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The Phylogeny of China Buffalo Analysis Based on Mitochondrial DNA D-Loop Sequence Variation

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Abstract: We sequenced mitochondrial DNA (mtDNA) control region (D-loop) sequence in 22 individuals from 5 Chinese buffalo local breeds/populations. Combined with other published data, we studied the matrilineal components of China buffalo based on the sequence variations. Fifty seven D-loop partial sequence haplotypes with 71 polymorphic sites were detected among 152 individuals. After phylogenetic analyses, all Chinese buffaloes could be divided into haplogroup A and haplogroup B; In haplogroup B, 2 sub-haplogroups were further discerned. Compared with haplogroup B, haplogroup A could represent a predominant matrilineal origin. We could not detect obvious breeds/populations specific haplotypes. The 10 swamp buffaloes (Carabao) from Brazil had been closely clustered into haplogroup A, which suggested there is some direct connection between haplogroup A of Chinese buffalo and outside gene pool. The genetic diversity of Chinese buffalo was relatively low compared with cattle and yak.

Key words: Chinese swamp buffalo, mtDNA, control region, genetic diversity, domestication

INTRODUCTION

Water buffalo (*Bubalus bubalis*) is one of the major large animals in China. There are about 22.75 million heads buffaloes, which ranks the 3rd in the world (Yang, 2006) and the number and distribution area are only second to cattle. As the development of Chinese milk industry, the buffalo will play a more and more important role in prompting agricultural economy. Based on the historical data, the domestication and selection of buffalo in China has more than 7,000 years old and has resulted in a kind of draft animals with good characteristics (Chen and Li, 1989). According to traditional taxonomy, there are 18 excellent local breeds/populations and all of them can be divided into Plateau, Foothill, Plain and Coast types based on their geographical distribution and phenotype characteristics (Zhang, 2001).

To develop rational breeding and genetic resource conservation programs, the identification, utilization and conservation of buffalo resources have recently become interesting in China. The phylogeny and genetic diversity of water buffalo had been studied by archaeological and morphological methods at first (Chen and Li, 1989) and then the molecular methods containing microsatellite loci and mitochondrial DNA (mtDNA) polymorphism had been introduced subsequently (Amano *et al*., 1994; Kierstein *et al*., 2004; Kumar *et al*., 2006; 2007; Lau *et al*., 1998; Lei *et al*., 2007; Tanaka *et al*., 1995). The modern domestic river and swamp buffalo could descend from artificial selection after one domestication (Kierstein *et al*., 2004), another idea is that the 2 types resulted from 2 independent domestication events (Kumar *et al*., 2007; Lei *et al*., 2007). The taxonomy of that all China buffalo local breeds/populations belonged to swamp buffalo type was widely accepted (Zhang, 2001). As to the domestication of China buffalo, there were 2 predominant hypotheses. Kierstein and his colleagues (Kierstein *et al*., 2004) thought that the Indus Basin would be one maternal domestication center for world buffalo, after this initial domestication, it spread south into the Philippines and north into China. The second allows for 2 domestication events, one in India and one in China with areas of introgression in between (Kumar *et al*., 2007; Lei *et al*., 2007). Chen and Li (1989) believed that modern Chinese buffalo was local origin and had an earlier domestication and then spread to South-East Asia along the rice cultivation area expanding. In addition, the suggestion that the buffalo (river and swamp) distributed in eastern islands of Borneo and Sulawesi and western islands of Indonesia were introduced from China via Taiwan and/or from Indian subcontinent via South-East Asia has been supported consistently (Kumar *et al*., 2006, 2007; Lau *et al*., 1998; Lei *et al*., 2007).

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In this study, we sequenced the mtDNA control region partial sequence of 22 Chinese swamp buffaloes and compared with the published data. The genetic diversity and phylogenetic relationship were subsequently studied.

