

## Recent Updates on Diversity of Buffalo (*Bubalus bubalis*) - A Review

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### Abstract

*Buffalo is an important livestock resource, playing a crucial part in the overall development of society in various South Asian and Mediterranean countries due to their contributions of milk, meat, skins, and draft power for agricultural activities. The buffalo species originated in India and is classified into river and swamp types. After domestication in the Indus valley, the river-type buffaloes spread west with greater phenotypic diversity, while the swamp-type buffaloes spread east with lesser phenotypic diversity. High-quality reference genomes and genotyping marker panels of buffalo have made numerous genome-based studies possible, providing important insights into the genetic basis of diversification. The present review highlights these high-throughput technologies linked to the diversity of buffaloes.*

**Keywords:** Buffalo, Diversity.

## Introduction

In India's rural sector, livestock is a vital source of income and plays important role in the Indian economy. Over the past few decades, the world buffalo population has rapidly increased and presently, it is approximately 208 million on the planet, with Asia accounting for 96.79 % of the population and the rest of the accounting for Africa (1.68 %), Americas (1.23 %) and Europe (0.22 %, FAOSTAT, 2019). India alone has a 109.85 million buffalo population with 19 registered breeds (Livestock Census, 2019).

Macgregor (1941) was the first to identify that domestic Asian water buffalo were of two types (swamp and river types) based on morphology and behaviour. Across southeast Asia, swamp buffaloes can be found in Bangladesh and Assam to the Yangtze River valley in China, whereas river buffaloes are indigenous to the Indian subcontinent and have expanded west to the Balkans, Greece, Egypt, and Italy. The swamp and river subspecies of buffalo have different chromosome numbers (Yilmaz *et al.*, 2012; 48 vs 50), morphology (body structure, body weight, horn shape, skin colour, etc.), and behaviour (i.e., mud vs. wallowing in water). Buffaloes are resistant to several tropical diseases and parasitic infestation, well-adapted to humid and harsh tropical climates, and can survive on poor quality feeds and fodders, but continued exposure to high temperatures and/or summer stress can cause dramatic changes in its biological functions that directly affect thermoregulation (Marai and Haebe, 2010).

The overall buffalo production and its efficient use calls for an extensive study worldwide on buffalo genetic resources to further develop and improve the available germplasm. Genetic characterization of buffaloes at a global scale is necessary for the development of a de novo reference assembly providing detailed annotations for the buffalo genome. Planning breeding strategies and executing genomic selection would require exploring genetics and an understanding of biology (Rehman *et al.*, 2021).

The present review highlights the molecular markers and candidate genes associated with diversity in buffalos.

## Evolutionary History, Domestication, Distribution, and Diversity of Buffalo Population

### *Evolution, Domestication, and Distribution*

Macgregor (1941) was the first to recognize that domestic Asian water buffalo were of two types, swamp and river, based on morphology and behaviour. They descended from the wild populations of the Asian water buffalo. Numerous studies have estimated this divergence time to be between 10 Kyr and 1.7 Myr BP (Tanaka *et al.*, 1996; 1997; Lau *et al.*, 1998; Kierstein *et al.*, 2004; Kumar *et al.*, 2007a; Lei *et al.*, 2007; Mishra *et al.*, 2015). Genes, mtDNA sequences, mutation rates, variations in the populations studied, and sample sizes likely contributed to this wide range. A recent estimate of the divergence time utilizing whole mitogenomes is between 900 and 860 Kyr BP (Wang *et al.*, 2017).

