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GENOME-WIDE *IN SILICO* ANALYSIS AND IDENTIFICATION OF *BOS TAURUS SOX* GENE FAMILY

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Abstract: The *SOX* (SRY-box transcription factors) gene family is defined by the presence of the HMG-box (High-mobility group) domain, which was first discovered in the SRY (sex-determining region on the Y chromosome) gene located on the Y chromosome, and plays a vital role in the initiation of male sex determination. The purpose of this study was to genome-wide identify and characterize *SOX* gene family members in silico in *Bos taurus*. A total of 15 *SOX* proteins were identified in the bovine genome. These proteins weigh between 26.49 (Bt-*SOX*-14) and 93.35 (Bt-*SOX*-6) kDa and have theoretical isoelectric points ranging from 4.95-9.85. As a result of phylogenetic analyses, Bt-*SOX* proteins and *SOX* proteins of *Mus musculus* and *Homo sapiens* species were clustered in 7 main groups. Segmental duplication was also detected between Bt-*SOX*-14/Bt-*SOX*-21, Bt-*SOX*-5/Bt-*SOX*-6, Bt-*SOX*-9/Bt-*SOX*-10 and Bt-*SOX*-4/Bt-*SOX*-11 gene pairs. On the other hand, according to in silico gene expression, Bt-*SOX* genes exhibited different expression profiles in different tissues and were found to have the highest expression in brain and testicular tissues. This indicates that the majority of Bt-*SOX* genes have tissue-specific expression. The findings of this research offer a valuable resource for gaining a deeper insight into the molecular foundation of the *SOX* gene family in bovine.

Keywords: *Bos taurus*, *SOX* gene, in silico, Bovine genome, Evolution, SRY specific

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1. Introduction

Farm animals contribute substantially to human nutrition, and the by-products derived from livestock serve as essential inputs in sectors such as pharmaceuticals, cosmetics, and various industrial processes (Rexroad et al., 2019).

The world population will reach approximately 10 billion by the year 2050 according to the estimations of the United Nations (UN) (Anonymous, 2025). In line with this increase, a growing demand for food -particularly animal-derived products- is anticipated. Meeting this demand should not rely solely on increasing livestock numbers, but rather on improving our understanding of animal biology and genetics and enhancing productivity per animal through advanced biotechnological methods. In this context, the reduction of antibiotic use will be necessary under consumer expectations and regulatory frameworks, which may, in turn, lead to heightened risks of life-threatening diseases associated with rising antimicrobial resistance. Considering all these factors, productivity in animal husbandry can be improved through the implementation of advanced production systems and precision livestock management practices (Fu et al., 2024; Rexroad et al., 2019).

The understanding of the genes and molecular mechanisms underlying biological processes is made possible through functional genetics. The *SOX* gene family refers to a group of transcription factors found in metazoans that bind to the minor groove of DNA rather than the major. Proteins encoded by the *SOX* gene family contribute to chromatin condensation and the regulation of multiprotein complex expression. Discovered in 1990, this gene family comprises approximately 40 *SOX* genes grouped into nine subfamilies across various species. From a reproductive perspective, *SOX* proteins play critical roles in the establishment of sexual characteristics, spermatogenesis, and gonadal development (Abdullah et al., 2023; Fu et al., 2024).

Numerous genome-based studies have been conducted on the characterization of the *SOX* gene family in various species; however, research specific to cattle remains limited. This *in silico* study aims to elucidate the evolutionary significance and potential mutations within the bovine *SOX* gene family (Abdullah et al., 2023; Akinyemi et al., 2022).

2. Materials and Methods

2.1. Retrieval of Genome Sequences

The accession numbers (NP_001071596.1, NP_001076940.1, NP_001178347.1, XP_024851815.1, XP_002698019.3, XP_024836864.1, NP_001180176.1, XP_024855327.1, XP_010809935.2, NP_001157253.1,

NP_001179004.1, NP_001193180.1, NP_001069257.1, XP_024855919.1, NP_001039894.1) of sequences of the *SOX* gene family member for representative species were provided from NCBI (https: //www.ncbi.nlm.nih.gov/genome) and Ensemble genomes (http://ensemblgenomes.org/).