MATERIALS AND METHODS

Sampling and sequencing: In this study, a total of 22 ear tissue samples of Chinese buffalo were collected from 5 local breeds/populations, containing 7 Dechang buffaloes from Xichang in Sichuan province, 5 Fuling buffaloes from Fuling in Chongqing municipality, 3 Jianghan buffaloes from Jianli in Hubei province, 5 Anhui buffaloes from Hefei in Anhui province and 2 Yunnan buffaloes from Simao in Yunnan province (Table 1 and Fig. 1). Genomic DNA was extracted by standard phenol/chloroform method. The mtDNA D-loop complete sequence was amplified and sequenced using primer pair: $tRNA^{Phe}$: (5'- AGGCATTTTCAGTGCCTTGC -3') and Cytb (5'-TAGTGCTAATACCAACGGCC-3') (Kierstein *et al*., 2004). Amplification of D-loop sequence was performed in a 50 μL reaction mixture containing 100 ng of DNA, 10 mM Tris-HCl (pH 8.3), 2.5 mM MgCl2, 50 mM Kcl, 10pM of each primer and 1 unit of Taq polymerase (S_{ABC}) following 35 cycles of 50 sec at 94°C, 50 sec at 57°C and 60 sec at 72°C. PCR products were purified on spin columns and directly sequenced with

Table 1: The distribution of breeds in different haplogroups and genetic diversity

Breeds/populations (Abbr.)	Gen Bank Accession Nos.	No. of samples (haplotypes)	Haplogroup A (%)	Haplogroup B (%)	Haplotype diversity (h)	Nucleotide diversity (π)
Wenzhou (WZ)	DQ364160-64; Q658067-91 (Lei *et al*., 2006)	30 (14)	23 (76.7)	7 (23.3)	0.834±0.064	0.01561±0.00341
Xinyang (XY)	DQ364165-69; Q658092-115 (Lei *et al*., 2006)	29 (12)	26 (89.7)	3 (10.3)	0.771±0.083	0.00994±0.00363
Xinglong (XL)	DQ364180-84; Q658116-39; AY702618 (Lei *et al*., 2006; Qian *et al*., 2004)	30 (17)	27 (90.0)	3 (10.0)	0.844±0.066	0.00944±0.00311
Fu'an (FA)	DQ364175-79; Q658055-66 (Lei *et al*., 2006)	17 (7)	10 (58.8)	7 (41.2)	0.831±0.065	0.02195±0.00266
Fuling (FL)	EF053543-47 (our study)	5 (2)	5 (100.0)	0 (0.0)	1.000±0.126	0.43572±0.11872
Hanzhong (HZ)	DQ364185-89; DQ658052 (Lei *et al*., 2006)	6 (3)	6 (100.0)	0 (0.0)	0.600±0.215	0.00109±0.00144
Jianghan (JH)	EF053548-50; DQ658053-54 (Our study; Lei *et al*., 2006)	5 (5)	4 (80.0)	1 (20.0)	0.600±0.215	0.01317±0.00823
Binhu (BH)	DQ364170-74; DQ658051 (Lei *et al*., 2006)	6 (3)	5 (83.3)	1 (16.7)	0.900±0.161	0.02270±0.00603
Anhui (AH)	EF053531-35 (Our study)	5 (4)	3 (60.0)	2 (40.0)	1.000±0.076	0.02195±0.00556
Dechang (DC)	EF053536-42 (Our study)	7 (7)	5 (71.4)	2 (28.6)	0.900±0.161	0.00582±0.00153
Yunnan (YN)	EF053551-51 (Our study)	2 (2)	2 (100.0)	0 (0.0)	0.711±0.117	0.00095±0.00023
Carabao (Car)	AF195596-99; AF197218-23 (Kierstein *et al*.,2004)	10 (2)	10 (100.0)	0 (0.0)	0.834±0.064	0.01561±0.00341
Haplogroup A	—	126 (40)	—	—	0.761±0.041	0.00213±0.00026
Haplogroup B	—	26 (17)	—	—	0.954±0.027	0.00807±0.00046
Total	—	152 (57)	126 (82.9)	26 (17.1)	0.833±0.031	0.01250±0.00153

