



Cytogenetic characterization of Sambalpuri and Manda buffaloes of Odisha

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Buffalo has a major place in Indian livestock as it contributes more than 49% to country's milk production as well as meat and also plays a major role in draft power. According to 19th Livestock Census, Indian buffalo population is estimated to be around 108.7 million; recording a growth of around 3.2% from the figures of previous census (<http://dahd.nic.in>). Water buffaloes are of two types, riverine and swamp type, differentiated based on chromosome number, as the riverine has 50 chromosomes and 48 in swamp type buffalo (Michelizzi *et al.* 2010). Riverine buffaloes reared mainly for milk, are spread all over the north, central and south India. Swamp buffaloes mainly found in north-east parts of the country are primarily used for draught purpose besides meat and to a lesser extent for milk (Mishra *et al.* 2010a, b). Among 13 registered buffalo breeds of India, Odisha with diverse buffalo populations, has two registered breeds- Kalahandi and Chilika (<http://dahd.nic.in/sites/default/files/Livestock%20%205.pdf>). The other populations yet to be characterized and documented include Sambalpuri, Manda, and Paralakhemundi. Among five major buffaloes, Sambalpuri and Manda are having unique phenotypes. The cytogenetic characteristics of Chilika, Paralakhemundi and Kalahandi buffaloes are reported to be riverine type (Mishra *et al.* 2009, Singh *et al.* 2017), whereas status of Sambalpuri and Manda buffaloes is not yet known. Therefore, this study was conducted to ascertain the status (riverine/swamp) of the Sambalpuri and Manda buffaloes using cytogenetics technique.

Blood samples (20 each) were collected from Sambalpuri (9 males and 11 female) (Fig. 1A, B) and Manda (10 males and 10 females) (Fig. 2A, B) buffaloes from the different

parts of their breeding tracts in Odisha into vacutainers containing sodium heparin. Samples were transported to the lab within 24 to 48 h under cold conditions before setting up the cultures for cytogenetic studies. The cultures were set up using RPMI 1640 culture medium described by Prakash *et al.* (2011) for lymphocyte culturing with slight modifications.

Mitosis was induced by the incorporation of 2% phytohemagglutinin (PHA) as mitogen, and the cultures were incubated at 37°C for 72 h. 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). Fixed lymphocyte cells from suspensions cultures were dropped onto clean glass slides from a certain height by micropipette or by dropper that will burst the cells and metaphase chromosome spread coming out onto the slide. After air drying, the cells were stained using 2% Giemsa stain and covered with a coverslip. The modifications from Prakash *et al.* (2011) were changing the incubation temperature from 37.5°C to 37°C and increasing incubation time from 15 to 20 min for RBC lysis and 2% phytohemagglutinin (PHA) was used as mitogen instead of pokeweed. The metaphase spreads were examined under 10× magnification and chromosome count recorded under 100× oil immersion and photographed. Screening of at least five spreads of each animal was attempted to confirm the results, taking chromosomal count and the structure. Each spread was analyzed critically to document chromosomal abnormality also.

The present study was carried out to ascertain the riverine/swamp status of two uncharacterized buffalo populations, viz. Sambalpuri and Manda. 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 riverine or swamp status of these two populations.

Cytogenetic screening of Sambalpuri and Manda buffaloes, revealed riverine status for both with a chromosome number $2n=50$ in both the populations

