INTERNATIONAL JOURNAL OF CONSERVATION SCIENCE

ISSN: 2067-533X Volume 8, Issue 1, January-March 2017: 105-112 www.ijcs.uaic.ro

PHYLOGENETIC ANALYSIS OF A THAI WILD WATER BUFFALO (BUBALUS ARNEE) THROUGH MITOCHONDRIAL CONTROL REGION

Nachai SARATAPHAN[1*], Wonganun NARONGWANICHGARN[1], Sompoch MANEERAT[2]

[1] Bureau of Biotechnology in Livestock Production, 91 Tiwanon road, Bang Kadi, Muang, Pathumthani 12000 Thailand
[2] Huai Kha Khaeng Wildlife Sanctuary, Department of National Parks, P.O. box 7, Lansak, Uthaithani 61160 Thailand

Abstract

Asiatic wild water buffaloes (Bubalus arnee) are categorized as endangered species on the IUCN Red List, and distribute only in Nepal, India, Sri Lanka, Myanmar, Bhutan, Cambodia and Thailand. The objective of this study was to verify the relationship between the maternal lineages of a Thai wild water buffalo in Huai Kha Khaeng Wildlife Sanctuary and other domesticated water buffaloes. Mitochondrial control region sequence were analysed in comparison with published sequences of domestic water buffaloes. The DNA fragment size of 396 bp indicated 55 polymorphic nucleotide sites with 50 transitions and 5 transversions. The maximum likelihood tree revealed that the Thai wild water buffalo located in lineage A of one major swamp water buffalo clade. The Thai wild water buffalo genetic material has more similarities with that of the swamp water buffalo from lineage A, than that from lineage B. Median-joining phylogenetic network and mutation position showed that the evolution of the Thai wild water buffalo happened prior to domesticate swamp water buffaloes. Analysis of mitochondrial control region sequence of the wild water buffalo showed independent maternal origin, separating from other domesticated swamp water buffaloes.

Keywords: *Thai wild water buffalo; Control region; mitochondrial DNA*

Introduction

Asiatic wild water buffaloes (*Bubalus arnee*) are categorized as endangered species on the IUCN Red List. The wild water buffalo is a highly endangered species, with a world population considered by FAO to be certainly fewer than 4,000 animals. This species has a limited distribution in Nepal, India, Sri Lanka, Cambodia, Myanmar, Bhutan and Thailand [1] (Fig. 1). In Thailand, wild water buffaloes can be found in the Huai Kha Khaeng wildlife sanctuary. The wild water buffalo was categorized as wildlife on the wildlife Protection act in 1960, in order to preserve the habitat for the only remaining population. A number of 35-40 wild water buffaloes were recorded in 2010 [2], decreased from the data of a first report in 1980 [3]. The wild buffaloes are recognized by the behavioral and phenotypic traits such as white chevron, socks and tip of tail, and larger, relatively straight, pale colored horns (similar to swamp buffalo). The identification criteria for wild buffalo from feral backcrosses were also used in earlier studies [4-8]. Moreover, observation of living buffaloes by measurement of available cranial material showed that there is an indigenous wild buffalo in Sri Lanka [9].

[*] Corresponding author: nachaisarataphan@gmail.com

Genetic identification by 10 microsatellite marker confirmed two of the 'wild' and seven of the 'domestic' as hybrids at the Koshi Tappu Wildlife Reserve in Nepal [10].

