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Cytogenetic analysis reveals swamp status of the indigenous '*Bhangor*' buffalo population from Tripura

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Water buffaloes, (*Bubalus bubalis*) were domesticated some 6300 years ago for milk and use in agriculture. It plays essential roles in agriculture, economy, and food production. The buffalo were incredibly important to the people and are widely distributed in many countries, including the Indian subcontinent, Southeast Asia, China, and across continents in Italy and Australia (Borghese *et al.* 2005, Kandeepan *et al.* 2009). In India, there are 19 registered defined breeds of buffalo (ICAR-NBAGR 2021) and various indigenous non-described populations that need further characterization both morphometric as well as genetic using molecular markers. So far buffaloes of the north-eastern states of India have been studied except for Tripura and are generally considered to be of swamp type based on their phenotypic resemblance to swamp type buffaloes. Here we have attempted to classify and characterize the indigenous buffalo population 'Bhangor' using cytogenetic tools.

The indigenous buffalo are known as 'bhangor' or 'manipuri' by local people. Tripura is a land-locked state and it share geographical boundary with international boundaries of Bangladesh and very narrowly it joins with the Indian states of Assam and Mizoram to the east. Covering an area of 10,491 km^2 (4,051 sq mi), it is the third-smallest state, situated between the river valley of Myanmar and Bangladesh. The terrain by and large consists of parallel hills and ridges running from the northwest to the southeast direction, with alternating narrow valleys. Swamp buffaloes are reared mainly for draught power in the paddy fields. Morphologically, swamp buffaloes are small in size and tend to wallow in water (Borghese *et al.* 2005). The present study aimed at assessing the status of the Bhangor buffalo population from Tripura employing cytogenetic studies. Karan *et al.* (2020) has shown the existence of swamp buffalo in Meghalaya.

Morphometric data collection: Information on indigenous buffalo population regarding its habitat, distribution, management practices, and socio-economic status of the farmers was collected through preset questionnaires during the pilot survey in collaboration with the State Animal Husbandry Department. Morphometric data on standard traits was recorded from adult buffaloes in West Tripura, Khowai, Dhalai, Unakoti, Gomati, and North Tripura districts of Tripura state. Blood samples from adult male, female and calves (20) were collected from unrelated animals from various villages in the breeding tract for cytogenetic analysis. Samples were transported to the lab within 24 to 48 h under cold conditions before setting up the cultures for cytogenetic studies.

Cytogenetic analysis: The cultures were set up using RPMI 1640 culture medium described by Prakash *et al.* (2011) for lymphocyte culturing standardised with slight modification, in 15 ml tubes under sterile conditions. Mitosis was induced by the incorporation of 2% phytohemagglutin in (PHA) as mitogen, and the cultures 72 h incubation at 37°C with intermittent shaking in the incubator. The cells were harvested after arresting chromosomes at metaphase using colchicine, followed by hypotonic treatment (0.075 M KCl) and fixed in methanol: acetic acid (3:1). The metaphase spreads were examined at 10× magnification and chromosome count recorded at 100× oil immersion and photographed. Screening of at least five spreads of each animal was attempted to confirm the results, each spread was analyzed critically to document chromosomal abnormality also.

The population of indigenous Tripura buffaloes is decreasing and as per the 20th Livestock Census 2019, it is around 7000, 50% of which is found in the Unakoti district alone followed by the North-Tripura and Dhalai districts. Morphometric body measurements of the Tripura buffaloes were recorded and evaluated. The Bhangor buffaloes are generally grey to greyish-black in colour. The horn is sickle-shaped (typical curved) with a broad base which is mostly corrugated and with the tip pointed upward or backward. The ears are horizontally placed. The forehead is mostly convex. The buffaloes are medium built and compact. The lower leg portion between hoofs and knee is white to greyish-white in colour (Figs 1, 2). Young calves have

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prominent white stripes, one in the neck and another slightly above the brisket, which is typical feature of these buffaloes, which fades in adult animals. The tail switch is generally black. They are primarily used for draught power in paddy fields and other agricultural operations but are also used for meat production; milk yield is 1.5–2.5 litres per day. Animals are also used in traditional festival sacrifice though it has been banned by Indian High Court order. Swamp buffaloes are stocky animals with marshy land habitats. Adult male or female buffaloes are mostly used as paired animals to plough fields with locally designed plough.