Molecular studies indicate that domestication happened from a swamp-like wild ancestor that was scattered across mainland Asia and has long since emerged into the existing population of *B. arnee* (Lei *et al.*, 2007; Colli *et al.*, 2018). It is believed that *B. arnee* originated in mainland south-east Asia and moved west to the Indian subcontinent, where river-type animals evolved. Meanwhile, swamp buffalo evolved from the 4p/9 chromosomal fusion in the population of mainland Southeast Asia (Lau *et al.*, 1998). Swamp buffalo were probably domesticated close to the border of China and Indochina between 3,000 and 7,000 years BP and eventually spread to Southeast Asia. Colli *et al.* (2018) provided evidence in favour of the dispersal patterns from the mainland south to Sumatra via Malaysia and the east to Nusa Tenggara, as well as to the Philippines by a northern route from China. Molecular studies reveal that swamp buffaloes lack gene flow, have considerable genetic divergence affected by geography and have a greater diversity of maternal and paternal lineages despite their strong phenotypic homogeneity (Sun *et al.*, 2020; Zhang *et al.*, 2020). Recently, a whole-genome analysis on buffalo revealed identical genetic patterns in cattle and buffalo, demonstrating convergent domestication resulting from the same mutation that occurred independently (Dutta *et al.*, 2020). Using phylogenetic analysis, two different lineages, A and B, could be recognized among Chinese swamp buffaloes. Lineage A was more common in all populations of these two lineages. South-western buffalo populations own the maximum genetic diversity for lineage A. It is hypothesized that South-western China is the most probable site for its domestication among the three postulated domestication centres (South-eastern, Central, and South-western China). Conversely, for lineage B, an intricate pattern of diversity is identified, which averts the clear-cut

location for its domestication centre (Dutta *et al.*, 2020).

Somewhere around 6,300 years BP, river buffaloes were domesticated in north-western India. From there, they moved west, passing through south-western Asia, Egypt and Anatolia before arriving in the Italian peninsula and the Balkans (Kumar *et al.*, 2007b). Populations of river buffalo exhibit greater phenotypic diversity and a weaker phylogeographic structure than swamp buffalo, resulting in more breeds (Zhang *et al.*, 2020). Later, in the eighth to fifteenth centuries, the water buffalo was introduced in Egypt, Italy, the Balkans, and Turkey.

## Diversity

### Genomics Level

Swamp and river buffaloes are distinctly different morphologically and cytogenetically. Based on morphology, swamp buffaloes are typically dark grey with white stripes on the throat, socks, and tip of the tail, with relatively straight pale-coloured horns, while river buffaloes, exception of native buffalo of Sri Lanka, are generally black with curved dark grey or black colour horns. Cytogenetically, they have different number of chromosomes (Fischer and Ulbrich, 1968; Yilmaz *et al.*, 2012), protein-coding loci i.e. allozymes (Amano *et al.*, 1980), autosomal DNA markers i.e. microsatellites (Barker *et al.*, 1997; Yindee *et al.*, 2010), mitochondrial (mt) DNA sequences (Zhang *et al.*, 2016) and single nucleotide polymorphism (SNP) markers (Colli *et al.*, 2018). The fusion of chromosomes 4p and 9 results in a different chromosome number.

### Microsatellite Markers

Data from microsatellite markers revealed a consistent relationship between the geographical distribution and historical spread of south-east Asian swamp buffalo populations (Barker *et al.*, 1997). A combined study of 26 Asian populations of swamp buffalo revealed that Thai buffaloes had the highest genetic variability and that there was significantly less genetic variation in Chinese populations than in those from southeast Asia (Zhang *et al.*, 2011; HO = 0.573). Model-based clustering of SNP marker or microsatellite data using the programs STRUCTURE (Pritchard *et al.*, 2000) or ADMIXTURE (Alexander *et al.*, 2009) split the Dehong buffalo into pure river-type, swamp-type and crossbred from far-south-west China, Thai populations, Indonesian and Philippino swamp populations (Zhang *et al.*, 2007, 2011; Colli *et al.*, 2018).