In this study, comparative studies were conducted using the genetic sequences of *Bos taurus* and other species, such as *Mus musculus* and *Homo sapiens*. To determine *SOX* genes from the genomic sequences, three methods were used. First, putative *SOX* homologous proteins were queried using the BLASTp tool with an e-value of cutoff_ 1e-5, utilizing the disclosed *SOX* protein sequences of *Bos taurus*. The local BLASTp tool was then used with an e-value threshold of 1e-4 to scan the local database against the conserved HMG-box domain (Pfam ID: PF00505) of 79 amino acids. Sequences that weren't relevant were manually removed. Lastly, the candidate sequences containing HMG-box domains were examined using the Pfam database (InterPro ID: IPR009071). The required data of amino acid numbers, chromosome locations and coding sequence (CDS) lengths were derived from Ensemblgenomes. For each *SOX* protein, the theoretical molecular weight and isoelectric points were calculated utilizing the pI/MW tool (http://www.expasy.org/tools/). The PROTPARAM tool (http://web.expasy.org/protparam/) was used to evaluate grand average of hydropathy (GRAVY) values. The MapChart tool was utilized to map the Bt-*SOX* genes (Voorrips, 2002).

The Multiple Expectation Maximization for Motif Elucidation (MEME) online tool was used to identify the conserved motifs in *Bos taurus SOX* protein sequences (Bailey et al., 2006). The maximum number of motifs was limited to 15, and the minimum/maximum width was 2 and 50, respectively. Motif sites were between 2 and 300. Site distribution was set as any number of repetitions. InterProscan was used to examine the described conserved motifs with default adjustment (Quevillon et al., 2005).

The Online Gene Structure Display Server program tool (GSDS) was used to make predictions for exon/intron organization of the *Bos taurus SOX* genes (Guo et al., 2007). Genomic DNA sequences and coding sequences of Bt-*SOX* genes were utilized.

The sequence logo of the HMG-box domains was constructed using the WEBLOGO online web tool (Crooks et al., 2004).

The parameters for gene duplication events among all putative Bt-*SOX* genes were as follows: the alignment of the coding nucleotide sequences covered 50 % of the longest genes, and the amino acid identity between the sequences was 50 % (Yang, 2008).

2.2. Phylogenetic Analysis

ClustalW was used to determine multiple sequence alignment of Bt-*SOX* proteins. MEGA v7 and Neighbor-joining (NJ) algorithm with 1000 replicated-bootstrap values were utilized to perform phylogenetic analysis (Tamura et al., 2013). The iTOL v4.2.3 was used to visualize the constructed files.

2.3. Gene expression analysis in silico

The European Bioinformatics Institute-Expression Atlas was utilized to retrieve the expression levels of Bt-*SOX* genes that were investigated in specific tissue libraries of plants at various stages of growth, including liver, colon, spleen, kidney, lung, heart, skeletal muscle tissue, brain, and testis (https://www.ebi.ac.uk/gxa/home). Transcripts Per Kilobase Million (TPM) units were used for the expression levels in silico. Log2 transformations were applied to TPM values, and the algorithm CIMMiner was used to produce the heatmap (http://discover.nci.nih.gov/cimminer).

3. Results

3.1. Definition of *SOX* Gene Family in *Bos Taurus* Genome

Protein sequences containing the HMG-box were identified using Pfam accession number. *SOX* gene family members obtained from *Bos taurus* genome are represented in Table 1. Molecular weights, amino acid numbers, theoretical isoelectric points, chromosomal locations and NCBI accession numbers of these genes are also given. Bt-*SOX* genes were distributed on chromosomes 1, 5, 7, 8, 11, 12, 13, 14, 15, 19, 23 and 25 of *Bos taurus*. The highest number of genes was obtained with 2 Bt-*SOX* genes on chromosomes 5, 13 and 19 (Figure 3).

Bt-*SOX* proteins were determined to have an amino acid range of 233-841. The Bt-*SOX*-6 gene had the longest amino acid sequence with 841, while Bt-*SOX*-15 had the shortest with 233. These proteins weighed between 26.49 (Bt-*SOX*-14) and 93.35 (Bt-*SOX*-6) kDa. The isoelectric point ranged from 4.95-9.85, varying from acidic to alkaline. The highest value was obtained from Bt-*SOX*-15, while the lowest value was detected in Bt-*SOX*-11. According to the analysis, Bt-*SOX*-14/Bt-*SOX*-21, Bt-*SOX*-5/Bt-*SOX*-6, Bt-*SOX*-9/Bt-*SOX*-10 and Bt-*SOX*-4/Bt-*SOX*-11 gene pairs were identified as segmentally duplicated genes in the evolutionary process.

