Cranial morphological homogeneity in two subspecies of water deer in China and Korea

Yung Kun KIM[1,3,4]**, Daisuke KOYABU[2]**, Hang LEE[1] and Junpei KIMURA[3]*

[1)]*Conservation Genome Resource Bank for Korean Wildlife, College of Veterinary Medicine, Seoul National University, Seoul 151–742, Korea*
[2)]*The University Museum, The University of Tokyo, 7–3–1 Hongo, Bunkyo-ku, Tokyo 113–0033, Japan*
[3)]*Department of Anatomy and Cell Biology, College of Veterinary Medicine, Seoul National University, Seoul 151–742, Korea*
[4)]*Marine Vertebrate Team, National Marine Biodiversity Institute of Korea, 101-75, Jangsan-ro, Janghang-eup, Seocheon-gun, Chungcheongnam-do, 33662, Korea*

ABSTRACT.   The water deer (*Hydropotes inermis*) has conventionally been classified into two subspecies according to geographic distribution and pelage color pattern: *H. i. inermis* from China and *H. i. argyropus* from Korea. However, the results of a recent molecular study have called this into question. To further reappraise this classification, we examined morphological variation in craniodental measurements of these 2 subspecies. Results of univariate and multivariate analyses demonstrated that these 2 subspecies are not well-differentiated, suggesting that individuals of the 2 populations share common morphological traits. Despite the distribution of the subspecies at different latitudes, no clear morphocline was detected, suggesting that Bergmann's rule does not apply in this case. Discriminant analysis indicated that the characteristics of individuals are shared by both populations, suggesting that not all individuals can be assigned to their original population. Results of principal component analysis showed that the two populations shared more than 75% of individuals, congruent with the "75% rule" of subspecies classification. In both the neighbor-joining and unweighted pair group methods with arithmetic mean cluster analyses, specimens of *H. i. argyropus* and *H. i. inermis* were highly mixed within the cladograms. These results suggest that the overall morphological variation in the 2 subspecies overlaps considerably and that there is no coherent craniofacial difference between the 2 groups. The present findings combined with prior observations from molecular biogeography point out that the taxonomic division of water deer into 2 subspecies should be revisited.

KEY WORDS: Bergmann's rule, biogeography, *Hydropotes inermis*, skull, taxonomy

The water deer (*Hydropotes inermis*) is the only species in the genus *Hydropotes*, subfamily Hydropotinae, family Cervidae. Two subspecies of water deer have traditionally been recognized. One is the Chinese water deer (*H. i. inermis*) [26], distributed in the lower Yangtze Basin, west to Hupeh in China [6]. The other is the Korean water deer (*H. i. argyropus*) [13], distributed throughout the whole of the Korean peninsula [1, 5]. The subspecies classification has been based solely on the pelage color differences between the two populations. The Korean subspecies is reported to have darker pelage, with more reddish coloring in the head region compared to the Chinese subspecies [28]. Otherwise, the 2 subspecies are very similar [28].

A recent molecular study has raised questions about this subspecies classification [17]. The authors studied the mitochondrial DNA (mtDNA) control region (927 bp) and cytochrome *b* gene (1,140 bp) sequences of both popula-

tions. A total of 30 samples from 3 sites in China and 45 samples from 5 sites in Korea were used. The authors demonstrated 2 sympatric mtDNA clades (a major clade from China and Korea and a minor clade from Korea) with an average genetic distance of 2.1% in the control region and 1.3% in the cytochrome *b* gene, respectively. A total of 35 haplotypes from the control region were detected with more than 50% bootstrap values; a major clade consisted of 27 haplotypes from China and Korea, and a minor clade had 8 haplotypes from Korea. Based on the cytochrome-*b* gene, 25 haplotypes were identified. A major clade had 17 haplotypes from China and Korea, and a minor clade had 8 haplotypes from Korea. From this finding, the authors concluded that the current subspecific classification based on pelage color cannot be supported and pointed out the need to morphologically reexamine the validity of the conventional subspecies classification.

In many cases, morphological variation related to adaptations to local climate is found between "subspecies" (i.e., Bergmann's rule) [4]. Bergmann's rule predicts that the average body size of a population in colder areas is generally larger than that in warmer regions due to physiological adaptations to colder environments. Numerous studies have tested Bergmann's rule, and the results have been equivocal, with some observations being consistent and others being inconsistent with the rule. According to Meiri and Dayan

*CORRESPONDENCE TO: KIMURA, J., Department of Anatomy and Cell Biology, College of Veterinary Medicine, Seoul National University, Seoul 151–742, Korea. e-mail: kimura@snu.ac.kr
**The first two authors contributed equally to this study.*

[23], 97 of 149 mammal species from 12 orders (65.1%) follow Bergmann's rule. They reported that the validity of Bergmann's rule differed depending on the taxon. For example, Artiodactyla (7 species), Carnivora (43 species), Cetacea (1 species), Chiroptera (13 species), Didelphimorphia (1 species), Diprotodontia (6 species), Hyracoidea (1 species), Insectivora (10 species), Primates (6 species) and Proboscidea (1 species) generally comply with the rule, whereas Rodentia (51 species) does not. Of the orders that do, some include fewer than 10 species or even only one species, which can be problematic for statistical analysis. That study included the order Artiodactyla, which includes the genus *Hydropotes*. The ranges of the two subspecies of water deer are at notably different latitudes (Chinese population: 30°N and Korean population: 35–38°N; Fig. 1), and the average lowest temperature differs considerably (about 2–8°C in January in the Zhoushan archipelago and about −10°C in January in Korea). In this study, we used skull size as an indicator of Bergmann's rule, instead of body mass, because skull size and body mass have high correlation [16]. If Bergmann's rule holds, we would expect to find larger individuals in the Korean population.