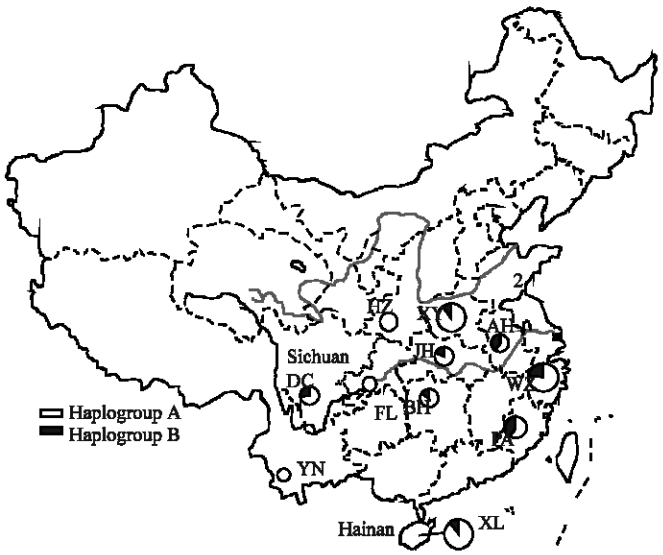

Fig. 1: The geographical distribution of the China buffalo samples adopted in this study. The area of circle is proportional to the sample size and the different colors represent individuals sorted in haplogroup A and haplogroup B

4 internal primers (5'-CCATCAACACACCTGACC-3'; 5'-GCGAGGACGGATTTGACT-3'; 5'-CCATTCGGAGTAGTAGGGTC-3'; 5'-CATAACATTAATGTAATAAGGGC-3') (Kierstein *et al.*, 2004) using Big Dye Terminator v3.1 Cycle Sequencing Kit (ABI) on an ABI PRISM® 3100 DNA sequencer according to the manufacturer's manual. These sequences were deposited in GenBank under accession nos.: EF053531-EF053552.

Data analysis: Total 130 swamp buffalo containing 120 individuals from 7 Chinese local breeds/populations and 10 individuals from Brazil (Carabao) mtDNA D-loop sequences were retrieved from GenBank and were compared together with our data (Table 1). After the sequences were aligned scoring relative to Haikou buffalo mtDNA D-Loop sequence (Accession No: AY702618), we exported the nucleotide variations in the segments using MEGA 3.1 (Kumar *et al.*, 2004) and excluded all gaps in the subsequent analyses. We first constructed a rooted neighbor-joining phylogenetic tree among swamp buffaloes using the Kimura 2-parameter model in MEGA 3.1 (Kumar *et al.*, 2004) and with river buffalo as outgroup (Accession No. AF197196). The reported alpha value of 0.20 was also used to define the gamma distribution (Kierstein *et al.*, 2004; Yang, 1994). To shade more lights on the phylogeny of swamp buffaloes from different religions and breeds, a network graph was further drawn according to Bandelt *et al.* (1999) and was subjected to confirmation using Network 4.1 (http://www.fluxus-engineering.com/sharenet.htm), in which transitions, transversions and insertion/deletions were evenly weighted. We also calculated the haplotype diversity (H) and nucleotide diversity (π) of the different populations or samples using DnaSP 4.10 to provide more information on genetic structure (Rozas *et al.*, 2003).

RESULTS

Out of 152 swamp buffalo individuals, 57 mtDNA D-loop sequence haplotypes holding 71 variations were determined (Fig. 2). Among the 57 haplotypes, the H1 being along with 61 individuals from 10 breeds/populations was the predominant haplotype. Forty-two haplotypes occurred in only 1 sample and 6 haplotypes presented twice. The others occurred in from 3-9 samples. Nine Carabao individuals out of 10 were along with H27 and the rest one resided in H1.

In the NJ-tree, all the swamp buffalo were clustered together and could be divided into 2 clades obviously with 28 mutations distance; according to Lei *et al.* (2007) proposition, the 2 clades corresponded to lineages A and B (Fig. 3). As a consequence, we adopted the term of "haplogroup" instead of "lineage" in this text as Naderi and his team suggested (Naderi *et al.*, 2007). Haplogroup A and B consisted of 40 and 17 haplotypes, respectively. In the haplogroup B, 2 sub-haplogroups (B1 and B2) could be further discerned supported by moderate bootstrap value. The network graph provided same cluster pattern with better resolution (Fig. 4). There were 28-mutations distance between haplogroup A and B. Haplogroup A had 126 individuals (40 haplotypes) from all 12 breeds/populations is seemed to the central haplogroup; whereas, haplogroup B owned much small sample (17 haplotypes from 8 breeds/populations). The haplogroup A is a star-like shape and with H1 as its central location. H1 contained 61 individuals of nine China local breeds and one India breed (Carabao), from which 28 haplotypes separated with only one/two mutation distance. These kinds of haplotypes, which are related by mutational steps to more than one haplotype and usually displaying a high frequency, are referred to as interior or ancestral haplotypes (Posada and Crandall, 2001). H36 and H38 had longer distance (more than 8-mutation) from H1 and linked to haplogroup B. Compared with haplogroup A, haplogroup B was line-like shape and with longer distance between different haplotypes. However, two sub-haplogroups were clearly discerned in haplogroup B. Seven individuals of Fu'an appeared in haplogroup B with the highest proportion (41.2%). Four breeds (Fuling, Hanzhong, Yunnan and Carabao) were absented from haplogroup B; despite this, we fell to detect breeds/populations specific haplotypes. The 2 haplotypes detected by 10 Carabao individuals were clustered together in H1 or with a single mutation step connection to H1.