3.1. Phylogenetic Analysis, Conserved Motifs, Gene Structure Of *SOX* Genes

To determine the relationship between Bt-*SOX* proteins, a phylogenetic tree was constructed with *SOX* genes of *Bos taurus*, *M. musculus* and *H. sapiens* species. The phylogenetic tree was drawn according to the Neighbor-joining method with 1000 replicate bootstrap values based on the amino acid sequence of *SOX* proteins (Figure 1). *SOX* proteins were grouped into 7 different groups as B2, C, D, E, F, G and H. The highest content of *SOX* protein determined in groups C, E and F and the lowest in groups G and H.

Table 1. The physicochemical properties of *SOX* genes in *Bos taurus*

Gene ID	GenBank accession number	Size (aa)	MW (kDa)	pI	Chromosome location	II	classifies	GRAVY
Bt-*SOX*-4	NP_001071596.1	481	47,79	7.2	23:36,520,669-36,522,538	58.41	unstable	-0.478
Bt-*SOX*-5	NP_001076940.1	728	80,13	6.13	5:86,028,631-86,627,007	63.71	unstable	-0.746
Bt-*SOX*-6	NP_001178347.1	841	93,35	7.98	15:35,833,980-36,542,301	61.60	unstable	-0.839
Bt-*SOX*-7	XP_024851815.1	387	41,82	6.23	8:8,558,547-8,565,452	58.24	unstable	-0.671
Bt-*SOX*-8	XP_002698019.3	534	56,24	7.77	25:788,526-793,121	59.49	unstable	-0.708
Bt-*SOX*-9	XP_024836864.1	524	57,17	6.31	19:58,919,579-58,923,174	80.78	unstable	-0.984
Bt-*SOX*-10	NP_001180176.1	469	50,02	6.19	5:109,757,715-109,768,623	58.52	unstable	-0.822
Bt-*SOX*-11	XP_024855327.1	451	47,15	4.95	11:90,953,818-90,955,173	65.27	unstable	-0.652
Bt-*SOX*-12	XP_010809935.2	314	34,00	5.14	13:60,693,347-60,694,291	67.74	unstable	-0.982
Bt-*SOX*-14	NP_001157253.1	240	26,49	9.68	1:131,371,915-131,372,637	53.51	unstable	-0.585
Bt-*SOX*-15	NP_001179004.1	233	25,14	9.85	19:27,319,235-27,321,042	69.49	unstable	-0.875
Bt-*SOX*-17	NP_001193180.1	410	43,06	5.91	14:22,229,596-22,232,109	65.47	unstable	-0.509
Bt-*SOX*-18	NP_001069257.1	389	41,34	8.42	13:53,823,431-53,825,284	78.07	unstable	-0.573
Bt-*SOX*-21	XP_024855919.1	277	28,67	9.74	12:69,202,956-69,203,789	58.51	unstable	-0.208
Bt-*SOX*-30	NP_001039894.1	766	83,74	8.81	7: 69,255,959-69,297,638	68.51	unstable	-0.607

aa= amino acids, MW= molecular weight, pI= isoelectric point, II= instability index, GRAVY= grand average of hydropathicity index.

