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Unique variations of *SRY* gene result in distinct patrilineal phylogeny of *Capra hircus* and other domestic *Bovidae**

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Patrilineal phylogeny of Beichuan White goat and other domestic *Bovidae* was inferred from 5´-UTR and coding region of *SRY* gene. Variation analysis revealed 208 variable sites, meanwhile, a 50-bp fragment inserted downstream of the initiation codon (ATG) of *SRY* genes modified the translational initiation process in *Bos* and *Bubalus*, while the mechanism of what should be explained in a further study. Amino acid sequence alignments of HMG-box region indicated a high degree of conservation among goats and other *Bovidae*. All the sequences of *Bovidae* clustered into *Bos, Bubalus* and *Capra*. *Bos indicus, Bos taurus, Bos javanicus, Bos frontalis, Bos grunniens* and *Bison bonasus* were comprised in genus *Bos,* while *Bubalus bubalis* and *Syncerus caffer* belonged to genus *Bubalus*. Beichuan white goats and other *Capra hircus* specimens were clustered into genus *Capra*. Patrilineal phylogeny of *Bovidae* exhibited a discrepancy from the earlier matrilineal analysis.

KEY WORDS: *Bovidae* / *Capra hircus* / *SRY* gene / Patrilineal phylogeny

The family *Bovidae* (suborder *Pecora*, order *Artiodactyla*) includes 128 extant species in 45 recent genera, which comprise domesticated forms (goats, sheep, and cattle), the large herding antelopes of the African plains, and the small solitary,

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territorial forms usually found in more forested areas [Allard *et al*. 1992]. Matrilineal phylogeny of *Bovidae* was well established based on mtDNA sequences. Allard *et al.* [1992] suggested that *Bovidae* family was monophyletic and included two clades: one including the tribes *Boselaphini, Bovini*, and *Tragelaphini,* and another for an *Antilopini/Neotragini* grouping. Further study based on mtDNA cyt *b* and 12S rRNA genes and two nuclear genes indicated that *Bovidae* consist of two major lineages, *i.e*. *Bovinae* which contain the tribes *Bovini, Boselaphini* and *Tragelaphini*, and Antilopinae which encompasses all other bovids. Within *Bovinae*, the tribe Bovini is divided into buffalo Bovini (*Bubalus* and *Syncerus*) and cattle Bovini (*Bos* and *Bison*) and Tragelaphini are possibly related to Boselaphini [Hassanin and Douzery 1999]. However, matrilineal analysis based on mtDNA is not adequate to depict the phylogenetic picture of *Bovidae*. Patrilineal investigation based on Y chromosome haplotypes should be another powerful tool to describe the veil of *Bovidae* phylogeny.

In mammals, *SRY* gene is located near the pseudoautosomal boundary of Y chromosome and encodes a nuclear factor-like protein harboring a DNA-binding domain known as the HMG box [Panyen and Cotinot 1993, Nagai 2001]. This gene is one of more conserved Y specific genes during evolution in a number of mammals due to its immunity to recombination with X chromosome in meiotic XY bivalent [Panyen and Cotinot 1993]. Therefore, *SRY* gene could be employed as one of the optimum molecular markers to investigate patrilineal phylogeny of *Bovidae* and other mammals. Cheng *et al*. [2001] cloned and sequenced the *SRY* genes of yak and Chinese native cattle. Their results showed that *SRY* genes in *Bovidae* were less divergent, especially in the coding and 3′regions. Nevertheless, the phylogeny of *Bovidae* based on sequence variation of *SRY* genes was poorly understood up to now. Here, we sequenced the 5′-UTR and coding region of *SRY* genes of Beichuan White goat from Sichuan province of China, and analysed the patrilineal phylogeny of the breed and other domestic bovids on the basis of the of *SRY* genes variation.