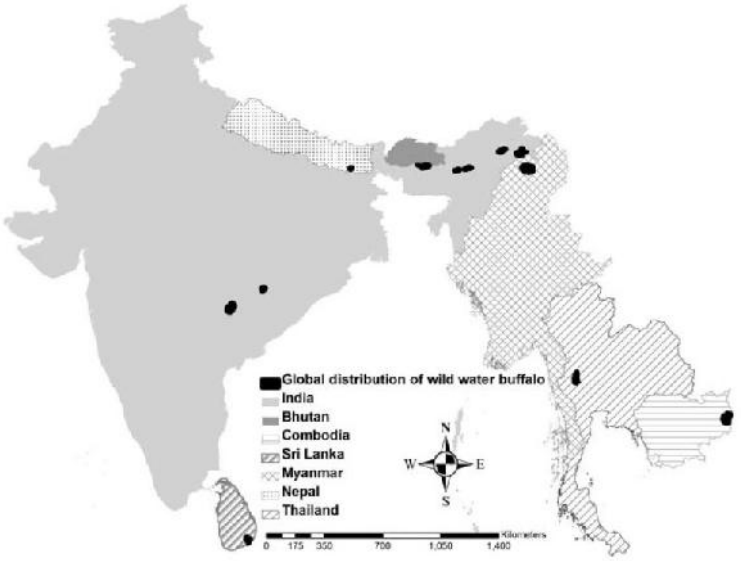

Fig. 1. Global distribution of wild water buffalo [30]

Mitochondrial DNA (mtDNA) analysis is well suited for evolutionary studies. The control region of mtDNA is particularly interesting due to the high variability level [11, 12]. The matrilineal transmission with the lack of recombination [13] and a moderate mutation rate estimated of one site every 6,000 years in humans [14]. Many mtDNA water buffalo studies based on the control region (D-loop) analysis were used to study phylogeny and evolution [15, 16]. The aim of this paper is to verify phylogenetic analysis of a Thai wild water buffalo in the Huai Kha Khaeng Wildlife Sanctuary and domesticated water buffaloes.

Materials and Methods

A skin sample of wild water buffalo in Huai Kha Khaeng wildlife sanctuary was submitted to analyze mtDNA. The D-loop region was amplified by the polymerase chain reaction (PCR) using two primers designed from a published water buffalo sequence (GeneBank DQ364160): forward 5'- CTTGCAACTTAACACTGACTTTAC -3' and reverse 5'- CCATAG CTGAGTCCAGCATC-3'. The PCR mixture contains 1x PCR buffer (50 millimolar of KCl, 10 millimolar of Tris-HCl, pH 8.3), 1.5 millimolar of $MgCl_2$, 200 micromolar of dNTPs, 0.4 picomole of each primer, 1 U *Taq* polymerase (Ampli Taq Gold^{TM}, Applied Biosystem, USA) and 100 nanogram of DNA template. The PCR reaction profiles included the following: denaturation at 94 °C for 10 min, followed by 30 cycles of denaturation at 94 degree Celsius for 30 sec, annealing at 56 degree Celsius for 1 min and extension at 72 degree Celsius for 1 min; a final extension at 72 degree Celsius for 10 min. The PCR products were initially electrophoresed at 150 Volts for 30 min. in 2% agarose gels, and viewed under UV light after staining with Ethidium bromide. The expected sizes of PCR products were determined in relation to a 100 bp DNA size standard. The PCR products were purified and sequenced using BigDye Terminator Kit (Applied Biosystems, USA) on an ABI PRISM 3010 DNA Sequencer equipped with Sequencing Analysis and Sequence Navigator (Applied Biosystems, USA). Mitochondrial DNA D-loop sequences were compared with the following mtDNA D-loop sequences selected in GenBank database (Table 1). To demonstrate the phylogenetic clusters,

the MEGA6.0 [17] was applied to construct Maximum likelihood (ML) trees, using Kimura 2-parameter distances. Median-joining network (MJ) and mutation position were generated using the NETWORK 4.6 program [18].

Table 1. Domesticated water buffalo used in this study

Buffalo type	Name/Breed	Country	GenBank accession	Ref.
River water buffalo	River buffalo	Egypt	EU268906	[31]
	Murrah	Brazil	AF197216	[21]
	Murrah	Brazil	AF197213	[21]
	Mediterranean	Italy	AF197208	[21]
	Mediterranean	Italy	AF197204	[21]
	Mediterranean	Italy	AF197202	[21]
	Jafarabadi	Brazil	AF197198	[21]
	Kundi	Pakistan	GQ166748	[32]
Swamp water buffalo				
	Carabao	Philippines	FJ873678	[33]
	Yunnan	China	EF053552	[34]
	JiangHan	China	EF053550	[34]
	FuLing	China	EF053547	[34]
	DeChang	China	EF053542	[34]
	AnHui	China	EF053535	[34]
	KhunthongCP	Thailand	KC817497	[35]
	Petchdum	Thaiand	KC817496	[35]
	SRS145/47	Thailand	KC817495	[35]
	YodrachanUT	Thailand	KC817494	[35]
	DaoCP	Thailand	KC817493	[35]
	SaenCP	Thailand	KC817492	[35]
	PetchCP	Thailand	KC817491	[35]
	SRS48/49	Thailand	KC817490	[35]
	Dao rungCP	Thailand	KC817489	[35]