The average haplotype diversity and nucleotide diversity of 152 swamp buffaloes were 0.833±0.031 and 0.01250±0.00153 (Table 1) and varied substantially among 12 breeds. The highest haplotype diversity occurred in Dechang and Jianghan (1.000). The Binhu and Hanzhong owned the lowest haplotype diversity (0.600). The nucleotide diversity ranges from 0.00109 (In Hanzhong) to 0.43572 (In Jianghan). Those breeds containing individuals from both haplogroup A and B would contribute to the bias of presenting high nucleotide diversity because of the large difference between the two clades. In addition to this point, the great difference on the sample size among different breeds could enlarge the bias. Because of this, we estimated genetic diversity for the samples assigned to haplogroup A and B, respectively. Haplotype diversity in haplogroup B (0.954±0.027) is higher than in haplogroup A (0.761±0.041). The Nucleotide diversity in haplogroup A and haplogroup B are 0.00213±0.00026 and 0.00807±0.00046, respectively.

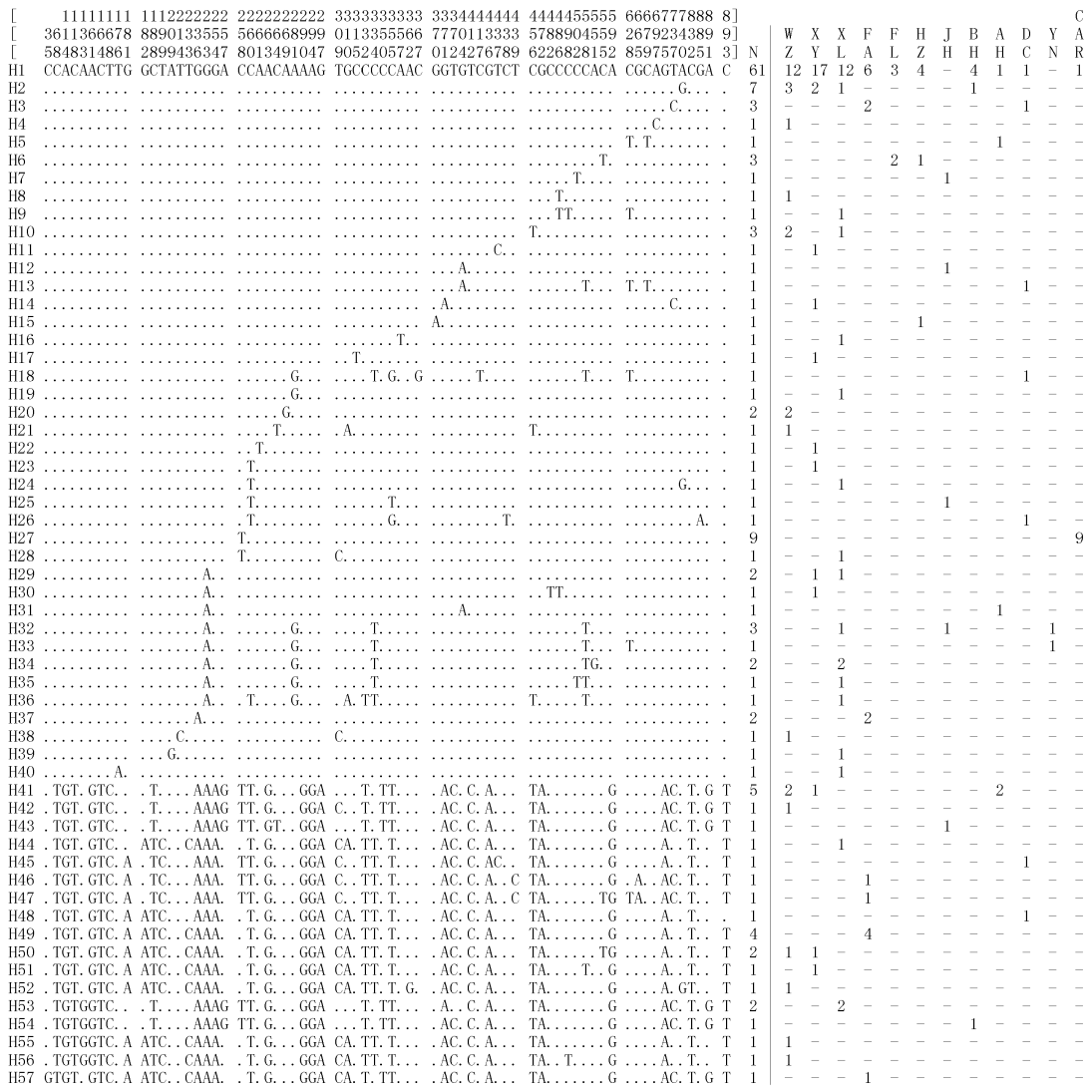

Fig. 2: Sequence variations of 57 haplotypes among 152 swamp buffaloes. The number of individuals sharing a haplotype in 12 breeds or populations is in the right vertical line. The sequence haplotypes were aligned to Haikou buffalo mtDNA complete sequence (Accession No. AY702618) and the number of individuals sharing the same haplotype in different breeds (the abbreviation of breeds were defined in Table1) are listed in left side of vertical line. Gaps had been excluded. Dots (●) denote identity with the reference sequence. Short line (-) represents the absence of one haplotype from a certain breed

DISCUSSION

The domestication of buffalo most likely took place in the civilization of the Indus, the Yangtze River, the Euphrates and Tigris (Chen and Li, 1989; Cockrill, 1981). Anoa buffalo, inhabiting India to date, is thought to be the founder of all the modern domestic buffalo, which are subdivided into swamp and river buffalo (Iannuzzi, 1994). However, the fact that the genetic division between swamp and river buffalo happened before or after the initial domestication remains controversial (Kierstein *et al*., 2004; Kumar *et al*., 2007; Lei *et al*., 2007). Only modern swamp buffalo has been distributed in China, with local domestication or introduced from other