Material and methods

Sample preparation and genomic DNA extraction

Blood samples of seven male Beichuan White (BW) goats (BWG01-BWG07) from Beichuan county, Sichuan province, were withdrawn and genomic DNA was extracted according to standard protocols.

Gene cloning

The sequence of 5'-UTR and coding region of *SRY* gene of BW goats was amplified by using the primers G-SRY-F (5'-TAAGTGGAGAAGCGGGGATAGT-3') and G-SRY-R (5'-AGCGT GCCTTTGTTAGCGAGAG -3') designed according to the *SRY* gene sequence of *Capra hircus* (acc. no. EU581862). PCR was performed in a 50 μL reaction mixture containing 200 ng of genomic DNA, 1.5 mM $MgCl_2$,

10 mM Tris-aHCl (pH 8.3), 50 mM KCl, 0.6 units of Taq polymerase (TaKaRa), 10 μM of each primer and 0.2 mM of each dNTP. For thermal cycling a PTC-200 thermocycler was used (MJ Reseach Inc.) under the following conditions: 4 min denaturation at 94°C, followed by 30 cycles of 30 s at 94°C, 30 s at 52°C, 30 s at 72°C, and final 7 min extension at 72°C, before cooling to 4°C for 10 min. PCR products were purified using a Qiagen QIAquick PCR purification kit and ligated to pMD™ 18-T Vector (TaKaRa). The subsequent transformation and clone screening were performed according to standard protocols. The positive clones identified were sequenced on an ABI 3730 automated sequencer at Shanghai Major BioTech Co. Ltd, Shanghai, China.

Polymorphic and phylogenetic analysis

The sequence of 5′-UTR and coding region of *SRY* gene of BW goats, and those of *Bos javanicus*, *Bos taurus*, *Bos indicus*, *Bos frontalis*, *Bison bonasus, Bos grunniens, Bubalus bubalis* and *Syncerus caffer* were retrieved from GenBank (Tab. 1). All sequences were aligned and edited in Clustal X [Thompson *et al.* 1997] with parameters set to default. Amino acid sequences of the coding region of *SRY*

Table 1. GenBank accession numbers of *SRY* genes for Beichuan White goats (BW), other *Bovidae* and *Sus scrofa* used for phylogenetic analysis

Animal	Genus	GenBank accession number
BWG01	*Capra*	JN561342
BWG02	*Capra*	JN561343
BWG03	*Capra*	JN561344
BWG04	*Capra*	JN561345
BWG05	*Capra*	JN561346
BWG06	*Capra*	JN561347
BWG07	*Capra*	JN561348
Capra hircus	*Capra*	EU581862
Capra hircus	*Capra*	D82963
Bos javanicus	*Bos*	DQ336528
Bos taurus	*Bos*	DQ336526
Bos taurus	*Bos*	AF148462
Bos indicus	*Bos*	DQ336527
Bos frontalis	*Bos*	DQ336530
Bison bonasus	*Bos*	DQ336533
Bison bonasus	*Bos*	DQ336532
Bos grunniens	*Bos*	DQ336531
Bos grunniens	*Bos*	AF148463
Bos grunniens	*Bos*	FJ373272
Bos grunniens	*Bos*	EU547257
Bubalus bubalis	*Bubalus*	GQ259332
Bubalus bubalis	*Bubalus*	DQ336535
Syncerus caffer	*Bubalus*	DQ336534
Sus scrofa	*Sus*	GU143249
Sus scrofa	*Sus*	GU143246

gene were explored by NCBI software ORF Finder (http://www.ncbi.nlm. nih.gov). Polymorphic sites of SRY protein sequences were analysed using MEGA 4 [Kumar *et al*. 2008]. The NJ tree [Saitou and Nei 1987] based on 5′-UTR and coding region of *SRY* gene sequences was reconstructed in MEGA using the corresponding sequences of *Sus scrofa* as outgroup, with the reliability of the tree topology assessed by 1000 bootstrap replications [Felsenstein 1988].

