

Intraspecific Phylogeny of the Korean Water Deer, *Hydropotes inermis argyropus* (Artiodactyla, Cervidae)

Hye Ri Kim¹, Eui Kyung Kim², Mi Gyung Jeon¹, Yung Chul Park^{1,*}

¹College of Forest and Environmental Science, Kangwon National University, Chuncheon 200-701, Korea

²Ecosystem Research Division, National Park Research Institute, Korea National Park Service, 171, Dangu-ro, Wonju 220-947, Korea

ABSTRACT

The water deer, *Hydropotes inermis* (Cervidae), is native to China and Korea and has two subspecies of the Chinese water deer (*Hydropotes inermis inermis*) and Korean water deer (*Hydropotes inermis argyropus*). To date, only the Korean water deer has been reported in South Korea. In this study, however, an intraspecific phylogeny and haplotype analysis based on mitochondrial cytochrome oxidase I indicated that both Korean and Chinese water deer are found in South Korea. The populations of the two Korean genetic lineages did not show distinct geographic distributions. Further morphological studies on the Korean water deer will be required to confirm its taxonomic status.

Keywords: water deer, *Hydropotes inermis argyropus*, intraspecific phylogeny, taxonomic status, Cervidae

INTRODUCTION

The water deer, *Hydropotes inermis* (Cervidae), is native to China and Korea and has two of subspecies of the Chinese water deer (*Hydropotes inermis inermis*) and Korean water deer (*Hydropotes inermis argyropus*) (Grubb, 2005; Harris and Duckworth, 2008). The Chinese water deer is distributed to only the lower Yangtze Basin (Xu et al., 1996; Wang, 1998; Hu et al., 2006), and it is listed as a vulnerable species in the IUCN Red List of Threatened Species (Harris and Duckworth, 2008) and the China Red Data Book of Endangered Animals (Wang, 1998). In contrast, the Korean water deer is widely distributed throughout the forests of South Korea, and it has been designated as a wildlife pest by the Korean Ministry of Environment.

According to morphological studies (Allen, 1940; Won, 1967; Won and Smith, 1999), only the Korean subspecies has been recognized in South Korea to date. However, a recent analysis of the mitochondrial *Cyt b* gene and its control region (Koh et al., 2009) suggested the presence of two genetic lineages in Korean water deer populations, and one of these lineages was grouped with the Chinese subspecies.

The objective of the present study was to confirm the presence of the two genetic lineages in the Korean populations

by analyzing mitochondrial cytochrome oxidase I (COI) gene sequences. We analyzed the intraspecific relationships in Korean water deer by using tissues collected from 18 road-killed individuals from two northern forests (SC: Sokcho, YG: Yanggu and HC: Hongcheon) and one southern forest (JR: Jirisan National Park) of South Korea (Table 1, Fig. 1A). Genomic DNA was extracted from the tissue samples by using the DNeasy blood and tissue kit (Qiagen, Valencia, CA, USA), and amplified using the Inno™ Taq DNA polymerase kit (Bookyung S·M, Korea). Primers for PCR amplification of mitochondrial COI gene was performed were Deer F (5'-TTCATTAACCGCTGATTATTTTC AAC-3') and Deer R (5'-CACGATATGAGAAATTATACC AAACC-3'). This amplification procedure yielded 680-bp fragments of the COI gene; these were aligned using the Clustal-X program package (Thompson et al., 1997) and manually edited using SeA 1.1.0a (Rambaut, 1996).