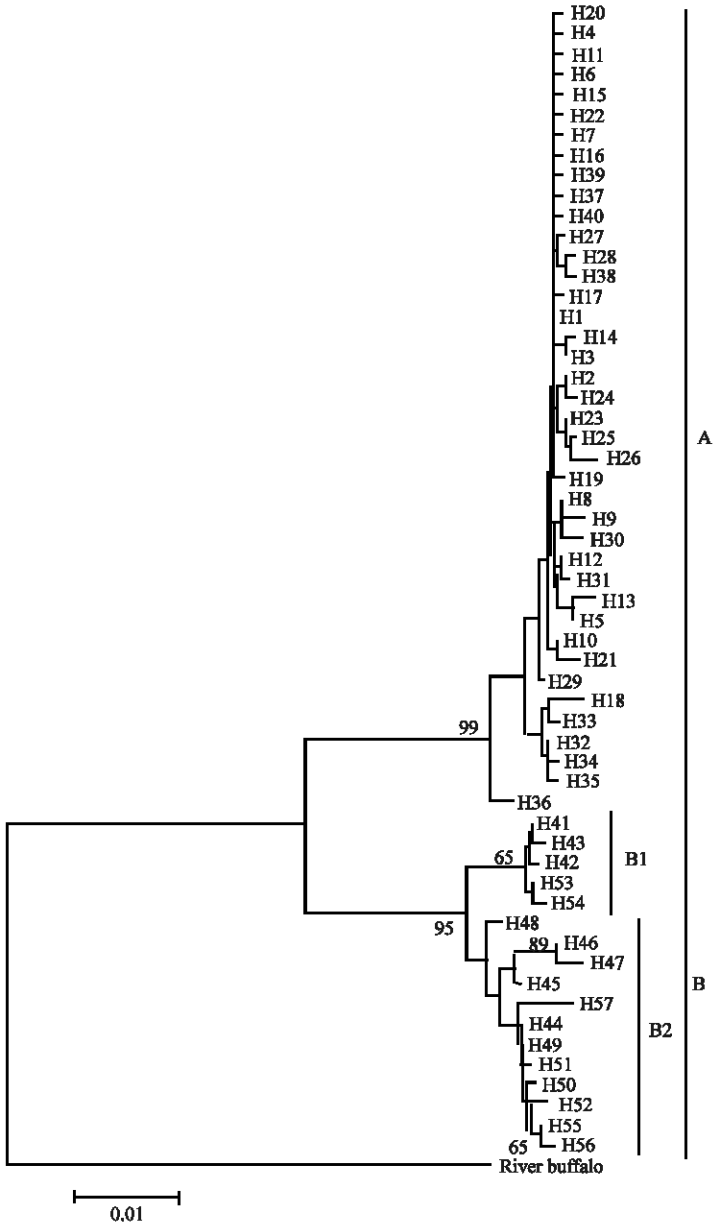

Fig. 3: Neighbor-joining (NJ) tree based on mtDNA control region partial sequence of 57 swamp buffalo haplotypes rooted with river buffalo (Accession No. AF197196). The values on the branch are bootstrap support based on 1000 replications and which lower 60% were excluded

places. Lots of fossilized bones of swamp buffalo excavated in South and North China, showed that there were wild species of buffalo dispersed in 100000-500000 years ago (Liu *et al*., 2006a). So, there presented the prime base for supporting the feasibility of local domestication of China buffalo (Chen and Li, 1989; Xie, 1985). However, Liu and colleagues found that there has significant divergence between Chinese modern buffalo and archaic buffalo fossil based on morphological and molecular data and concluded that all the old indigenous buffaloes in China were wild species; while the domestic buffalo was most likely to be introduced from South Asia round the first millennium BC, into Yunnan province at first and then diffused along with rice cultivation (Liu *et al*., 2006a). In addition, the independent domestication for Chinese modern buffalo was further supported based on mtDNA D-loop sequence variation (Lei *et al*., 2007).