Results and discussion

Each sequence of 5′-UTR and coding region of *SRY* gene of seven BW goats amplified was 1184 bp in length (Fig. 1). After removing the primer sequences and editing by multiple alignments, we have obtained seven sequences with a length of 1098 bp which covered 5′-UTR (371 bp) and coding region of *SRY* gene (723 bp). Each ORF sequence of *SRY* gene for BW goat was 723 bp in length and the predicted SRY protein was composed of 240 amino acids (GenBank accession numbers: JN561342-JN561348). However, the coding regions of *SRY* gene for 14 *Bos* and *Bubalus* animals, including *Bos javanicus*, *Bos taurus*, *Bos indicus*, *Bos frontalis*, *Bison bonasus, Bos grunniens, Bubalus bubalis* and *Syncerus caffer,* were 690 bp in length and the predicted SRY proteins were composed of 229 amino acids (aa). These results are in accordance with the study of Cheng *et al.* [2001], who reported the SRY protein sequences from Chinese native cattle, yak, Japanese cattle and bison to be 229 aa and those from sheep and goats of 240 aa in length.

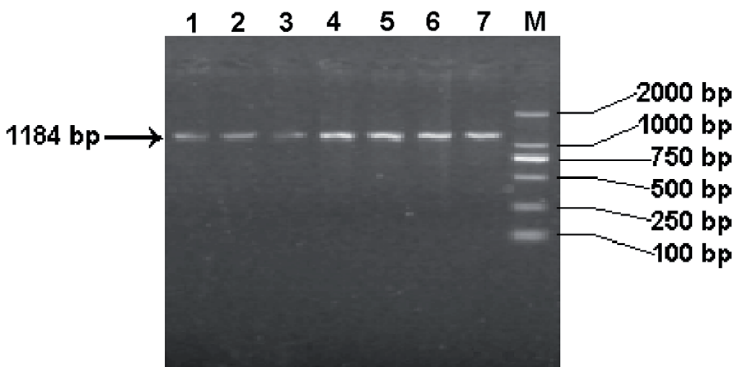

Fig. 1. PCR amplification of 5′-UTR and coding region of *SRY* genes from seven Beichuan White goats. M stands for DL2000 DNA Marker and the numbers 1-7 indicate different individuals of Beichuan White goats.

Variation analysis of 5′-UTR and coding region of *SRY* gene from nine *Capra* animals (including seven BW males) together with those from 14 *Bos* and *Bubalus* representatives revealed 208 variable sites. Meanwhile, a 50-bp fragment insertions were examined downstream the initiation codon (ATG) of *SRY* genes from *Bos* and