Intraspecific phylogeny was inferred by neighbor-joining (NJ) implemented in PAUP* 4.0b10 (Swofford, 2003) and Bayesian inference (BI) using MrBayes 3.2 (Ronquist et al., 2012). The NJ tree was generated using the K2P model, and the robustness of the tree was tested with 1,000 pseudoreplications. For the BI analysis, the HKY model was selected as the best-fit model under the Akaike Information Criterion

© This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/3.0/>) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

***To whom correspondence should be addressed**
Tel: 82-33-250-8366, Fax: 82-33-257-8361
E-mail: parky@kangwon.ac.kr

Table 1. List of the samples used in this study

Common name	Scientific name	Sampling locality	Species abbreviation	Genbank accession No.	
Korean water deer	<i>Hydropotes inermis argyropus</i>	Yanggu, Gangwon Province, South Korea	YG1	KP100311	
	<i>H. i. argyropus</i>	Yanggu, Gangwon Province, South Korea	YG3	KP100312	
	<i>H. i. argyropus</i>	Yanggu, Gangwon Province, South Korea	YG4	KP100313	
	<i>H. i. argyropus</i>	Yanggu, Gangwon Province, South Korea	YG5	KP100314	
	<i>H. i. argyropus</i>	Yanggu, Gangwon Province, South Korea	YG6	KP100315	
	<i>H. i. argyropus</i>	Yanggu, Gangwon Province, South Korea	YG7	KP100316	
	<i>H. i. argyropus</i>	Yanggu, Gangwon Province, South Korea	YG8	KP100317	
	<i>H. i. argyropus</i>	Yanggu, Gangwon Province, South Korea	YG9	KP100318	
	<i>H. i. argyropus</i>	Jiri Mountain Province, South Korea	JR16	KP100320	
	<i>H. i. argyropus</i>	Jiri Mountain Province, South Korea	JR17	KP100321	
	<i>H. i. argyropus</i>	Jiri Mountain Province, South Korea	JR18	KP100322	
	<i>H. i. argyropus</i>	Jiri Mountain Province, South Korea	JR19	KP100323	
	<i>H. i. argyropus</i>	Jiri Mountain Province, South Korea	JR20	KP100324	
	<i>H. i. argyropus</i>	Sokcho, Gangwon Province, South Korea	SC15	KP100307	
	<i>H. i. argyropus</i>	Sokcho, Gangwon Province, South Korea	SC21	KP100308	
	<i>H. i. argyropus</i>	Sokcho, Gangwon Province, South Korea	SC22	KP100309	
	<i>H. i. argyropus</i>	Sokcho, Gangwon Province, South Korea	SC24	KP100310	
	<i>H. i. argyropus</i>	Hongcheon, Gangwon Province, South Korea	HC23	KP100319	
	Chinese water deer ^a	<i>H. i. inermis</i>	Jingsu, China	J1	GQ329030
		<i>H. i. inermis</i>	Zhejiang, China	ZH1	GQ329027
<i>H. i. inermis</i>		Zhejiang, China	ZH2	GQ329028	
Tufted deer ^a	<i>Elaphodus cephalophus</i>	Unknown	–	DQ873526	
Eurasian elk ^a	<i>Alces alces</i>	Unknown	–	EU835716	

^aThe sequences of Chinese water deer and Eurasian elk were obtained from the GenBank.