Cytogenetic characteristics of Bhangor buffaloes: The protocol followed for cytogenetic analysis helped in arresting of chromosomes at metaphase and spreads could clearly define the chromosomes structure and numbers to ascertain the riverine or swamp status of animals investigated. The typical buffaloes karyotype standardized internationally for identifying the riverine, swamp and hybrid buffaloes (Iannuzzi *et al.* 1994) was taken as a reference for confirming the riverine or swamp status. The animals have distinctive karyotypic features established for swamp type buffaloes. Samples of the animals exhibited a diploid count of 2N=48 chromosomes (Fig. 3), which is typical for swamp buffalo features (Bongso *et al.* 1984, Sat 1987). The karyotype comprised of 23 pairs of autosomes and a pair of sex chromosomes. The 23 pairs of autosomes contain 5 biarmed (metacentric/submetacentric) and 18 acrocentric chromosomes. The large Swamp buffalo chromosome 1 originated from tandem fusion translocation between the River buffalo chromosome 4 (telomeres of p-arm) and 9 (centromere) (Di Berardino *et al.* 1981). Cytogenetic analyses thus confirmed the swamp status of Bhangor buffaloes of north-east India. No morphological or chromosomal abnormality was observed in the populations during cytogenetic analysis.

Fig. 1. Adult male Bhangor buffalo.

Fig. 2. Adult female Bhangor buffalo with calf.

In the taxonomic classification of the 'Bovini' tribe, three groups have been distinguished: Cattle, Asian buffalo and African buffalo. African buffalo with chromosome number of 52 belongs to a different genus (*Syncerus*) with respect to the genus *Bubalus* of Asia and Europe. Asian buffalo includes two subspecies known as the River and Swamp types. The River buffalo has 50 chromosomes of which five pairs are sub-metacentric, while 20 are acrocentric: the Swamp buffalo has 48 chromosomes, of which 19 pairs are metacentric.

Buffalo population from North-east Indian state has been studied previously and one swamp breed '*Luit*' has been registered by ICAR-NBAGR, which is found in the found in upper Assam Brahmaputra valley and bordering areas of Manipur and Nagaland. Mishra *et al.* (2010a) characterized buffalo samples from Manipur and found pure swamp type animal. The Assamese buffalo cytogenetic characterization revealed the presence of both the pure riverine and hybrid buffalo but no swamp type (Mishra *et al.* 2010b).

Cytogenetic characterization in Bhangor buffalo is the first confirmed documentation of the existence of pure swamp-type buffaloes in the Tripura state of India. Cytogenetic analysis even though mostly used for the evaluation of chromosomal abnormality by arresting the

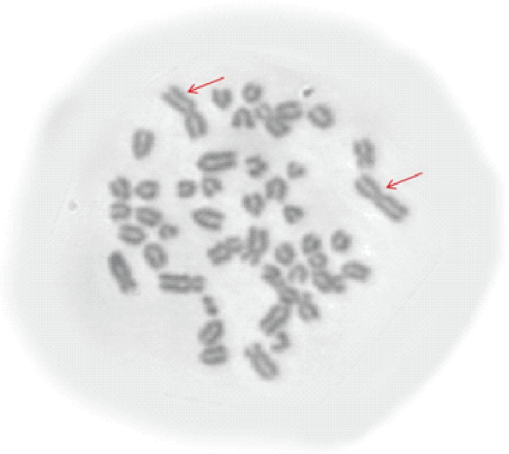

Fig. 3. Typical metaphase chromosome spread of Bhangor swamp buffalo, Swamp (2N=48 chromosomes).

cell division at metaphase stage, but is also helpful to ascertain the riverine/swamp status of buffaloes. North-east is the region where admixture of both riverine and swamp along with hybrids are present. There is a severe decline in the swamp buffalo population, due to various anthropogenic reasons. This unique germplasm needs immediate intervention for breeding and conservation program.

SUMMARY

The domestic water buffalo (*Bubalus bubalis*) is classified into the swamp and riverine. However, their hybrids are also found in some parts of Assam (Brahmaputra Valley) in North-east India. Swamp buffaloes have a typical phenotypic appearance, like the shape of horns, small body size as compared to riverine breeds, and body-colour, etc. This study characterizes the indigenous non-descript 'Bhangor' buffalo population from the Tripura state using karyotype analyses. Blood samples were collected from unrelated animals of both sexes phenotypically identified as swamp buffaloes from the breeding tract in Unakoti district which has >50% of indigenous buffalo population in Tripura. The blood leukocytes were cultured, terminated, and harvested using conventional karyotype protocol as standardised in lab to determine the number of chromosomes present in the metaphase chromosome spreads after 72 h incubation at 37°C with intermittent shaking in the incubator. Chromosomal spread showed presence of 2N=48 chromosomes, comprised of 23 pairs of autosomes and a pair of sex chromosomes with clearly distinct size of fourth pair of metacentric chromosome. And for the first time, non-descript indigenous buffalo population 'Bhangor' from Tripura has been characterized using karyotypic analysis.

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