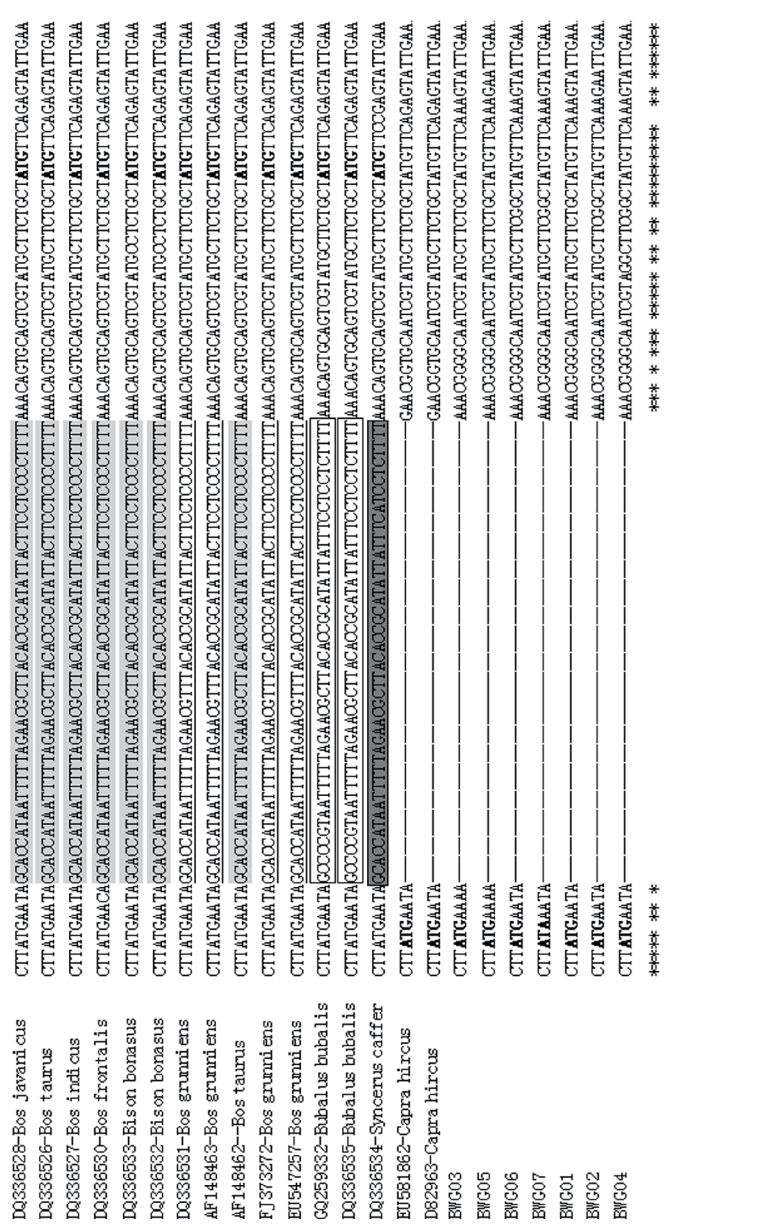

Fig. 2. The specific inserted sequences approximating to the initiation codon of *SRY* genes from *Bos* and *Bubalus* animals compared to those from *Capra*. *Bos javanicus*, *Bos taurus*, *Bos indicus*, *Bos frontalis* and *Bison bonasus* shared the identical sequences showed in shade. The underlined sequences are specific to *Bos grunniens* and the boxed specific to *Bubalus bubalis*. The shaded and boxed sequence is specific to *Syncerus caffer*. The initiation codons are bolded.

Bubalus groups compared to those from *Capra* group (Fig. 2). We suggest that it was these 50-bp insertion fragments between the initiation codon (ATG) and the immediately following codon (ATG) that modified the translational initiation process in *Bos* and *Bubalus*, while the mechanism of what should be explained in a further study. On the other hand, these 50-bp insertion fragments exhibited some specific characteristics. *Bos javanicus*, *Bos taurus*, *Bos indicus*, *Bos frontalis* and *Bison bonasus* groups shared the identical insertion fragment, as shown on Figure 2. The insertion fragments shared by *Bos grunniens*, *Bubalus bubalis* and *Syncerus caffer* occurred special enough to distinguish them from another (Fig. 2). Therefore, we could conclude that 5′-UTR of *SRY* genes were more divergent in *Bovidae*, what was in contrast with the conclusion that SRY genes in *Bovidae* were less divergent, especially in the 3′-UTR regions [Cheng *et al.* 2001].

The high-mobility-group protein HMG of Bovidae is composed of 77-residues termed HMG box (Fig. 3), which is conserved motif representing a functional protein

