SHORT COMMUNICATION

The Journal of Animal & Plant Sciences, 25(3): 2015, Page: 892-895
ISSN: 1018-7081

COMPARATIVE ANALYSIS OF HIGHLY CONSERVED *ZFX* GENE SEQUENCES OF PAKISTANI RIVER BUFFALO (*BUBALUS BUBALIS*) WITH OTHER BOVIDS

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ABSTRACT

ZFX gene which encodes Zinc Finger Protein of X chromosome and homologous to ZFY gene of Y chromosome, is considered to be a highly conserved gene in vertebrates and has a great potential in assessing the genetic diversity and differentiation among different species and within their respective breeds. In this pioneering study on buffalo, we investigated the genetic variation in *ZFX* gene exon 5 in Nili-Ravi and Kundi buffalo breeds of Pakistan and made comparison with other bovid. We sequenced this gene in 50 animals (25 of each breed). The sequence alignment revealed that 153 bp long nucleotide sequence of buffalo's *ZFX* exon 5 gene was homogenous to that of *Bos taurus and Ovis aries* with 99% homology. Nucleotide variation was observed at position 77 and 131 in *Ovis aries* and *Bos taurus* respectively. Both the SNPs were found to be synonymous. Phylogenetic analysis was also carried out using MEGA 6.1 with *Bos taurus* and *Ovis aries* along with other reported species. We conclude that the *ZFX* gene is highly conserved in bovine species. Moreover, *ZFX* can be very helpful to explicit the relatedness between different buffalo breeds and other bovids.

Keywords: *ZFX*, Pakistani buffalo, Sequence homology, Phylogeny.

INTRODUCTION

Previous understanding of ZFY gene role as Testes Determining Factor (TDF) has now been strengthened by many experimentations. Evidential illustration of functionality of this gene has depicted the conserved nature of gene across different species. Zinc-finger genes comprise of 13 members that function as transcription activators (Eizirik *et al*. 2001). Two well-studied members of this family are *ZFX* and ZFY. In many species of Mus, there are two copies of Y chromosome: ZFY1 and ZFY2, resulting from a recent intra-chromosomal gene duplication event, as well as an autosomal copy: ZFa, a pseudogene located on MMU10, resulting from a recent transposition (via RNA) of a processed ZFX transcript (Page *et al*. 1987; Mardon and Page 1989; Ashworth, Swift, and Affara 1989; Nagamine *et al*. 1989; Mitchell *et al*. 1989; Mardon *et al*. 1989; Page *et al*. 1990; Mardon *et al*. 1990). Present study was designed to determine the evolution pattern of *ZFX* gene in different bovine species. This novel report about *ZFX* genetic diversity is the first step to disclose the Phylogenetic aspect of this gene in different bovine species. In this study, we investigated the genetic variation in *ZFX* gene locus exon 5 between the two buffalo breeds of Pakistan: Nili-Ravi and Kundi and made comparison with other bovids.

MATERIALS AND METHODS

Sampling: Taxonomic source for this project was two indigenous buffalo breeds (Nili-Ravi and Kundi). A total of 50 animals (25 animals from each breed) for two breeds of river buffalo were selected for this study. 10 mL of blood was collected in sterile EDTA coated vacutainers and kept in cold storage at the Molecular Biology and Genomic Laboratory of the University of Veterinary and Animal Sciences, Lahore, Pakistan.

Genomic DNA Extraction: DNA was isolated by using inorganic method of DNA extraction reported by Maryam *et al*. (2012). DNA was quantified by using Agarose gel and NanoDrop and all samples were brought to same concentration of 50ng/uL.

PCR Amplification of Exon-5 of the *ZFX* gene: Polymerase Chain Reaction was used for the amplification of targeted region of exon 5 of the *ZFX* gene. For this purpose specific primers were used in pair. Sequencing of amplified region was performed bi-directionally by Sanger's chain termination method using Big Dye terminator kit from Life Technologies.

Alignment and Phylogenetic Analysis: Sequenced samples were compared with published sequences of *Bos taurus* and *Ovis aries* by using Codon Code Aligner (www.codoncode.com/aligner/). Phylogenetic analysis among these species was also carried out by using MEGA6 (www.megasoftware.net/). Neighbour-Joining

tree was constructed by including mammalian species with 1000 bootstrap value (Fig 1). Pair-wise distance matrix between DNA sequences was constructed using "BH87" model (Barry and Hartigan., 1987) in Bioconductor (Fig 2).