Results and Discussions

In this study, a 405-bp DNA fragment was amplified from a sample of Thai wild water buffalo. The mtDNA D-loop sequence was located in D-loop position 197 to 602 which was hypervariable region (HVR) in the mitochondrial control region. The sequence was aligned with 23 mtDNA D-loop sequences of domesticated swamp and river buffaloes in the GenBank database (Table 1). The result showed that 55 polymorphic nucleotide sites in comparison with the reference sequences. The detected mutations corresponded to 50 transitions and 5 transversions, as shown in Figure 2.

The maximum likelihood tree clearly showed two clades of swamp and river water buffalo similarly to the results of previous studies [19-28]. However, the majority of swamp water buffalo in China and Thailand was lineage A [24, 29]. In this study, the Thai wild water buffalo belongs to lineage A (Fig.3).

According to pairwise genetic distances, the Thai wild water buffalo genetic material has more similarities with that of the swamp water buffalo from lineage A, than that from lineage B. In lineage A, one Thai swamp water buffalo (SaenCP) showed similar mitochondrial control region sequences to two Chinese swamp water buffaloes (Fuling and Anhui). In lineage B, one Thai swamp water buffalo (DaorungCP) showed similar mitochondrial control region sequences to a Chinese swamp water buffaloes (Dechang), as shown in Figure 4.

Median-joining phylogenetic network with mutated position in the mtDNA D-loop sequences showed that the Thai wild water buffalo has two mutated positions from ancestral node, whereas domesticated swamp water buffaloes have more than 5 mutated positions, as shown in Figure 5.

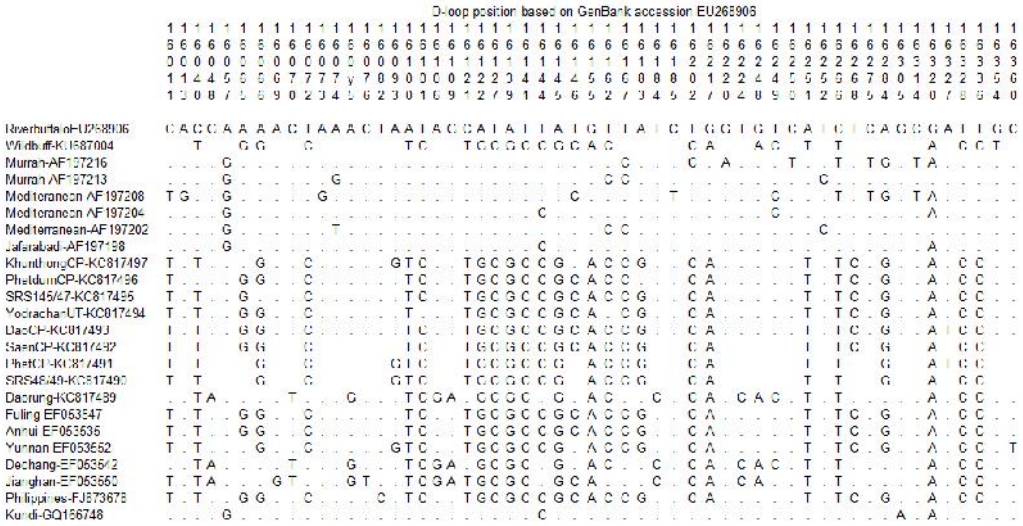

Fig. 2. Nucleotide polymorphisms in D-loop mtDNA sequences (396 bp) of 24 buffaloes are shown, vertically oriented numbers indicating the D-loop position. The sequences are only the variable sites. Dots (.) indicate identity with river buffalo sequence (GenBank accession number EU268906).