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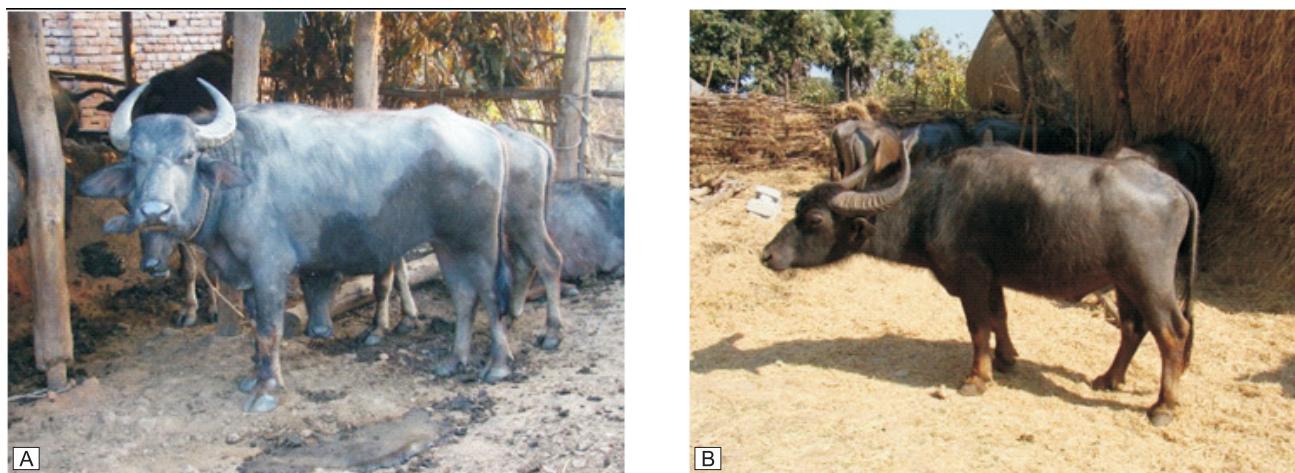


Fig. 1. Typical Sambalpuri male (A) and female (B) buffalo in the breeding tract.



Fig. 2. Typical Manda male (A) and female (B) buffalo in the breeding tract.

(Fig. 3 A, B; Fig. 4A, B). 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 cytogenetic constitution of animals of both the populations showed total 25 chromosome pairs, of which 24 were somatic and 1 pair of sex chromosomes XX or XY depending upon the sex of the animal. Both types of animals had 5 pairs of bi-armed or sub-metacentric and 19 pairs of acrocentric, including both X and Y sex chromosomes, typical for riverine buffalo (Berardino and Iannuzzi 1981, Bidhar *et al.* 1986, Iannuzzi 1994). The results showed that both the breeds have 48 somatic chromosomes and two sex chromosomes. The X chromosome was larger in size, whereas Y was the smallest chromosome, both the sex chromosomes being acrocentric. The results thus confirmed the riverine status of both Sambalpuri and Manda, since they displayed typical karyotype of the riverine buffalo.

This is the first report on cytogenetic status of both the buffalo populations of Odisha, i.e. Sambalpuri and Manda. The other populations include Chilika, Kalahandi and Paralakhemundi, among which Chilika had been reported as riverine type (Mishra *et al.* 2009). Bidhar *et al.* (1986)

reported Paralakhemundi as swamp type buffalo but recent reports have shown both Kalahandi and Paralakhemundi populations of Odisha, to be riverine type (Singh *et al.* 2017). The contrasting results could be due to difference in sample collection place and also possibility of shifting of population from swamp to riverine by mixing with other riverine buffaloes.

Chromosomal abnormalities like structural and numerical were not observed in karyotyping studies of both Sambalpuri and Manda buffaloes. These results were in agreement with the previously conducted experiments, in which number of chromosomes in riverine buffaloes of Brazil (Pires *et al.* 1998), Pakistan (Ali *et al.* 2012), Thailand (Kenthao *et al.* 2012), Turkey (Yavasoglu *et al.* 2014), Greek (Nicolae *et al.* 2015) and India (Agrawal 2005, Murali *et al.* 2009, Pandya *et al.* 2016, Singh *et al.* 2017) had been reported as 50.

The present study of cytogenetic characterization of both Sambalpuri and Manda buffaloes of Odisha thus revealed their riverine status, without any chromosomal abnormalities. This concludes the status of major Odisha buffaloes as riverine type similar to previous reports about Chilika, Paralakhemundi and Kalahandi (Mishra *et al.* 2009, Singh *et al.* 2017).