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                                1 1111111112 2222222223 3333333334 4444444445 5555555556 6666666667 7777777
                       1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 1234567
DQ336527-Bos indicus   HVKRPMNAFI VWSRERRRKV ALENPKMKNS DISKQLGYEW KRLTDAEKRP FFEEAQRLLA IHRDKYPGYK YRPRRRA
DQ336533-Bison bonasus ..........  ..........  ..........  ..........  ..........  ..........  ..........  .......
DQ336530-Bos frontalis ..........  ..........  ..........  ..........  ..........  ..........  ..........  .......
EU547257-Bos grunniens ..........  ..........  ..........  ..........  ..........  ..........  ..........  .......
FJ373272-Bos grunniens ..........  ..........  ..........  ..........  ..........  ..........  ..........  .......
DQ336531-Bos grunniens ..........  ..........  ..........  ..........  ..........  ..........  ..........  .......
AF148463-Bos grunniens ..........  ..........  ...Y......  ..........  ..........  ..........  ..........  .......
AF148462—Bos taurus    ..........  ..........  ...Y......  ..........  ..........  ..........  ..........  .......
DQ336528-Bos javanicus ..........  ..........  ..........  ..........  ..........  ..........  .....H...  .......
DQ336526-Bos taurus    ..........  ..........  ..........  ..........  ..........  ..........  ..........  .......
DQ336532-Bison bonasus ..........  ..........  ..........  ..........  ..........  ..........  ..........  .......
GQ259332-Bubalus bubalis .I.......  L.......L.  ..........  E.........  ..........  .........S  ..........  .....K.
DQ336535-Bubalus bubalis .I.......  L.......L.  ..........  E.........  ..........  .........S  ..........  .....K.
DQ336534-Syncerus caffer .I.......  .........L  ..........  E.........  ..........  .........S  ...E......  .....K.
EU581862-Capra hircus  ..........  ..........  ......LQ..  E.........  ..........  ..........  ..........  .....K.
D82963-Capra hircus    ..........  ..........  ......LQ..  E.........  ..........  ..........  ..........  .....K.
BWG05                  ..........  ..........  ......LQ..  E.........  ..........  ..........  ..........  .....K.
BWG06                  .........L  ..........  ......LQ..  E.........  ..........  ..........  ..........  .....K.
BWG07                  ..........  ..........  ......LQ..  E.........  ..........  ..........  ..........  .....K.
BWG01                  ..........  ..........  ......LQ..  E.........  ..........  ..........  ..........  .....K.
BWG03                  ..........  ..........  ......LQ..  E.........  ..........  ..........  ..........  .....K.
BWG04                  ..........  ..........  ......LQ..  E.........  ..........  ..........  ..........  .....K.
BWG02                  ..........  ..........  ......LQ..  E.........  ..........  ..........  ..........  .....K.
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Fig. 3. Amino acid sequence alignments of HMG-box region from Beichuan White goats and other *Bovidae*. Mutations are scored relative to the reference sequence (acc. no. DQ336527). Sequence identity is indicated by points and the differences are noted. Numbers at the top of the Figure indicate the amino acid sequence position.

domain necessary for DNA binding activity of SRY [Wright and Dixon 1988]. The mutations in the SRY gene associated with sex inversion have been located within the HMG box [Hawkins *et al*. 1992, McElreavey *et al*. 1992]. Amino acid sequence alignments of HMG-box region indicated a very high degree of conservation among BW goats and the other representatives of *Bovidae* (Fig. 3). Among the genus *Bos* (including *Bos javanicus*, *Bos taurus*, *Bos indicus*, *Bos frontalis* and *Bison bonasus*) only two variable sites were observed and more than 97% of homology was exhibited, while the sequences from the genus *Bubalus* showed seven variable sites and more than 90% of homology (Fig. 3). All representatives of BW goat together with two "extra" sequences (GenBank accession no. D82963, EU581862 of the genus *Capra* also displayed higher degree of conservation, with five variable sites and more than 93% homology.