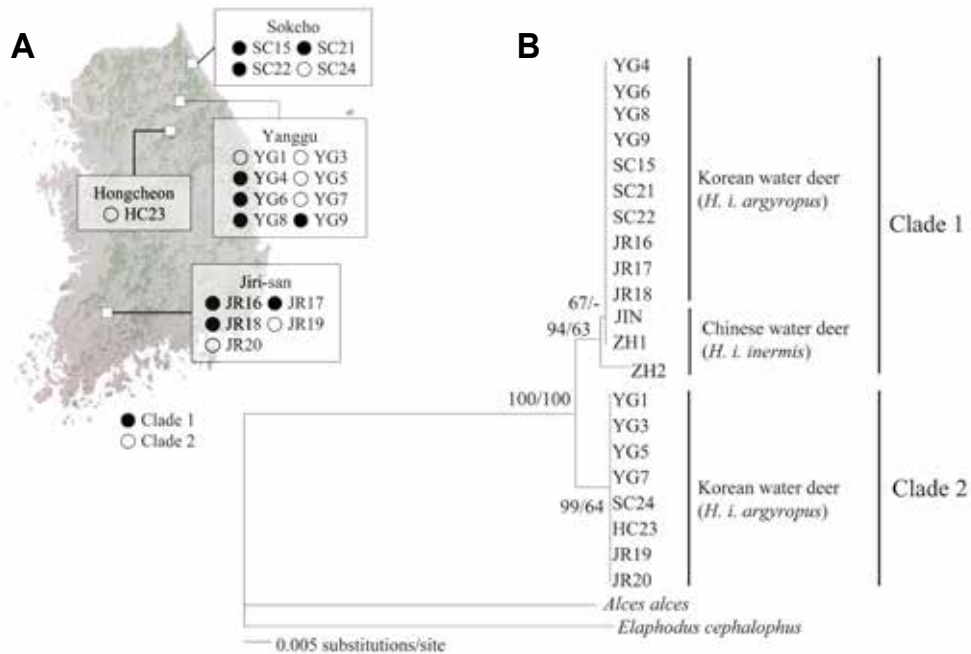


Fig. 1. A, Collection localities for the Korean water deer. Further information on the samples is provided in Table 1; B, Intraspecific phylogeny of Korean and Chinese water deer inferred from the neighbor-joining (NJ) and Bayesian inference (BI) analyses, based on mitochondrial cytochrome oxidase I (COI) gene sequences (680 bp). Only the NJ tree is shown in the figure, and the numbers at each node indicate the bootstrap values for the NJ tree (left) and posterior probabilities (shown as percentages) for the BI tree (right). *Alces alces* and *Elaphodus cephalophus* were used as outgroup species. The Korean water deer formed two separate clades, regardless of the collection localities.

Clade	Subspecies	Population	Nucleotide position													Haplotype				
			1	2	3	3	4	4	4	4	4	6	6	6	6					
Clade 1	<i>H. i. argyropus</i> (Korean water deer)	JR16	T	G	A	A	T	T	A	A	C	T	C	G	C	C	T	T	KH1	
		JR17		
		JR18		
		YG4		
		YG6		
		YG8		
		YG9		
		SC15		
		SC21		
		SC22		
Clade 2	<i>H. i. inermis</i> (Chinese water deer)	JIN	?	CH1		
		ZH1	?		
		ZH2	.	A	.	.	C	C	?	CH2	
Clade 2	<i>H. i. argyropus</i> (Korean water deer)	YG1	C	.	G	G	C	.	A	T	.	C	C	KH2	
		YG3	C	.	G	G	C	.	A	T	.	C		C
		YG5	C	.	G	G	C	.	A	T	.	C		C
		YG7	C	.	G	G	C	.	A	T	.	C		C
		JR19	C	.	G	G	C	.	A	T	.	C		C
		JR20	C	.	G	G	C	.	A	T	.	C		C
		SC24	C	.	G	G	C	.	A	T	.	C		C
		HC23	C	.	G	G	C	.	A	T	.	C		C

Fig. 2. Haplotype patterns of *Hydropotes inermis*. In Korean water deer, two different haplotypes KH1 and KH2 were found; there was no sequence difference between KH1 and the Chinese CH1 haplotype.

Table 2. Pairwise genetic distances between the Korean and Chinese water deer haplotypes

Population	Haplotype	Chinese water deer		Korean water deer	
		CH1	CH2	KH1	KH2
Chinese water deer	CH1	–	–	–	–
	CH2	0.00466	–	–	–
Korean water deer	KH1	0.00000	0.00466	–	–
	KH2	0.01079	0.01555	0.01191	–

(AIC) by using Modeltest 3.7 (Posada and Crandall, 1998). The BI analysis was performed using the Markov chain Monte Carlo technique (MCMC) and one sample every 500 generations was used. The first 25% from each run was discarded as burn-in.