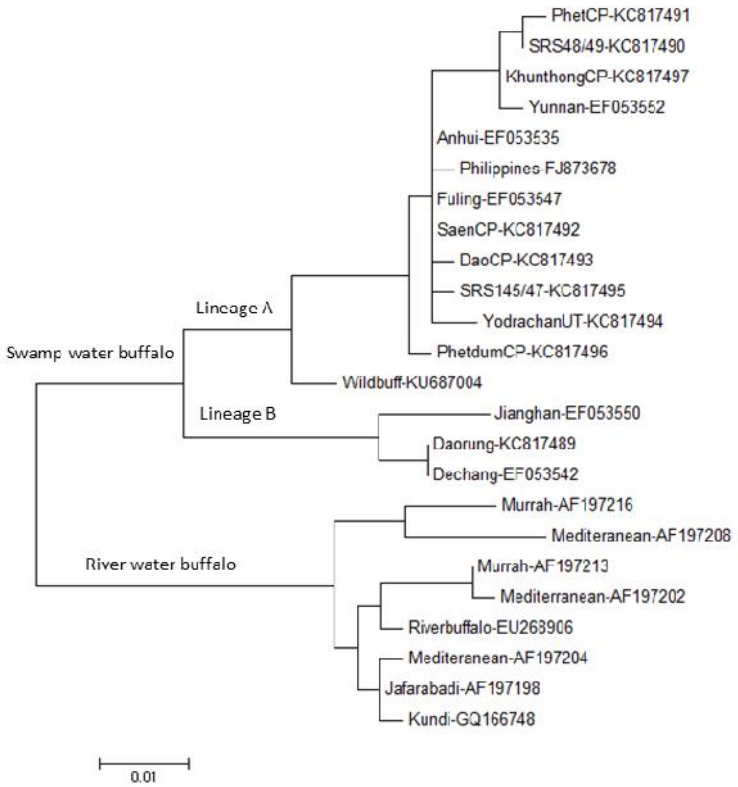

Fig. 3 Maximum likelihood (ML) tree based on D-loop mtDNA sequences of a Thai wild water buffalo-KU687004 and domesticated swamp and river water buffaloes in this study

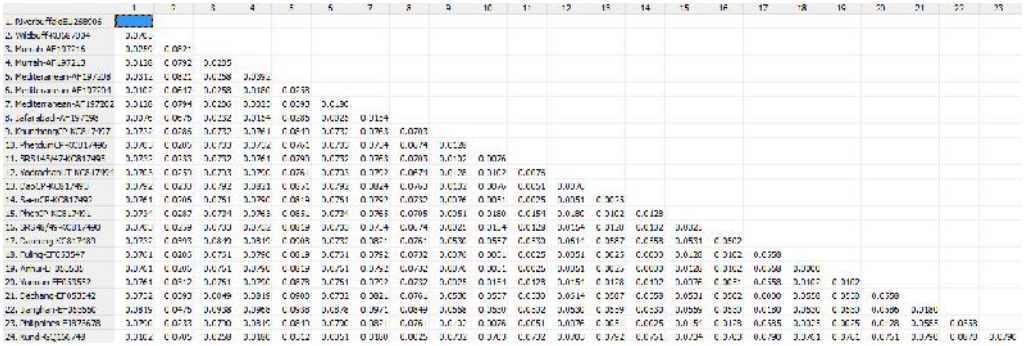

Fig. 4. Pairwise distances among a Thai wild water buffalo-KU687004 and domesticated swamp and river water buffaloes in this study