The genetic diversity of the local population of river buffalo has been described using highly polymorphic microsatellite loci (Zhang *et al.*, 2011; Saif *et al.*, 2012; Mishra *et al.*, 2015). Murrah, Nili-Ravi, and Kundi groups of Indian breeds show the highest diversity in values of expected heterozygosity (HE = 0.71–0.78, Kumar *et al.*, 2007b; Vijn *et al.*, 2008). However, the Mediterranean (Elbeltagy *et al.*, 2008; Saif *et al.*, 2012) and Italian populations (HE = 0.58–0.68) exhibit a gradual decrease in heterozygosity. In silico mining for microsatellite loci has been made easier by the availability of water buffalo WGS data on the BuffSatDb Buffalo MicroSatellite Database (<http://webapp.cabgrid.res.in/buffsatdb/>), a web-based searchable STR database which has found thousands of genome-wide markers (Sarika *et al.*, 2013).

### Mitochondrial DNA and Y-chromosome Variations

Riverine and swamp buffalo have independent maternal origins is supported by various studies based on mitochondrial D-loop sequence (~910 bp) and Cyt b (Tanaka *et al.*, 1996; Lau *et al.*, 1998; Kumar *et al.*, 2007a; Lei *et al.*, 2007). Zhang *et al.* (2016) analyzed the complete mitochondrial D-loop and Cyt b sequences from 913 swamp buffaloes and reported five haplogroups (A1, A2, B1, B2, B3) composed of three that were rare and highly divergent (C, D, E). In another study, two novel haplogroups, SA3 and SB4, were also discovered using the mitochondrial genomes of 107 swamp buffalo (Wang *et al.*, 2017). Remarkably, the 16 kb mitogenome sequences allowed the refinement of the swamp buffalo mtDNA subhaplogroups for SA1 (SA1a, SA1a1, SA1a2, SA1a3), SB1 (SB1a, SB1a1, SB1a2, SB1b), SB2 (SB2a, SB2b), SB3 (SB3a, SB3a1) and SD (SD1, SD2), and the reconstruction of the demographic history of swamp buffalo matrilineal lineages. The mtDNA pattern of swamp buffaloes from China, Indochina and Bangladesh on phylogeographic analysis revealed that the maximum diversity was found in south China and north Indochina on both banks of the Mekong River (Zhang *et al.*, 2016).

As compared to swamp buffalo, only three major categories of haplogroups (R1, R2, R3) have been reported in mitochondrial DNA D-loop variation of riverine buffalo. These haplogroups were distributed throughout the region

of Asia and the Mediterranean (Kumar *et al.*, 2007a; Zhang *et al.*, 2016). Among three haplogroups, R1 founds the major and greatest frequent lineage (75.4%), followed by R2 which was less frequent (116.4%) and R3 which was rare (8.2%, Zhang *et al.*, 2016). Maximum and minimum values of haplotype diversity was recorded in the population of India and the Mediterranean, respectively (Nagarajan *et al.*, 2015). The evidence from D-loop sequences of swamp and river buffaloes from a wide geographic area revealed that the former had a stronger phylogeographic structure and a lower haplotype diversity than the latter (Zhang *et al.*, 2016). The absence of geographic distribution of diversity was also established which was already reported locally (in Pakistan, India, Egypt and the Mediterranean; Hassan *et al.*, 2009; Babar *et al.*, 2012; Mishra *et al.*, 2015), and added that there were no significant population bottlenecks during the post-domestication migration from India to western Asia and Europe (Zhang *et al.*, 2016).

The origin of river and swamp buffalo from distinct wild populations is demonstrated by the clear divergence seen from sequencing of Y-chromosome portions of the DBY, ZFY and SRY gene segments in the water buffalo (Yindee *et al.*, 2010).

## Conclusion

In addition to their socio-cultural significance, domesticated water buffaloes are an efficient source of nutrient-rich products, particularly milk and meat, and they can survive exceptionally well on limited resources in harsh environmental conditions. The present-day domesticated water buffalo (*Bubalus bubalis*) originated in the northeastern region of India from the wild buffalo (*Bos arnee*), but presently, they have spread across 67 countries on the planet.

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## Contribution by Authors

All authors contributed to the conception and design of the study. Material preparation, data collection and analysis were performed by Asit Jain, Akansha Singh and Tripti Jain. The first draft of the manuscript was written by Asit Jain and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

## Conflict of Interests

There is no conflict of interest.

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