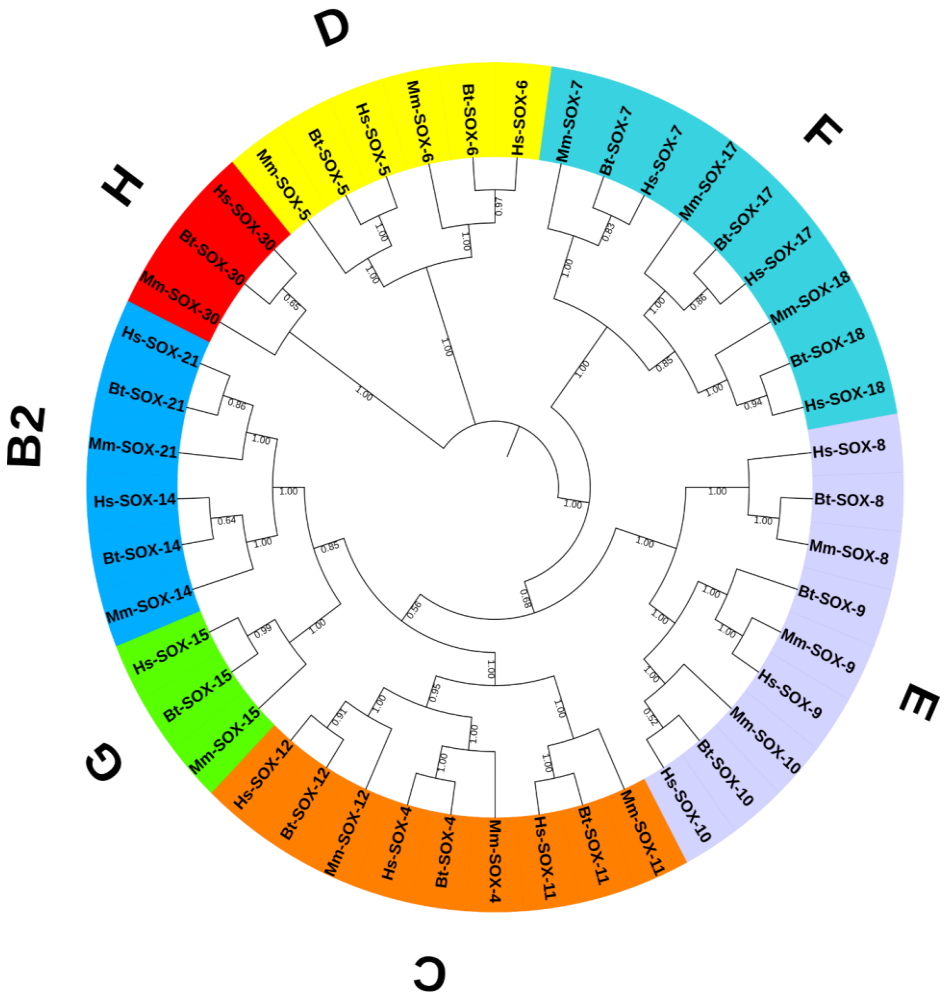

Figure 1. Phylogenetic tree of *SOX* gene family in *Bos taurus* and related species (*Mus musculus* and *Homo sapiens*) constructed using neighbor joining method with a bootstrap value of 1000.

The structural features of Bt-*SOX* genes, including gene structure, were analyzed. The number of introns varied between *SOX* genes; the highest length of introns was found in *SOX*-5 while *SOX*-4 gene had the shortest. Furthermore, *SOX*-11, *SOX*-12, *SOX*-14 and *SOX*-21 had no introns at all and, *SOX*-8, *SOX*-9 and *SOX*-30 contain two or more introns (Figure 2). We can speculate that intronless structures in genes are supposed to come from prokaryotic cell genome origin (Ilhan et al., 2018).

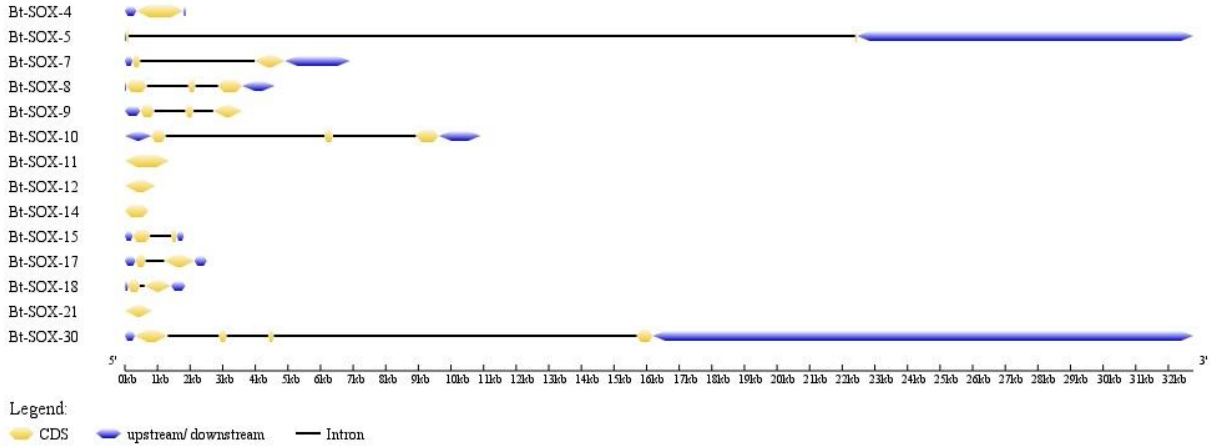

Figure 2. Gene structures of Bt-*SOX* gene family members. Yellow colors, grey lines and, blue colors represent exons, introns and, represent untranslated regions (UTRs), respectively.

