± 0.21	13.36± 0.34	13.88± 0.21	13.42± 0.32	13.37± 0.31	13.66± 0.19	13.80± 0.18
BL (cm)	46.14± 0.76	46.64± 0.27	46.23± 0.44	46.65± 0.27	46.44± 0.41	46.81± 0.27	46.45± 0.34	46.39± 0.35	43.94± 0.28	43.44± 0.24
HG (cm)	54.04± 0.65	54.13± 0.59	54.34± 0.62	54.30± 0.45	55.16± 0.57	55.30± 0.39	56.92± 0.45	54.04± 0.54	54.52± 0.38	54.20± 0.36
PG (cm)	60.14± 0.61	60.67± 0.54	60.83± 0.61	61.23± 0.41	62.83± 0.45	61.54± 0.41	64.70± 0.56	58.78± 0.51	63.03± 0.38	62.72± 0.41
BH (cm)	46.64± 0.46	46.28± 0.28	46.74± 0.46	46.34± 0.28	46.89± 0.46	46.53± 0.28	61.39± 0.57	46.66± 0.48	46.04± 0.26	45.46± 0.24
HL (cm)	15.06± 0.16	15.06± 0.10	15.05± 0.15	15.05± 0.09	15.06± 0.14	15.06± 0.08	18.11± 0.26	16.09± 0.25	14.43± 0.08	14.53± 0.08
TL (cm)	11.89± 0.25	9.97± 0.21	11.89± 0.25	9.97± 0.23	11.89± 0.25	9.98± 0.22	11.57± 0.25	9.76± 0.22	11.37± 0.25	9.56± 0.22
EL (cm)	14.03± 0.31	10.76± 0.21	14.03± 0.31	10.87± 0.21	14.03± 0.31	10.97± 0.21	13.93± 0.31	10.84± 0.21	13.67± 0.31	10.64± 0.21
EW (cm)	6.98± 0.21	6.03± 0.19	6.98± 0.21	6.03± 0.19	6.98± 0.21	6.04± 0.19	6.92± 0.21	5.96± 0.19	6.75± 0.21	5.84± 0.19
DBE (cm)	11.87± 0.28	10.86± 0.23	11.87± 0.26	10.87± 0.23	11.88± 0.26	10.88± 0.23	11.62± 0.26	10.45± 0.23	11.34± 0.26	10.65± 0.23

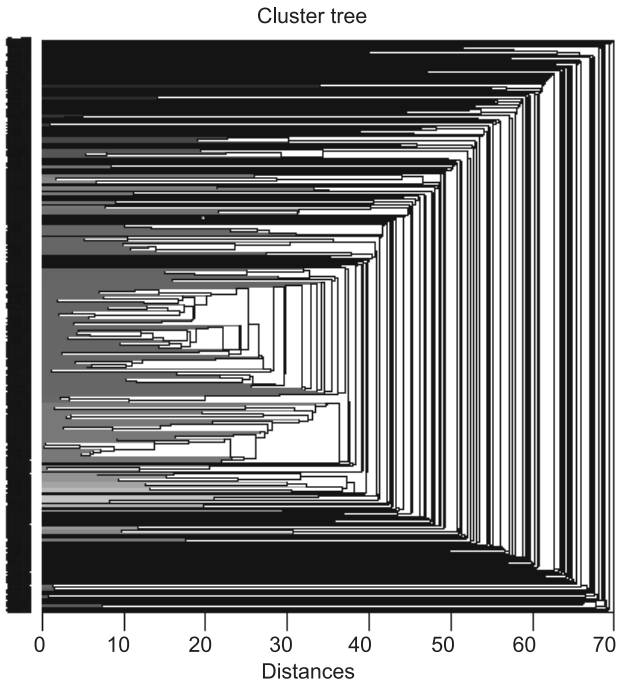

Fig. 5. Phylogenetic tree for goats reared under different agroclimatic zone of West Bengal.

throughout the state. Thus it indicates that the goats under present study were true to breed. The present findings agreed with the earlier observations carried out at Pakistan (Khan and Naznin 2013). It gives us an idea that there may be absence of any evolutionary force, in terms of inbreeding or crossbreeding in the existing goat population. However further studies are needed, preferably with animal model to establish the facts, when estimated inbreeding co-efficient would have been zero.

Reproductive traits: The reproductive performances studied for BB goat reveal no significant differences for age at first kidding and age at first service (Table 9). Goats reared at coastal region mature early and have better age at first kidding, compared to goats reared at alluvial region or dry arid region. It indicates that a better germplasm of Black Bengal goat is present in coastal region, which needs an immediate attention and strategic conservation. Higher fecundity was also recorded for BB goat at coastal region compared to that of other regions of West Bengal.

Genetic diversity is prevailing within the existing sheep breeds of West Bengal. Sheep reared at dry arid region of the Birbhum district exist as a new breed with distinctly better growth, reproductive traits, wool characteristics and disease resistance. Phylogenetic analysis both at phenotypic and molecular level also reckoned this population as a breed with distinct genetic identity. These sheep were originated in the Birbhum district of West Bengal and they are known for more than 200 years, reared mostly for meat purpose. Body colour ranges from white, grey, to deep brown, either intact or patchy. They have slightly convex head, with horizontal ears, two horns (blackish-grey coloured) present only in ram but not in ewe; and absence of wattles. The head, face and legs were almost devoid of wool, whereas

Table 9. Reproductive performance of Black Bengal goat at different agroclimatic regions of West Bengal

	Terai region	New-alluvial region	Old-alluvial region	Coastal region	Dry arid region
Age at 1st kidding (days)	548.62± 16.9[a]	550.89± 13.9[a]	554.62± 15.9[a]	465.58± 15.89[b]	544.62± 14.9[a]
Litter size (No.)	1.23± 0.03	1.3± 0.03	1.25± 0.03	1.6± 0.04	1.24± 0.03
Age at 1st service (days)	394.25± 14.34[a]	398.25± 14.34[a]	401.25± 14.34[a]	341± 15.78[b]	396.25± 15.34[a]
Gestation period (days)	144± 12.89	144± 13.67	145± 12.78	144± 11.56	145± 11.45
Kidding interval (days)	183.73± 15.98	182.87± 14.87	181.35± 13.45	179.98± 13.78	180.45± 13.56
Weight at first kidding (kg)	14.52± 0.84	14.62± 0.75	14.72± 0.35	14.65± 0.29	14.70± 0.27

*Mean values bearing different superscripts a,b in a row differ significantly (P<0.05)

rest of the body remain covered by wool of medium staple length, non-lustrous, straight or low crimp. The wool obtained as by-product may be utilized for carpet production. Beard is absent and tail is drooping downwards. The peculiarity of the Birbhum sheep is that about 30.6% population had rudimentary ear with high disease resistance and better litter size. Rudimentary ear seems to be one of the breed characteristics of these sheep.

Black Bengal breed of goat is reared throughout West Bengal and reveals reed identity primarily from the samples under study.

ACKNOWLEDGEMENT

Funding provided by Department of Biotechnology, Ministry of Science and Technology, Govt. of India and necessary support extended by the Director, Animal Resource Development Department, Govt. of West Bengal, India is gratefully acknowledged.

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