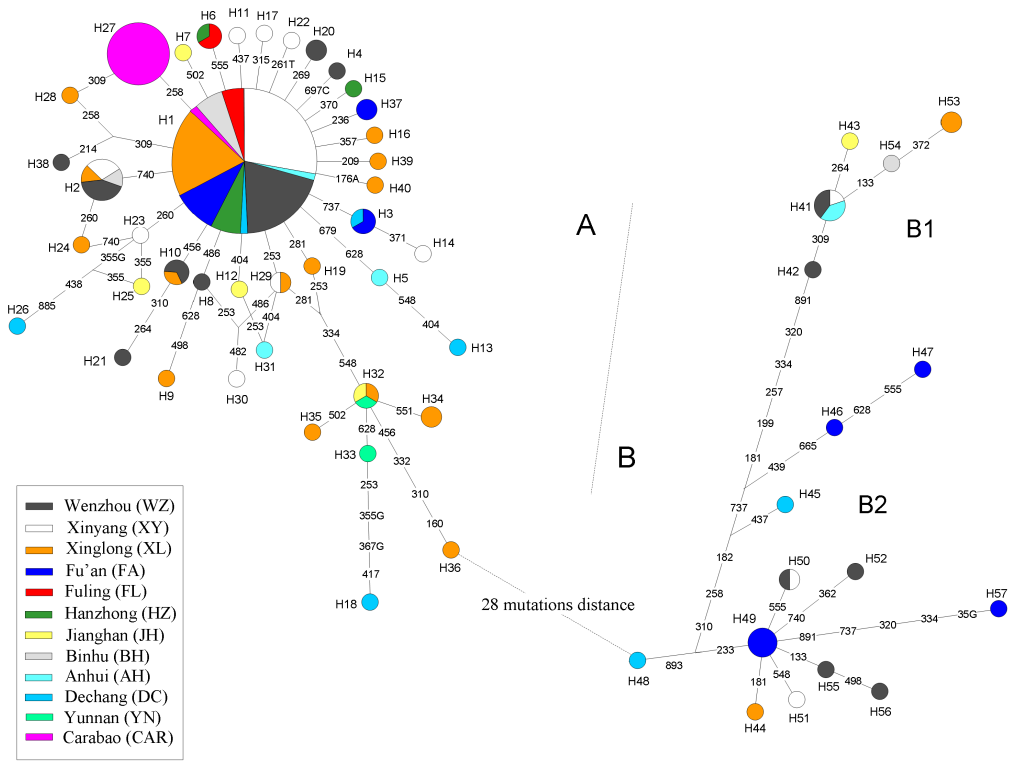

Fig. 4: Network graph of the 57 haplotypes among 152 swamp buffalo samples based on nucleotide substitutions in mtDNA control region sequences. The links are labeled by the nucleotide positions to designate transitions; Transversions are specified adding suffixes A, G, C and T. The order of the mutations on branches is arbitrary. Circle areas are proportional to haplotype frequencies and 12 colors were distributed to different breeds or populations

Two haplogroups (A and B) were discerned in Chinese buffalo, which is consistent with other farm animals in China (Lai *et al*., 2007; 2006; Liu *et al*., 2006b; 2006c). Compared with haplogroup A showing star-like shape, the haplogroup B presented longer distance among these haplotypes and could further be divided into 2 sub-haplogroups. Owing to the relatively limited information given only by mtDNA D-loop sequence, we could not affirm that whether the 2 sub-haplogroups represent another independent haplogroup. Although, 4 China buffalo local breeds/populations were absent from haplogroup B, we thought there were no obvious breeds/populations specific haplotypes. The result is same to Lai and Shi (Lai *et al*., 1994; Shi *et al*., 1996), which were studied by blood proteins polymorphism. As mentioned above, haplogroup A being along with the highest frequency and star-like shape should be regarded as their chief ancestor. Of interesting, the 10 Carabao individuals from Brazil were closely clustered into haplogroup A. In this study, we did not intend to deduce whether the Chinese modern buffalo was local domestication or exotic introduction; but we can affirm that there has a direct connection between Chinese buffalo and outside gene pool. As