A total of 15 conserved MOTIFs were identified from Bt-*SOX*, of which MOTIF-1 and MOTIF-10 had the highest number of amino acids. Besides, MOTIF-1 is explained as an HMG domain after the Pfam search (Table 2, Figure 5). A total of 15 preserved motifs were determined in Bt-*SOX* genes using MEME (Bailey et al., 2006). The highest number of motifs was obtained in Bt-*SOX*-5 and -6, while the lowest number of motifs was obtained in Bt-*SOX*-15. All Bt-*SOX* genes were determined to include MOTIF-1 and -2. Figure 6 represents the sequence logo, which displays the relative frequencies of each conserved domain together with their respective places. Bt-*SOX* protein sequence logo plots illustrating the most conserved domain and amino acid locations. WEBLOGO is used to represent 13 multiple sequence alignments in total.

The expression levels of 10 Bt-*SOX* genes in various tissues (liver, colon, spleen, kidney, lung, heart, skeletal muscle tissue, brain and testis) of *Bos taurus* were performed (Figure 4). The level of expression exhibited that tissue and expression patterns differed among each *SOX* gene. The liver, colon and spleen showed the lowest expressions for all genes whereas the testis and brain relatively exhibited the highest. Bt-*SOX*-5, -6, -9 and -12 genes were expressed highest in testis tissue and Bt-*SOX*-5, -8, -9, -10 and -12 genes were mostly expressed in brain tissue. The expression level of Bt-*SOX*-17 and -18 genes were highest in lung tissue.

Table 2. Differentially conserved motifs in *Bos taurus SOX* gene family

ALT ID	Width	Best possible match	Domain
MOTIF-1	50	IKRPMNAFMVWAKIERRKIMQQNPDMHNAEISKMLGKRWKLLTESEKRPF	High mobility group box
MOTIF-2	29	IEEAERLRVQHMKDYPDYKYRPRRKKKCK	N/A
MOTIF-3	36	IDFGNVDIGEISHEVISNMETFDVHEFDQYLPPNGH	N/A
MOTIF-4	33	SHFEFPDYCTPEVSEMIAGDWLESNISDLVFTY	N/A
MOTIF-5	32	DDKFPVCIREAVSQVLKGYDWTLVPMPVRVNG	N/A
MOTIF-6	29	GEIKKIMESQIEKMRQFMNMINQQQIQIE	N/A
MOTIF-7	41	RQQQQLLQQQHKINLLQQQIQVQGQMPPLMIPIFPHDQRTL	N/A
MOTIF-8	47	HTHTHPSPGNPGYMIPCNCTAWPAPGLQPPVAYILFPGMGKTGIDPY	N/A
MOTIF-9	12	HWEQPVYTTLTR	N/A
MOTIF-10	50	VTFGTPERRKGSLADVVDTLKQKKMEEMIKNEQEDTPCIEKLLSKDWKDK	N/A
MOTIF-11	17	ELWGDVDRTEFDQYLNC	N/A
MOTIF-12	8	WCKTPSGH	-
MOTIF-13	25	HSGQSHGPPTPPTTPKTDLQHGKAD	N/A
MOTIF-14	43	NNMGLNNCRNEKEKTRFENLGQQLTVKQNEDGKFGHGMIDFNM	N/A
MOTIF-15	38	QIAYTDFSLPHYGGPFPGITRGQYDYNDFQNSGRYYAH	N/A