RESULTS AND DISCUSSION

The BLAST alignment results revealed that 153 bp long nucleotide sequence of buffalo's *ZFX* exon 5 gene was homogenous to that of *Bos taurus* (NC007331) *and Ovis aries ZFX* exon 5 genes (NC019484) with a percentage similarity of 99%. Nucleotide variation was observed at 131 bp and 77 bp position in *Bos taurus* and *Ovis aries*, respectively. Both the SNPs were found to be synonymous. On the basis of these sequences, Neighbour-Joining tree (Fig 1) was constructed where a strict consensus pattern was observed for members of bovid (Tibetan antelope XM005972902, Goat XM005700995, Wild yak XM005904032, Minke whale XM007185147, Sperm whale XM007118657, Bottlenosed dolphin XM004322112, Killer whale XM004283088, Fresh water dolphin XM007463969, Armadillo XM004463759, Domestic cat EU879984, Pacific walrus XM004416055, Crab-eating macaque XM005593187, White-tufted-ear marmoset XM002762731, Olive baboon XM003917511, Horse XM003365768, White rhinoceros XM004435207, Florida manatee XM004382859, Light brown bat XM006085638, Pig XM003134981, Bactrian Camel XM006193214, Alpaca XM006213010, and Korean hare EF110884) as they were sharing same cluster with considerable boot strap score for each divergence. While Pair-wise distance matrix (Fig 2) indicated that genetic distance between sequences of different species indicating how distantly related they are from each other.

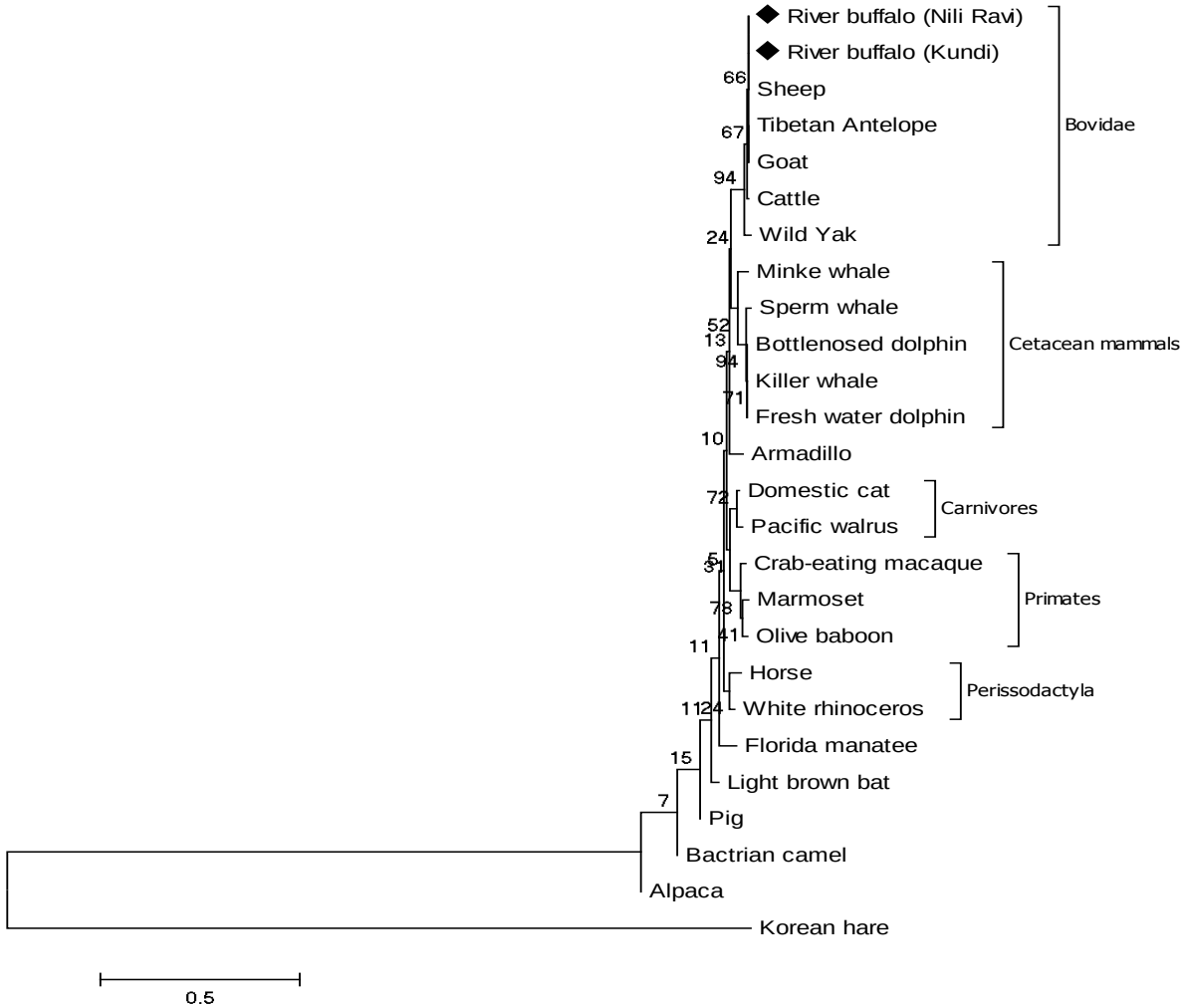

Fig-1: Neighbour-Joining Tree for *ZFX* gene in Mammalian Species using MEGA 6.1 software package with 1000 boot strap values