well, we could not conclude whether the haplogroup B is specific to China due to the low sample number from other place out of China. The Chinese buffalo hold relatively low genetic diversity compared with cattle and yak (Lai *et al*., 2007, 2006), which is consistent with former reports (Lai *et al*., 1994; Shi *et al*., 1996).

REFERENCES

Amano, T., Y. Miyakoshi, T. Takada, Y. Kikkawa and H. Suzuki, 1994. Genetic variants of ribosomal DNA and mitochondrial DNA between swamp and river buffaloes. Anim. Genet., pp: 29-36.

Bandelt, H.J., P. Forster and A. Röhl, 1999. Median-joining networks for inferring intraspecific phylogenies. Mole. Biol. Evol., pp: 37-48.

Chen, Y.S and X.H. Li, 1989. New evidence of the origin and domestication of the Chinese swamp buffalo (*Babalus bubalis*). J. Buffalo., pp: 51-55.

Cockrill, W.R., 1981. The water buffalo: A review. J. Br. Vet., pp: 8-16.

Iannuzzi, L., 1994. Standard karyotype of the river buffalo (*Bubalus bubalis* L., 2n = 50). Report of the committee for the standardization of banded karyotypes of the river buffalo. Cytogenet. Cell. Genet., 67: 102-113.

Kierstein, G., V. Marcelo, S. Artur, L. Iannuzzi and B. Brenig, 2004. Analysis of mitochondrial D-loop region casts new light on domestic water buffalo (*Babalus bubalis*) phylogeny. Mol. Phylogenet. Evol., pp: 308-324.

Kumar, S., J. Gupta, N. Kumar, K. Dikshit, N. Navani, P. Jain and M. Nagarajan, 2006. Genetic variation and relationships among eight Indian riverine buffalo breeds. Mole. Ecol., pp: 593-596.

Kumar, S., M. Nagarajan, J.S. Sandhu, N. Kumar, V. Behl and G. Nishanth, 2007. Mitochondrial DNA analysis of Indian water buffalo support a distinct genetic origin of river and swamp buffalo. Anim. Genet., pp: 227-232.