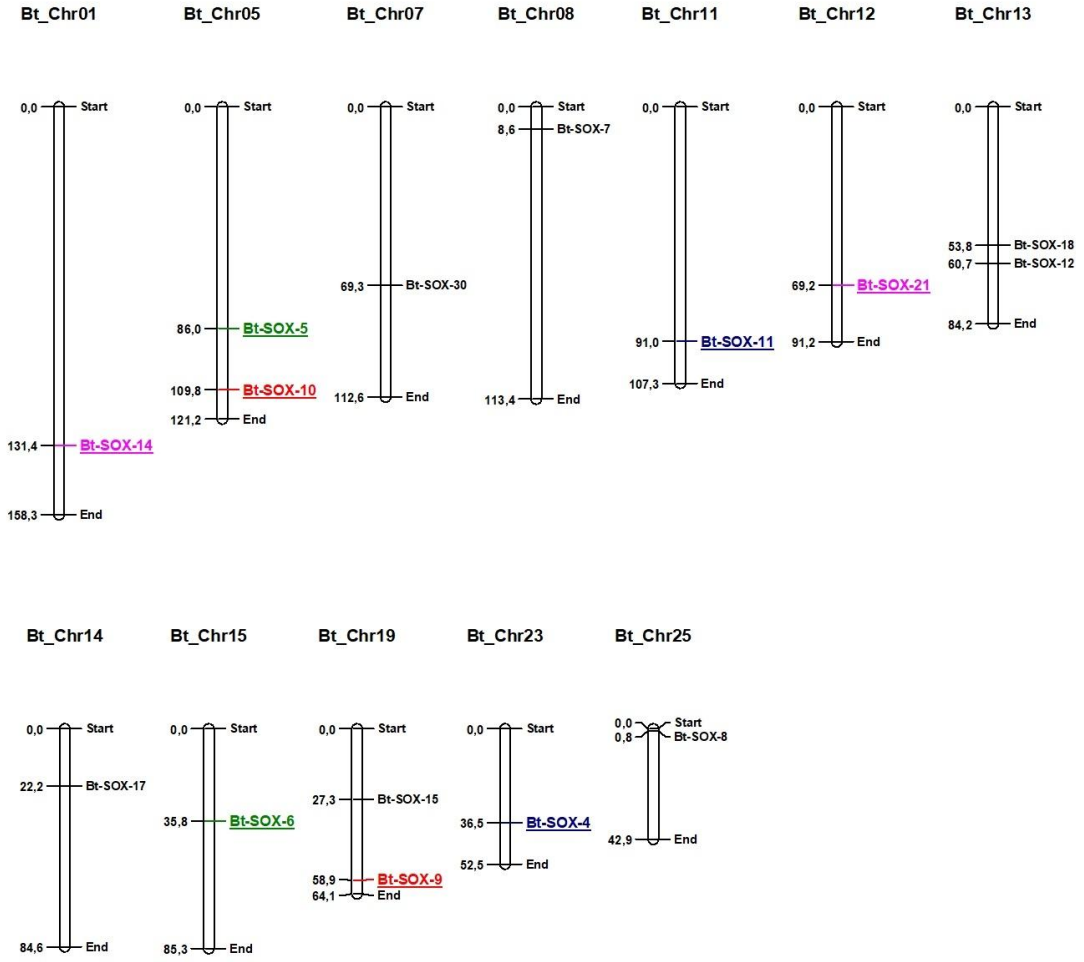

Figure 3. Distribution of *SOX* genes on *Bos taurus* chromosomes. Similar colors represent segmentally duplicated genes.