Neighbour-joining tree was constructed from 5'-UTR and coding region of *SRY* genes on the basis of Kimura two-parameter distances, with *Sus scrofa* as the outgroup. All the sequences were reasonably clustered into phylogenetic clades representing different genus with more than 93% bootstrap values, namely *Bos*, *Bubalus, Capra* and *Sus* (Fig. 4). Therefore, the *Bovidae* family compromised *Bos*, *Bubalus* and

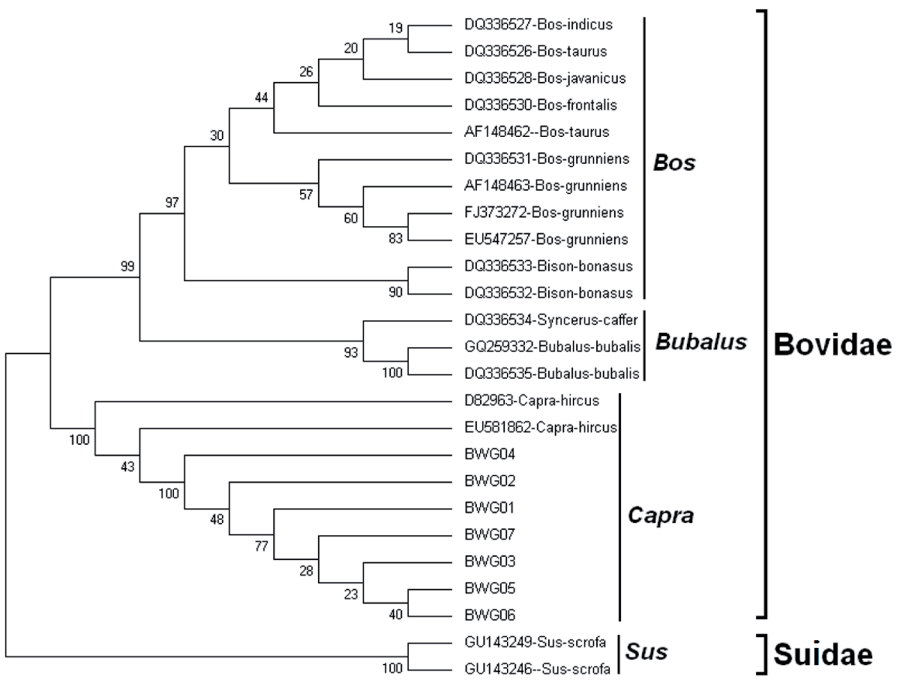

Fig. 4. Neighbour-joining tree of Beichuan White goats and other *Bovidae* as constructed from 5′-UTR and coding region of *SRY* genes on the basis of Kimura two-parameter distances, with *Sus scrofa* as the outgroup. The numbers at the branches stand for bootstrap values for 1000 replications.

Capra examined in the present study. The sequences of *Bos indicus*, *Bos taurus*, *Bos javanicus*, *Bos frontalis, Bos grunniens* and *Bison bonasus* were clustered into genus *Bos*, while *Bubalus bubalis* and *Syncerus caffer* to genus *Bubalus*. This result is in accordance with the view of Hassanin and Douzery [1999] who claim that *Bovini* should be divided into buffalo *Bovini* (*Bubalus* and *Syncerus*) and cattle *Bovini* (*Bos* and *Bison*). All individuals of BW goats together with two sequences of *Capra hircus* were clustered into genus *Capra*. Within the phylogeny of *Bos*, *Bos taurus* and *Bos indicus* were the most closely related species commonly known as cattle. *Bos javanicus* and *Bos frontalis* were more closely related to *Bos taurus* and *Bos indicus* than to *Bos grunniens*, while more divergent from *Bison bonasus*. This was not in accordance with the results we obtained from the phylogeny of *Bovidae* inferred from mtDNA cyt *b* gene that *Bos javanicus* and *Bos grunniens* were found to be more divergent from *Bos taurus* and *Bos indicus* than the European bison was from the two lineages [Cai *et al*. 2007]. In this case, we conclude that using of different molecular markers may lead to inconsistent results of phylogenetic inferences. Therefore, comprehensive markers, matrilineal, patrilineal and nuclear should be employed to more accurately investigate the phylogeny of *Bovidae*.

