



Cytogenetic analysis reveals existence of swamp buffalo population in Meghalaya

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The best milch breeds of buffalo in the world are found in Indian subcontinent; majority of which are riverine type. Indian is home to diverse well established riverine buffalo breeds from high-yielding dairy type (Murrah and Nili-Ravi) to draught type buffaloes (Assamese and Toda). Water buffaloes are distributed widely around the world including the Indian subcontinent, South-east Asia, China and across continents in Italy and Australia. There are 16 well defined registered buffalo breeds in India. Out of which one is of swamp breed 'Luit' found in North-eastern Indian states of Manipur, Nagaland, Mizoram and upper Assam valley. However, buffalo populations in the north-eastern states are yet to be precisely classified. The North-east region of India is the home of the Asiatic wild buffalo (*Bubalus arnee*), a small population surviving in Balpakram National Park in south Garo hills (Meghalaya), which is thought to be the progenitor of the domesticated water buffalo (Mathur *et al.* 1995, Lei *et al.* 2007) and is possibly the region for the river-swamp division. Majority of the buffaloes of the north-eastern region of India resemble swamp type buffaloes in their external morphological features with compact body and medium size. The buffaloes from Assam along the Brahmaputra River have generally been described as swamp type, based on their morphological characters (Tamuly *et al.* 1982, Das *et al.* 2005). The buffaloes of Manipur were described as pure swamp type by Mishra *et al.* (2010a) based on cytogenetic studies. The existence of swamp type buffaloes in north-eastern part of India had been reported earlier (Bidhar *et al.* 1986, Yadav *et al.* 1988, Mishra *et al.* 2010). Besides India's north-eastern region, native buffaloes of China, Bangladesh and South-east Asian countries are of swamp types. However there is no demarcation line in the distribution of riverine and swamp type buffaloes in the north-east region within India.

Indian state of Meghalaya has sizeable number of buffalo population spread across the state; these are mainly reared for draught and meat purposes. The information collected from 8 districts covering the state revealed differences in their body measurements. Karyotyping has been used to identify animal species. Systematic study to characterize the unexplored buffalo germplasm of the north-east region

of India has been taken up by few researchers. This is also essential for conservation plan to be conceived for these populations. We have attempted to characterize buffaloes of Meghalaya state of India based on cytogenetic profiling to ascertain the riverine/swamp status of uncharacterized buffalo populations. The method involved inhibition of cell division at metaphase by adding colchicine and resolving the chromosomal spread at metaphase. The typical buffaloes karyotype standardized internationally for identifying the riverine, swamp and hybrid buffaloes (Iannuzzi 1994) was taken as a reference for confirming the status.

Information on buffalo population regarding its habitat, distribution, management practices and socio-economic status of the farmers was collected through preset questionnaires during pilot survey in collaboration with state animal husbandry department. Morphometric data on standard traits as described in breed descriptor of ICAR-NBAGR was recorded from adult buffaloes from different areas of Meghalaya. Blood samples were collected from unrelated animals of both sexes from various villages in the breeding tract. Survey for blood sample was done in the 8 out of 11 districts of Meghalaya which are East Khasi Hills, Ri-Bhoi, West Khasi Hills, South West Khasi Hills, East Garo Hills, West Garo Hills, South West Garo Hills and South Garo Hills, which has a good number of buffalo populations according to the animal husbandry department information (AH&VD, Meghalaya 2019).

The method described by Prakash *et al.* (2011) was used for blood leukocyte cultures with slight modifications using RPMI 1,640 medium with 2% phyto-haemagglutinin (PHA) as mitogen in 15 ml tubes under sterile conditions. The cultures were harvested using standard procedure for the preparation of metaphase chromosome spreads after 72 h incubation at 37°C with intermittent shaking in an incubator. Screening of at least five spread of each animal was attempted to confirm the results.

Meghalaya buffaloes are distributed in the hilly as well as valley/plain regions of different parts of the state. The population of Meghalaya buffaloes is estimated around 0.70 lakh (Livestock Census 2012). Morphometric body measurements of the Meghalaya buffaloes were recorded and evaluated. The Meghalaya buffaloes are generally grey to greyish-black in colour. The horn is sickle shaped (typical

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curved) with broad base which is mostly corrugated and with tip pointed upward or backwards. The ears are horizontally placed. The forehead is mostly convex. The buffaloes are medium built and compact. Lower leg portion between hoofs and knee is of white to greyish-white in colour. The tail switch is generally black. The udder is bowl shaped and poorly developed, with small, cylindrical teats with pointed tips. The Meghalaya buffaloes have typical white markings on either side of muzzle and lower jaw. Some animals had one white patch on the lower part of the neck region. The temperament of these animals is aggressive and active. Breeding of buffaloes is purely by natural service.