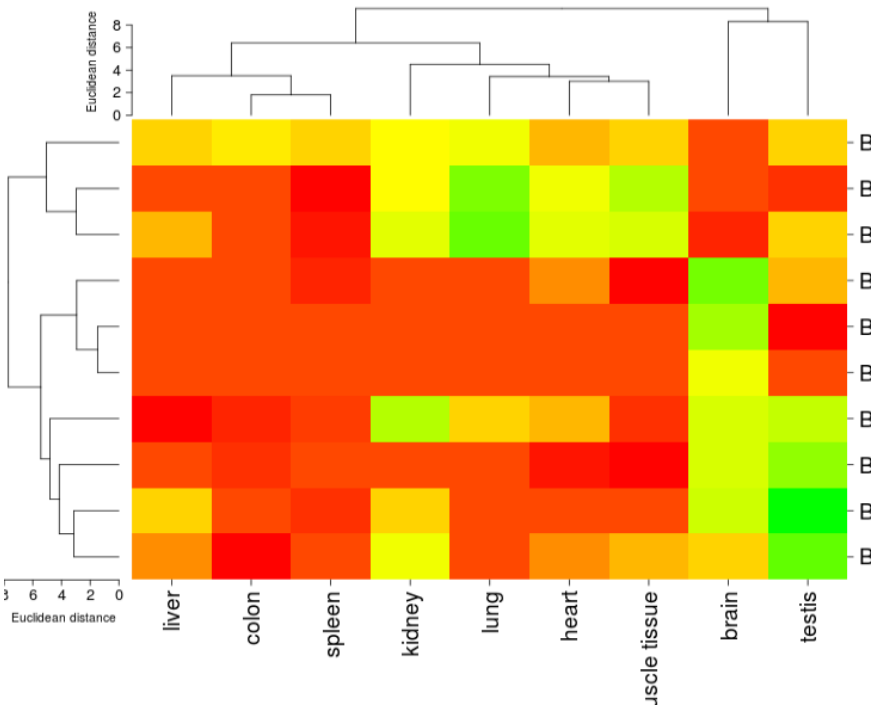

Figure 4. Hierarchical clustering of differentially expressed Bt-*SOX* genes in nine different tissues of bovine. To be drawn the heat map was used CIMminer web-based tool.

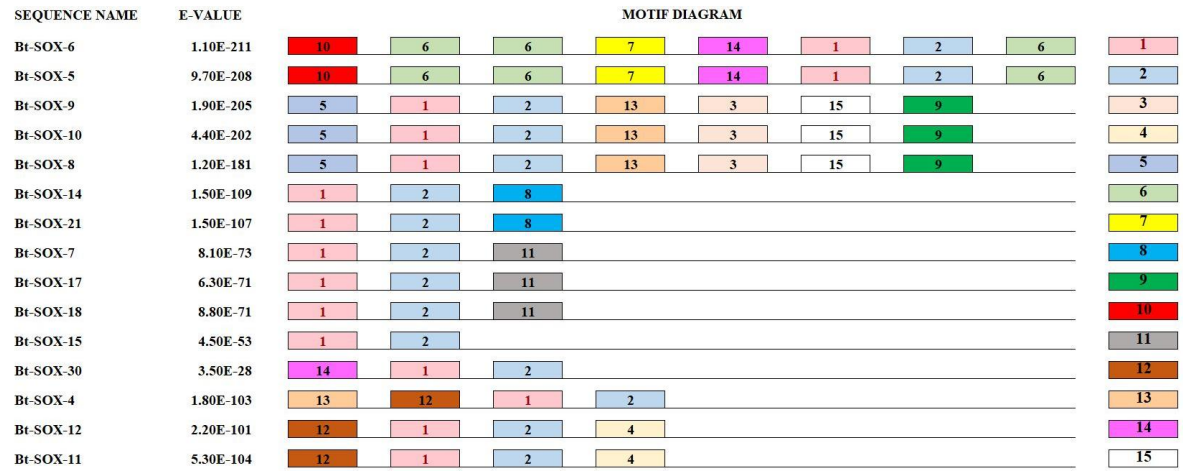

Figure 5. Distribution of 15 putative motifs in Bt-*SOX* genes. The 15 putative motifs were assorted utilizing MEME Suite v5.0.1. Each motif was given in different colored boxes. The numbers in the boxes were shown as MOTIF 1 – MOTIF 15, respectively.