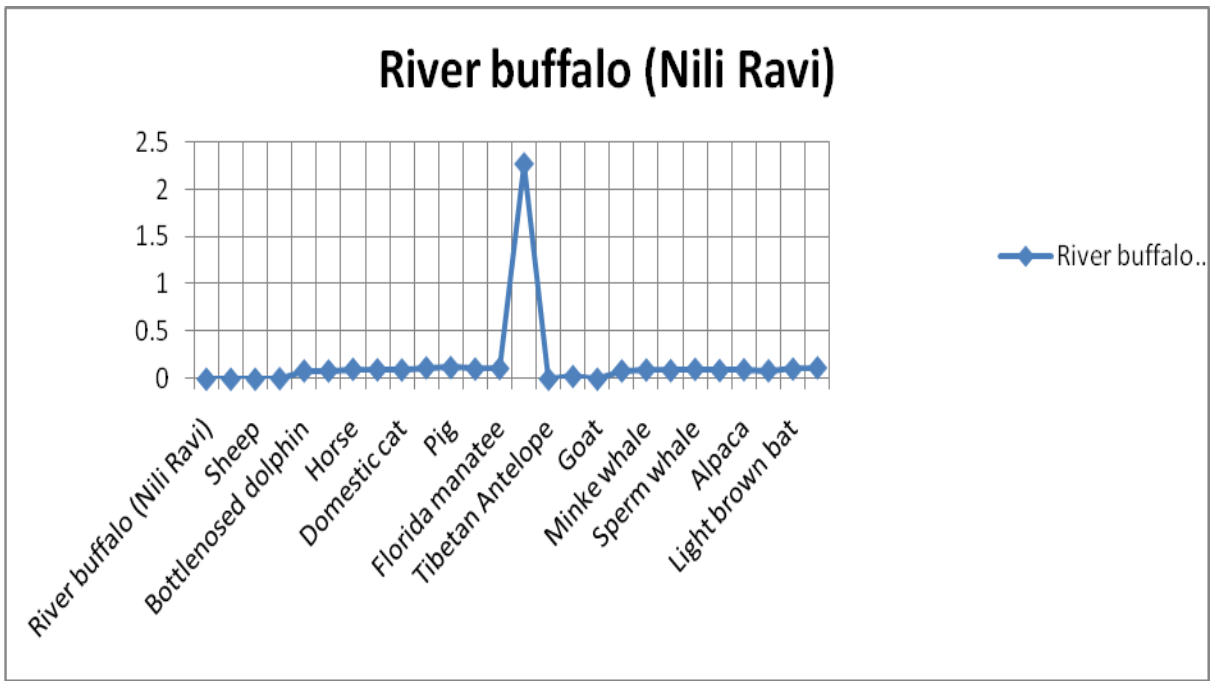

Fig-2: Pair-Wise Genetic Distance Matrix for *ZFX* gene exon 5. Evolutionary distance was calculated between selected buffalo breeds and other mammalian species by making pairwise comparisons among them. Species were plotted around horizontal while phylogenetic distance they achieved with the passage of time was placed as "Evolutionary Distance" along vertical axis. The graph shows the distance pattern for river buffalo with respect to other selected mammalian species.

Conclusions: The existence of bovid in same cluster supported the idea of strict consensus in the structural attributes of *ZFX* gene in bovine group. This pattern is comparable to another Y-chromosome–linked functional gene, the male sex– determining locus, SRY (Tucker and Lundrigan 1993; Whitfield, Lovell-Badge, and Good fellow 1993; Pamilo and O'Neill 1997; Wang, Zhang, and Zhang 2002, Hussain *et al*. 2013). We conclude that the *ZFX* gene exon 5 is highly conserved in bovine species. Moreover, *ZFX* can be very helpful to explicit the relatedness between different buffalo breeds and other bovids as the *Bubalus bubalis* genome shares a common parentage with *Ovis aries* after the separation of subfamily Bovinae from the Bovidae family. The phylogenetic tree reconfirmed the biological classification of different mammalian species.

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