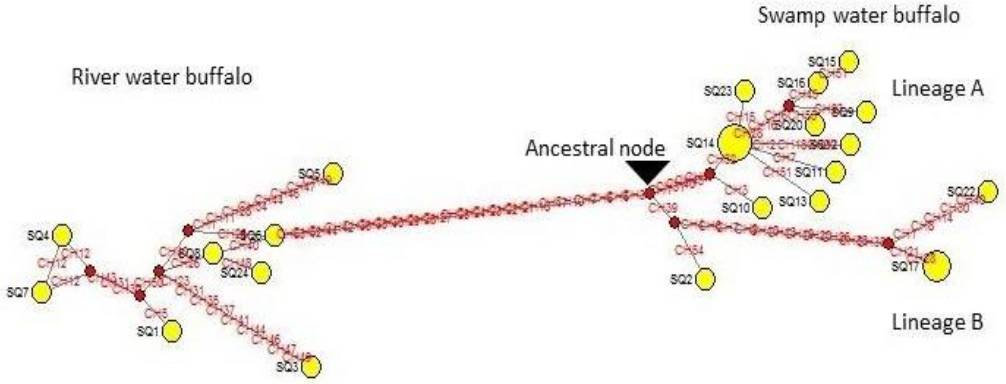

Fig. 5. Median- joining network construction from 396 bp D-loop of 24 sequences in this study, mutated position is shown by number of character (CH) and the area of the circle is proportional to sample sizes: (SQ1: River buffalo-EU268906, SQ2: Wild buffalo-KU687004, SQ3: Murrah-AF197216, SQ4: Murrah-AF197213, SQ5: Mediteranean buffalo-AF197208, SQ6: Mediteranean buffalo-AF197204, SQ7: Mediteranean buffalo-AF197202, SQ8: Jafarabadi-AF197198, SQ9: KhunthongCP-KC817497, SQ10: PhetdumCP-KC817496, SQ11: SRS145/47-KC817495, SQ12: YodrachanUT-KC817494, SQ13: DaoCP-KC817493, SQ14: SaenCP-KC817492, SQ15: PhetCP-KC817491, SQ16: SRS48/49-KC817490, SQ17: Daorung-KC817489, SQ18: Fuling-EF053547, SQ19: Anhui-EF053535, SQ20: Yunnan-EF053552, SQ21: Dechang-EF053542, SQ22: Jianghan-EF053550, SQ23: Philippines-FJ873678, SQ24: Kundi-GQ166748)

The result indicated that evolution of the Thai wild water buffalo happened prior to the swamp water buffalo from lineage A. These results provide strong evidence supporting the independent maternal origin of the wild water buffalo in Thailand. Furthermore, large sample sizes are required to identify genetic diversity in both maternal and paternal lineages of the Thai wild buffalo in Huai Kha Khaeng wildlife sanctuary.

Conclusions

This is the first study of phylogeny of a Thai wild water buffalo using mtDNA D-loop sequences. The results of the current study support to the independent maternal origin and earlier evolution than domestic swamp water buffalo. Wildlife conservation of not only Thai

wild water buffalo, but also Asian wild water buffalo should be taken to ensure its genetic integrity.

Acknowledgements

This research was supported by the Department of Livestock Development, Bangkok, Thailand under the livestock diversity activity budget in 2015. We would like to thank the Department of National Parks, Ministry of Environment and Science for providing tissue sample that was used in this study.

Sequence data from this article had been deposited with GenBank accession KU687004.

References

[1] B.D. Scherf, **World Watch List for Domestic Animal Diversity**, 3rd Edition, Food and Agriculture Organization of the United Nations, Rome, Italy, 2000, p.746.

[2] Anonymous, **Wildlife Research Section, Department of National Parks, Wildlife and Plant Conservation,** Ministry of Natural Resources and Environment, 2010, http://www.dnp.go.th/index_eng.asp [accessed on 15.01.2016].

[3] R. Chaiyarat, *Ecology and Habitat Utilization of Wild Water Buffalo (Bubalus bubalis) in Huai Kha Khaeng Wildlife Sanctuary, Thailand,* **Ph.D. Thesis**, Kasetsart University, Thailand, 2001.

[4] T.D. Dahmer, *Status and distribution of the Wild Asian Buffalo (Bubalus bubalis) in Nepal,* **MS Thesis**, University of Montana, Missoula, USA, 1978.

[5] J.T. Heinen**,** *Population viability and management recommendations for wild water buffalo (Bubalus bubalis) in Koshi Tappu Wildlife Reserve, Nepal,* **Biological Conservation, 65**, 1993, pp. 29–34.