Herein, we concluded that the sequences of 5′-UTR of *SRY* genes from BW goats and other *Bovidae* exhibited abundant variations. The 50-bp fragment inserted downstream of the initiation codon (ATG) of *SRY* genes modified the translational initiation process in *Bos* and *Bubalus* groups while the mechanism of what should be explained in a further study. Amino acid sequence alignments of HMG-box region indicated a high degree of conservation among goats and other *Bovidae*. All the sequences were reasonably clustered into phylogenetic clades representing different genus with more than 93% bootstrap values, namely *Bos*, *Bubalus, Capra* and *Sus*. However, patrilineal phylogeny of *Bovidae* exhibited a discrepancy from the previous matrilineal analysis, for different molecular markers may lead to inconsistent inference of phylogeny.

REFERENCES

1. ALLARD M.W., MIYAMOTO M.M., JARECKI L., KRAUS F., TENNANT M.R., 1992 – DNA systematics and evolution of the artiodactyl family Bovidae. ***Proceedings of the National Academy of Sciences of the USA*** 89, 3972-3976.
2. CAI X., CHEN H., LEI C., WANG S., XUE K., ZHANG B., 2007 – mtDNA diversity and genetic lineages of eighteen cattle breeds from Bos taurus and Bos indicus in China. ***Genetica*** 131, 175-183.
3. CHENG H.H., SHI H.F., ZHOU R.J., GUO Y.Q., LIU L., LIU J.D., JIANG Y., KUDO T., SUTOU S., 2001 – Characterization of Bovidae sex-determing gene *SRY*. ***Genetics Selection Evolution*** 33, 687-694.
4. FELSENSTEIN J., 1988 – Phylogenies from molecular sequences: inference and reliability. *Annual Review* ***of Genetics*** 22, 521-565.
5. HASSANIN A., DOUZERY E.J.P., 1999 – Evolutionary affinities of the enigmatic saola (Pseudoryx

nghetinhensis) in the context of the molecular phylogeny of Bovidae. ***Proceedings of the Royal Society*** *B* 266, 893-900.

6. HAWKINS J.R., TAYLOR A., BERTA P., LEVILLIERS J., VAN DER AUWERA B., GOODFELLOW P.N., 1992 – Mutational analysis of SRY: nonsense and missense mutations in XY sex reversal. ***Human Genetics*** 88, 471-474.
7. KUMAR S., DUDLEY J., NEI M., TAMURA K., 2008 – MEGA: A biologist-centric software for evolutionary analysis of DNA and protein sequences. ***Briefings in Bioinformatics*** 9, 299-306.
8. MCELREAVEY K.D., VILAIN E., BOUCEKKINE C., VIDAUD M., JAUBERT F., RICHAUD F., FELLOUS M., 1992 – XY sex reversal associated with a nonsense mutation in SRY. ***Genomics*** 13, 838-840.
9. NAGAI K., 2001 – Molecular evolution of Sry and Sox gene. ***Gene*** 270, 161-169.
10. PANYEN E.J., COTINOT C.Y., 1993 – Comparative HMG-box sequences of the SRY gene between sheep, cattle and goats. ***Nucleic Acids Research*** 21, 2772.
11. SAITOU N., NEI M., 1987 – The neighbor-joining method: a new method for reconstructing phylogenetic trees. ***Molecular Biology Evolution*** 4, 406-425.
12. THOMPSON J.D., GIBSON T.J., PLEWNIAK F., JEANMOUGIN F., HIGGENS D.G., 1997 – The Clustal X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tool. ***Nucleic Acids Research*** 25, 4876-4882.
13. WRIGHT J.M., DIXON G.H., 1988 – Induction by torsional stress of an altered DNA conformation 5' upstream of the gene for a high mobility group protein from trout and specific binding to flanking sequences by the gene product HMG-T. ***Biochemistry*** 27, 576-681.