RESULTS AND DISCUSSION

The NJ and BI analyses generated similar trees with identical topologies, with greater support for the NJ tree than the BI tree. The analyses confirmed that Korean water deer could be separated into two phylogroups (Fig. 1B), one of which clustered with the Chinese water deer (JIN, ZH1, and ZH2). This result is consistent with that of a previous study that analyzed the mitochondrial cytochrome *b* gene and its control region and concluded that some Korean populations

might belong to the Chinese water deer (Koh et al., 2009). Two haplotypes (KH1 and KH2) were found in Korean water deer (Fig. 2). The nucleotide sequence of the KH1 haplotype did not differ from that of the Chinese CH1 haplotype. The K2P distance between the Korean KH1 and KH2 haplotypes was 0.012, and it was considerably higher than the distance (0.000–0.005) between the Korean KH1 and two Chinese haplotypes (CH1 and CH2) (Table 2). Thus, the Korean water deer has at least two genetic lineages, namely, *H. i. argyropus* and another that may belong to the Chinese water deer, which was previously thought to be restricted to China. The populations of the two Korean genetic lineages did not show distinct geographic distributions (Fig. 1A).

We conclude from our intraspecific phylogeny and haplotype analysis based on the mitochondrial COI gene sequence that both the Chinese and Korean water deer are distributed across South Korea. Further morphological studies of

the Korean water deer will be required to confirm this reassessment of its taxonomic status.

ACKNOWLEDGMENTS

This work was supported by the Brain Korea 21 PLUS Project in 2014.

REFERENCES

- Allen GM, 1940. Mammals of China and Mongolia. Natural history of central Asia. Vol. 11. American Museum of Natural History, New York, pp. 1126-1261.
- Grubb P, 2005. Artiodactyla: Cervidae: Capreolinae. In: Mammal species of the world: a taxonomic and geographic reference (Eds., Wilson D, Reeder D). The Johns Hopkins University Press, Baltimore, MD, pp. 652-653.
- Harris RB, Duckworth JW, 2008. *Hydropotes inermis*. The IUCN red list of threatened species, version 2014.2 [Internet]. International Union for Conservation of Nature and Natural Resources, Cambridge, Accessed 6 Nov 2014, <<http://www.iucnredlist.org>>.
- Hu J, Fang SG, Wan QH, 2006. Genetic diversity of Chinese water deer (*Hydropotes inermis inermis*): implications for conservation. *Biochemical Genetics*, 44:161-172.
- Koh HS, Lee BK, Wang J, Heo SW, Jang KH, 2009. Two sympatric phylogroups of the Chinese water deer (*Hydropotes inermis*) identified by mitochondrial DNA control region and cytochrome b gene analyses. *Biochemical Genetics*, 47:860-867.
- Posada D, Crandall KA, 1998. Modeltest: testing the model of DNA substitution. *Bioinformatics*, 14:817-818.
- Rambaut A, 1996. Se-AI: sequence alignment editor [Internet]. <<http://evolve.zoo.ox.ac.uk/>>.
- Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP, 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology*, 61:539-542.
- Swofford DL, 2003. PAUP*, phylogenetic analysis using parsimony (*and other methods). 4th ed. Sinauer Associates, Sunderland, MA.
- Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG, 1997. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research*, 25:4876-4882.
- Wang S, 1998. China red data book of endangered animals (mammal volume). Science Press, Beijing, pp. 335-338 (in Chinese).
- Won C, Smith KG, 1999. History and current status of mammals of the Korean peninsula. *Mammal Review*, 29:3-36.
- Won PH, 1967. Illustrated encyclopedia of fauna and flora of Korea, Vol 7. Mammals. Ministry of Education, Seoul, pp. 180-187 (in Korean).
- Xu H, Lu H, Liu X, 1996. The current status and habitat use of Chinese water deer in the coast of Jiangsu Province. *Zoological Research*, 17:217-224.

Received November 27, 2014
 Revised March 24, 2015
 Accepted April 15, 2015