The buffaloes in the hilly region are mainly used for draught and meat purpose while the animals in the plains are used primarily for agricultural operations and to some extent milk production. Adult male or female buffaloes are mostly used as paired animal to plough fields with locally designed ploughs. Animals are reared primarily under zero-input system. The housing for keeping animals is separately constructed. In most cases it is covered with greens. Temporary field shelters are also found, which is open on all sides except for roof.

In the hilly region, buffaloes are largely let loose in the forests for grazing, and during cultivation season and for work animals travel to nearby villages on foot. The log pulling is the major work in hilly areas, these buffaloes can easily pull logs of wood from the jungles to bring it near the road sides; these buffaloes are efficient climbers and can walk in thick forest. Load carrying capacity is about 5 to 6 quintal and one adult male buffalo can pull around 20–25 feet logs easily.

This is the first report on cytogenetic status of Meghalaya state buffalo population. Distinctive karyotypic features established for swamp and riverine buffaloes were studied and exploited to ascertain whether buffaloes samples collected belong to the riverine or swamp category. Blood samples (40) of both sexes, collected from various locations of the Meghalaya state, were utilized for cytogenetic analysis. Samples of the animals exhibited a diploid count of $2N=48$ chromosomes (Fig. 1), which is typical of swamp type buffaloes (Bongso *et al.* 1984, Sat 1987).

The karyotype comprised 23 pairs of autosomes and 1 pair of sex chromosomes, of the 23 pairs of autosomes, 5 were biarmed (metacentric/submetacentric) and 18 were of acrocentric. The size of the fourth pair of metacentric

chromosome in all the swamp buffaloes samples were typical of swamp type resulting from the tandem fusion of chromosomes 4 (4p) and 9 (Di Berardino and Iannuzzi 1981, Chowdhary *et al.* 1989) of riverine type. Cytogenetic analyses thus confirmed the swamp status of Meghalaya buffaloes of north-east India in some pockets. Chromosomal abnormalities like structural and numerical were not observed in karyotyping studies.

The samples collected from the location close to international border with Bangladesh were of riverine type and this could be due to abundance of buffaloes transported from West Bengal and other parts of the country for milk production and for marketing of spent animals for meat. During survey of the regions close to international boundary it was observed that farmers keep animals for short duration of 2–3 years for milk production and then sell them for meat.

Northeast is the region where admixture of both riverine and swamp along with hybrids are present. Mishra *et al.* (2010a) characterized buffalo samples collected from Manipur and found pure swamp type animal; phylogenetic analysis based on mt DNA D-loop sequences with published sequences clustered all Manipuri buffalo samples into one clade with Chinese buffalo which further supports the origin of swamp buffalo from Chinese domestic swamp buffalo. The Assamese buffalo cytogenetic characterisation revealed presence of both the pure riverine and hybrid buffalo but no swamp type (Mishra *et al.* 2010b). Since there is a severe decline in the swamp buffalo population, it needs immediate intervention for breeding and conservation program for this unique germplasm.

SUMMARY

The present study aimed at assessing the status of the Meghalaya buffalo population from North-East India employing cytogenetic studies. So far buffaloes of the north-eastern states of India have been studied except for Tripura and Meghalaya and are generally considered to be of swamp type based on their phenotypic resemblance to swamp type buffaloes. A pilot survey was conducted in the Indian State of Meghalaya for collection of morphometric data from adult animals. The Meghalaya buffaloes investigated cytogenetically possessed a somatic chromosome count of $2N=48$, identical to that of typical swamp buffalo, 23 pairs of autosomes and a pair of sex chromosomes. The distinctive karyotypic feature of swamp buffalo observed was due to the distinct size of the fourth pair of metacentric chromosome. No morphological or chromosomal abnormality was observed in any of the populations. This is the first confirmed documentation of existence of pure swamp type buffaloes in Meghalaya state of India.

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Fig. 1. Metaphase spread of male and female Meghalaya buffaloes. Swamp $2N=48$ chromosomes.

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