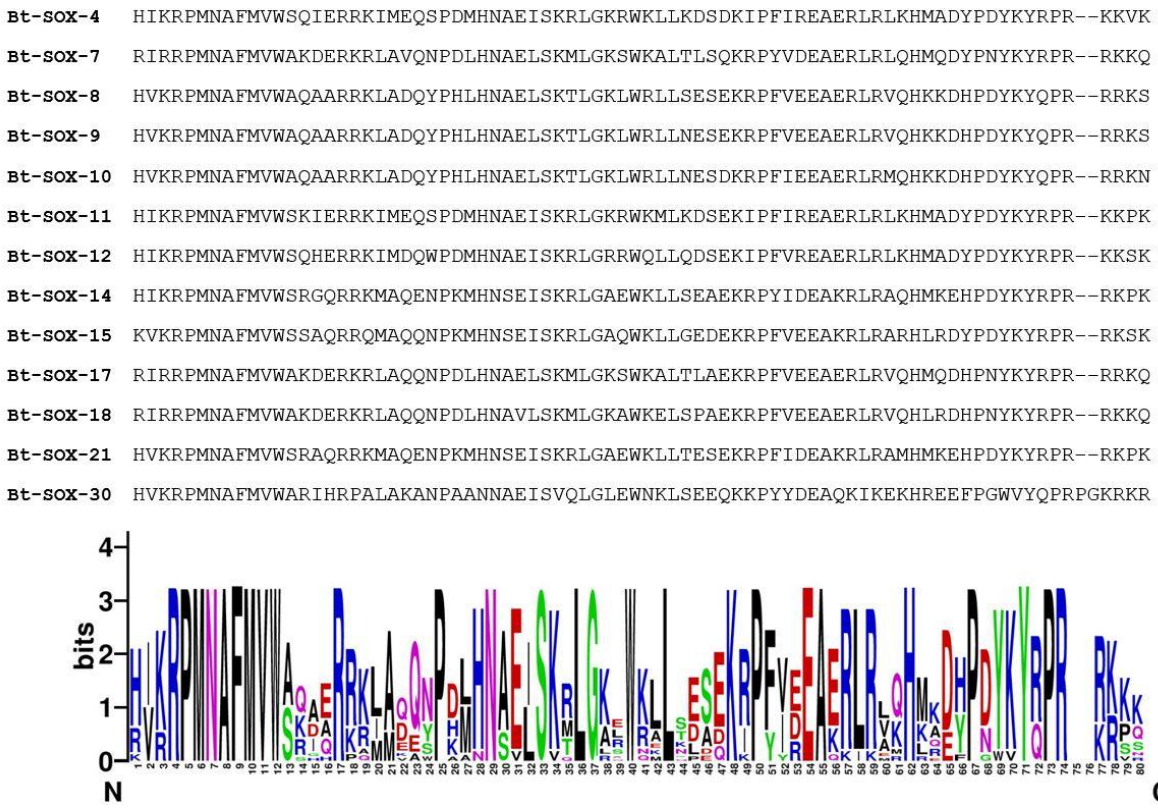

Figure 6. Alignment of selected *SOX* gene sequences (upper) and sequence logo (lower). The direction of gene sequences is from the N-terminal to the C-terminal. The height of the letters in bits is proportional to their frequency.

4. Discussion

The *SOX* gene family displays various tissue-specific patterns during the early stages of embryonic development (Akinyemi et al., 2022). This research was conducted to provide a better understanding of the function of *SOX* genes family in the evolution of Bovidae. We identified 7 main clusters including the genes within the same group exhibited greater sequence similarity. The *SOX*-15 subfamily had the lowest amino acid number and molecular weight whereas, *SOX*-6 had the highest. GRAVY values of all *SOX* proteins were negative which indicates a hydrophilic nature (Jiang et al., 2019; Zhang et al., 2018). Phylogenetic studies indicated that *SOX*4, *SOX*11 and *SOX*12 proteins are highly related but information on *SOX* 12 is relatively limited. Furthermore, *SOX*4 and *SOX*11 are significantly involved in the formation of cardiac ventricles and limb buds, as well as in the development of the enteric nervous system and the proliferation of glial cells. *SOX*4 and *SOX*11 have been implicated in the differentiation of lymphocytes, the development of osteoblasts, the growth of neural and glial cells, and regulating progenitor development and facilitating communication with other cells to promote

the growth and maturation of skeletal structures (Akinyemi et al., 2022). The knockout of *SOX*5 and *SOX*6 may cause skeletal abnormalities and deficiency of chondrogenesis in specific species (Smits et al., 2001). *SOX*8, *SOX*9 and *SOX*10 play a significant role in transactivation (Haseeb and Lefebvre, 2019). *SOX*14 and *SOX*21 are very similar proteins and are associated with the control of mesenchymal stem cell differentiation. *SOX*15, which is found only in mammals and plays a role in the self-renewal of mouse stem cells (Akinyemi et al., 2022).

In conclusion, this study exhibits a detailed molecular evolution and functions of 15 *SOX* genes in *Bos taurus* to provide a better understanding of genetic variation in *SOX* gene family in *Bos* sp.

Author Contributions

Percentages of the authors' contributions are presented below. All authors reviewed and approved the final version of the manuscript.

	E.İ.	Y.Z.G.
C	90	10
D	50	50
S	100	
DCP	100	
DAI	100	
L	50	50
W	40	60
CR	70	30
SR	20	80
PM	80	20
FA	50	50

C= concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The authors declare no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because of there was no study on animals or humans.

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