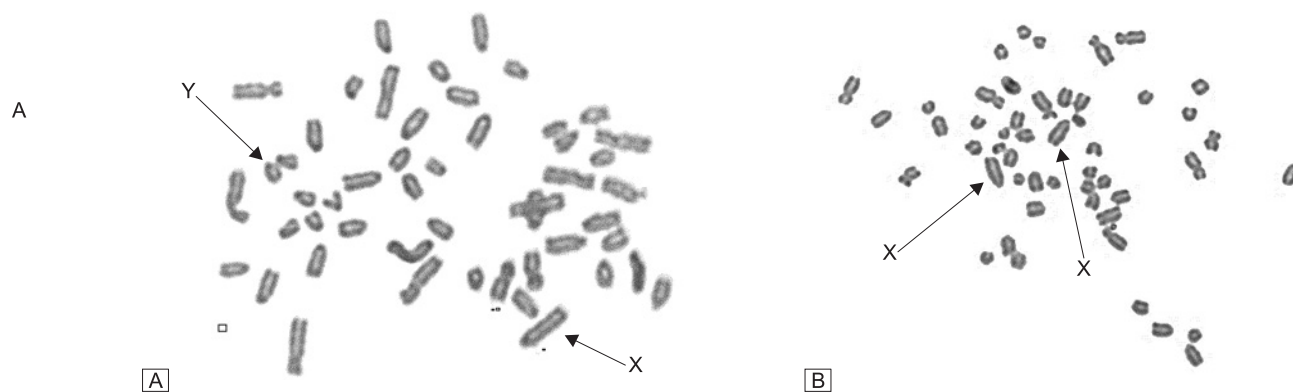


Fig. 3. Typical riverine metaphase spread of male (A) and female (B) Manda buffalo.

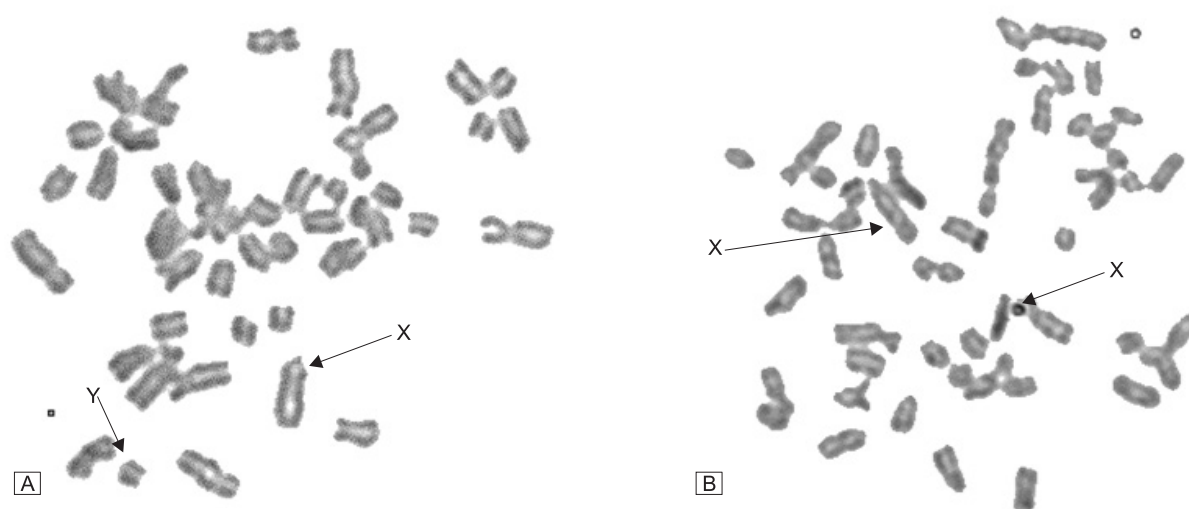


Fig. 4. Typical riverine metaphase spread of male (A) and female (B) Sambalpuri buffalo.

SUMMARY

Cytogenetics analysis even though mostly used for the evaluation of chromosomal abnormality by arresting the cell division at metaphase stage, but is also helpful to ascertain the riverine/swamp status of buffaloes. The study was conducted to characterize two buffalo populations, viz. Sambalpuri and Manda of Odisha cytogenetically, to establish their riverine or swamp status. Metaphase chromosome spreads of both, Sambalpuri and Manda buffaloes showed a chromosome number of $2n=50$. There were five pairs of metacentric or sub-metacentric and 19 pairs acrocentric along with a pair of sex chromosomes in both the populations, typical of riverine buffalo. No morphological or chromosomal abnormality was observed in any of the populations. This study thus confirms the riverine status of both the Odisha buffalo populations.

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