[6] J.T. Heinen, G.R. Singh, *A census and some management implications for wild buffalo in Nepal,***Biological Conservation, 101**, 2001, pp. 391–394

[7] J.T. Heinen, *Phenotypic and behavioral characteristics used to identify wild buffalo from feral backcrosses in Nepal,* **Journal of the Bombay Natural History Society, 99**, 2002, pp.173–183.

[8] J.T. Heinen, R. Kandel, *Threats to a small population: A census and conservation recommendation recommendations for Wild Buffalo Bubalus arnee in Nepal,* **Oryx, 40**, 2006, pp. 324–330.

[9] C. Groves, J. Jayawardene, *The wild buffalo of Sri Lanka,* **Tarobanica, 01,** 2009, pp. 55-62.

[10] J.R.B. Flamand, D. Vankan, G.P. Ghaire, H. Duong, J.S.F. Barker, *Genetic identification of Wild Asian Water Buffalo in Nepal,* **Animal Conservation, 6**, 2003, pp. 265–270.

[11] C.F. Aquadro, B.D. Greenberg, *Human mitochondrial DNA variation and evolution: analysis of nucleotide sequences from seven individuals*, **Genetics, 103**, 1983, pp. 287–312.

[12] D.R. Wolstenholme, *Animal mitochondrial DNA: structure and evolution*, **International Review Cytology, 141**, 1992, pp. 173–215.

[13] L. Vigilant L, R. Pennington, H. Harpending, T.D. Kocher, A.C. Wilson, *Mitochondrial DNA sequences in single hairs from a southern African population,* **Proceeding National Academic Science USA, 86**, 1989, pp. 9350–9354.

[14] M. Stoneking, S.T. Sherry, A.J. Redd, L. Vigilant, *New approaches to dating suggest a recent age for the human mtDNA ancestor,* **Philosophical transaction of the Royal Society of London, B 337**, 1992, pp. 167–175.

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

[16] G. Kierstein, M. Vallinoto, A. Silva, M.P. Schneider, L. Iannuzzi, B. Brenig, *Analysis of mitochondrial D-loop region casts new light on domestic water buffalo (Bubalus bubalis) phylogeny*, **Molecular Phylogenetic Evolution, 30**, 2004, pp. 308-324.

[17] K. Tamura, G. Stecher, D. Peterson, A. Filipski, S. Kumar, *MEGA6: Molecular evolutionary genetics analysis version 6.0*, **Molecular Biology Evolution, 30,** 2013, pp. 2725-2729.

[18] H.J. Bandelt, P. Forster, A. Rohl, *Median joining networks for inferring intraspecific phylogenies*, **Molecular Biological Evolution, 16**, 1999, pp. 37-38.

[19] T. Amano, Y. Miyakoshi, T. Takada, Y. Kikkawa, H. Suzuki, *Genetic variants of ribosomal DNA and mitochondrial DNA between swamp and river buffaloes*, **Animal Genetics, 25**, 1994, pp. 29-36.

[20] K. Tanaka, T. Yamagata, J.S. Masangkay, M.O. Faruque, D. Vu-Binh, Salundik, S.S. Mansjoer, Y. Kawamoto, T.Namikawa, *Nucleotide diversity of mitochondrial DNAs between the swamp and the river types of domestic water buffaloes, Bubalus bubalis, based on restriction endonuclease cleavage patterns*, **Biochemical Genetics, 33**, 1995, pp. 137–148.

[21] G. Kierstein, M. Vallinoto, A. Silva, M.P. Schneider, L. Iannuzzi, B. Brenig, *Analysis of mitochondrial D-loop region casts new light on domestic water buffalo (Bubalus bubalis) phylogeny*, **Molecular Phylogenetics Evolution, 30**, 2004, pp. 308–324.

[22] S. Kumar, M. Nagarajan, J.S. Sandhu, N. Kumar, V. Behl, G. Nishanth, *Mitochondrial DNA analyses of Indian water buffalo support a distinct genetic origin of river and swamp buffalo*. **Animal Genetics, 38**, 2007, pp. 227–232.