Kumar, S., K. Tamura and M. Nei, 2004. Integrated software for Molecular Evolutionary Genetics Analysis and sequence alignment. Brief. Bioinformatics., 5: 150-163.

Lai, S.J., S.Y. Chen, Y.P. Liu and Y.G. Yao, 2007. Mitochondrial DNA sequence diversity and origin of Chinese domestic yak. Anim. Genet., pp: 77-80.

Lai, S.J., Y.P Liu, Y.X. Liu, X.W. Li and Y.G. Yao, 2006. Genetic diversity and origin of Chinese cattle revealed by mtDNA D-loop sequence variation. Mole. Phyl. Evol., pp: 146-154.

Lai, S.J., R.X. Shi, W.M. Zheng and F.Y. Zuo, 1994. A study on transferring polymorphism of China buffaloes. Sichuan Anim. Vet. (in Chinese), pp: 2-5.

Lau, C.H., R.D. Drinkwater, K. Yusoff, S.G. Tan, D.J.S. Hetzel and J.S.F. Barker, 1998. Genetic diversity of Asian water buffalo (*Bubalus bubalis*): mitochondrial DNA D-loop and cytochrome b sequence variation. Anim. Genet., 29: 253-264.

Lei, C.Z., W. Zhang, H. Chen, F. Lu, R.Y. Liu, X.Y. Yang, H.C. Zhang,Z.G. Liu, L.B.Yao, Z.F. Lu and Z.L. Zhao, 2007. Independent maternal origin of Chinese swamp buffalo (*Bubalus bubalis*). Anim. Genet., pp: 97-102.

Liu, L., Y.D. Yang and X.C. Chen, 2006a. On the origin of the Bubalus Bubalis in China. Acta. Arch. Sini. (in Chinese), pp: 141-178.

Liu, Y.P., G.S. Wu, Y.G. Yao, Y.W. Miao, G. Luikart, M. Baig, A. Beja-Pereira, Z.L. Ding, M.G. Palanichamy and Y.P. Zhang, 2006b. Multiple maternal origins of chickens: Out of the Asian jungles. Mole. Phyl. Evol., pp: 12-19.

Liu, Y.P., Q. Zhu and Y.G. Yao, 2006c. Genetic relationship of Chinese and Japanese gamecocks revealed by mtDNA sequence variation. Biochem. Gene., pp: 19-29.

Naderi, S., H.R. Rezaei, P. Taberlet, S. Zundel, S.A. Rafat, H.R. Naghash, M.A. El-Barody, O. Ertugrul and F. Pompanon, 2007. Large-scale mitochondrial DNA analysis of the domestic goat reveals six haplogroups with high diversity. PLoS. ONE 2., e1012.

Posada, D. and K.A. Crandall, 2001. Intraspecific gene genealogies: Tree grafting into networks. Trends. Ecol. Evol., pp: 37-45.

Rozas, J., J.C. Sachez-delbarrio, X. Messeguer and R. Rozas, 2003. DNA SP, DNA polymorphism analyses by the coalescent and other methods. Bioinformatics., pp: 2496-2497.

Shi, R.X., S.J. Lai and W.M. Zheng, 1996. A study on blood protein polymorphism of China buffaloes. J. Sich. Agri. Univ. (in Chinese), pp: 586-598.

Tanaka, K., J.S. Masangkay, M.O. Faruque and D. Vu-Binh, 1995. Nucleotide diversity of mitochondrial DNAs between the swamp and the river types of domestic water buffaloes (*Bubalus bubalis*) based on restriction endonuclease cleavage patterns. Biochem. Genet., pp: 137-148.

Xie, C.X., 1985. The development of cattle and sheep breeding in China. Agriculture Publishing House, Beijing, China (in Chinese).

Yang, B.Z., 2006. The development of studying on Chinese buffalo and industrialization. China central party translation Publishing House, Beijing, China (in Chinese).

Yang, Z., 1994. Maximum likehood phylogenetic estimation from DNA sequences with variable rates over sites: Approximate method. J. Mol. Biol., pp: 306-314.

Zhang, C.X., 2001. Science and technology in Chinese buffalo. Guangxi Science and Technology Publishing House, Nan Ling, China (in Chinese).