[23] C.Z. Lei, W. Zhang, H. Chen, F. Lu, Q.L. Ge, R.Y. Liu, R.H. Dang, Y.Y. Yao, L.B. Yao, Z.F. Lu, Z.L. Zhao, *Two maternal lineages revealed by mtDNA D-loop sequences in Chinese native water buffaloes (Bubalus bubalis)*, **Asian-Australasian Journal of Animal Sciences, 20**, 2007, pp. 471–476.

[24] C.Z. Lei, W. Zhang, H. Chen, F. Lu, R.Y. Liu, *Independent maternal origin of Chinese swamp buffalo (Bubalus bubalis)*, **Animal Genetics, 38**, 2007, pp. 97–102.

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

[26] Y.C. Chen, X.H. Li, *New evidence of the origin and domestication of the Chinese swamp buffalo (Bubalus bubalis)*, **Buffalo Journal, 1**, 1989, pp. 51–55.

[27] Y. Kikkawa, H. Yonekawa, H. Suzuki, T. Amano, *Analysis of genetic diversity of domestic water buffaloes and anoas based on variations in the mitochondrial gene for cytochrome b.*, **Animal Genetics, 28**, 1997, pp. 195–201.

[28] X.P. Yue, R. Li, W.M. Xie, P. Xu, T.C. Chang, L. Liu, F. Cheng, R.F. Zhang, X.Y. Lan, *Phylogeography and Domestication of Chinese Swamp Buffalo*, **PLoS ONE, 8**(2), 2013, pp. e56552.

[29] M. Yindee, *Reproduction and genetic diversity of swamp buffalo*, **Ph.D. Thesis**, Utrecht University, The Netherland, 2010.

[30] A. Aryal, T. K. Sherestha, A. Ram, W. Frey, C. Groves, H. Hemmer, M. Dhakal, R.K. Koirala, J. Heinen, D. Raubenheimer, *Call to conserve the wild water buffalo (Bubalus arnee) in Nepal*, **International Journal of Conservation Science, 2**, 2011, pp. 261-268.

[31] A.A Hassan, S.M. El Nahas, S. Kumar, P.S. Godithala, K. Roushdy, *Mitochondrial D-loop nucleotide sequences of Egytian river buffalo: Variation and phylogeny studies*, **Livestock Science, 125**, 2009, pp. 37-42.

[32] T. Hussain, M. Ellahi Babar, M. Imran, A. Nadeem, A. Ali, M. Abdul Jabbar, **Genetic Characterization of Pakistani Buffalo Breeds by the Analysis of Mitochondrial D-loop Region**, UVAS Library catalog, https://www.*opac.uvas.edu.pk/cgi-bin/koha/opac-detail.pl?biblionumber*.

[33] L.A.M. del Barrio, C.B. dela Vina, C.A.S. Estrella, Herrera,J.R.V., A.N. del Barrio, M.G.N. Jr. Yebron, **Molecular Characterization of the Philippine Carabao from the Major Island Groups of the Philippines using Molecular Cloning and Sequence Analysis of the D-loop of Mitochondrial DNA**, UVAS Library catalog, https://www.ncbi.nlm.nih.gov/nuccore/FJ873678.

[34] S.J. Lai, S.Y. Chen, Y.P. Liu, S.Z. Xu, **The Genetic Diversity and Phylogeny of Swamp Buffalo Analysis Based on Mitochondrial DNA D-loop Sequence Variation**, UVAS Library catalog, https://www.ncbi.nlm.nih.gov/nuccore/EF053552.

[35] N. Sarataphan, S. Cheunsuk, M. Apimeteetamrong, W. Yiengvisawakul, W. Wara-Asawapati, W. Thongteing, J. Chansaad, **Genetic Diversity of Maternal Lineages in Thai Swamp Buffaloes by Mitochondrial DNA Analysis**, UVAS Library catalog, https://www.ncbi.nlm.nih.gov/nuccore/KC817497.

Received: March 15, 2016
Accepted: February 10, 2017