Here, we report the first detailed morphological study of water deer. We examined geographical variation in the skull using 36 measurements and tested the validity of the conventional classification. The difference in sexual dimorphic patterns between the 2 populations was also examined. Based on the results, we suggest the need to reconsider the subspecies classification of water deer.

MATERIALS AND METHODS

Sample collection: In total, 95 crania were examined: 50 *H. i. inermis* (♂=30, ♀=20) and 45 *H. i. argyropus* (♂=28, ♀=17) (Table 1). The specimens were from museums including East China Normal University (ECNU), Shanghai; the Shanghai Science and Technology Museum (SSTM), Shanghai; and the Chinese Academy of Sciences (CAS), Beijing. As locality information for some of the Chinese specimens was missing, we considered all of these specimens as one Chinese population. For the Korean water deer, all specimens were collected by the Conservation Genome Resource Bank for Korean Wildlife (CGRB) and kept in the Department of Anatomy and Cell Biology, College of Veterinary Medicine, Seoul National University. Specimens were limited to adults with fully erupted teeth to avoid age-related bias.

Measurements and statistical analyses: Following the definitions of von den Driesch [9], 36 linear measurements (Fig. 2 and Table 2) were taken on the right side of each skull by one of the authors (Y.K.K.) to the nearest 0.01 mm with digital vernier calipers (Mitutoyo, Tokyo, Japan).

As geographical differences have not yet been reported in this species, we examined the differences in each skull measurement between males and females of both subspecies with a Student's *t*-test using PASW Statistics v18 program (IBM, Chicago, IL, U.S.A.). All data were log-transformed before following multivariate analyses. Principal component analysis (PCA) and subsequent VARIMAX rotation were at-
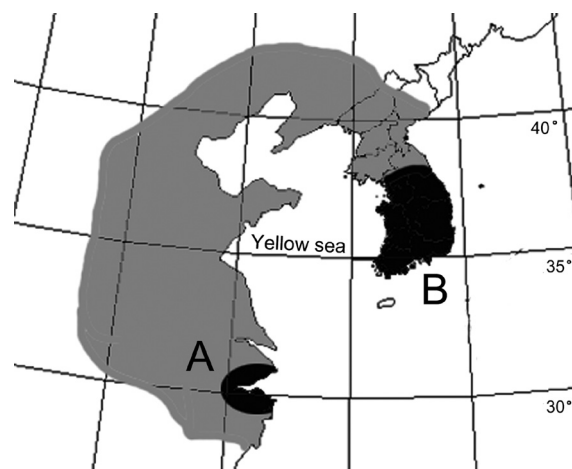

Fig. 1. Range map of water deer. Gray: original range map, black: distribution map of individuals used in this research. A: Chinese population, B: Korean population (redrawn from Whitehead, 1993).

tempted to analyze the variation pattern using PASW Statistics v18 program (IBM) [8]. Standardized Cronbach's alpha value was estimated to assess the reliability of the principal component analysis. The statistical certainty of assignment for individuals into their reference populations was evaluated by discriminant analysis (DA). These analyses were conducted using PAST version 2.12 for DA [11]. Results of PCA were applied to the "75% rule" that defines the criteria for subspecies classification [2].

The overall morphological similarities between and within the two populations were calculated using a Euclidean distance matrix by PopTools [14]. Each Euclidean morphological distance value (*Ed*) was recalculated with the formula $1/(1+Ed)$ to set maximum and minimum values. Using this formula, all morphological distance values were converted into the range 0–1. Here, the pairwise similarity value approaches 1 with increasing morphological similarity between the 2 populations. The hierarchical cluster diagram was drawn using measurement data in PAST version 2.12 [11]. In this clustering analysis, the neighbor-joining (NJ) clustering and the unweighted pair group method with arithmetic mean (UPGMA) clustering methods were conducted to test hierarchical topology among these specimens and were assessed by 1,000 bootstrap replicates.

RESULTS

Univariate analysis: Mean values of 7 skull measurements from female specimens (BFA, LMPR, GBOC, GBB, LFB, GBO and BC) and male specimens (GLN, LMPR, LPR, GBB, GBFM, LFB and BC) were significantly larger for *H. i. argyropus* than *H. i. inermis* (Table 3). In contrast, one measurement from females (BCA) and from males (GBN) was significantly larger for *H. i. inermis* than *H. i. argyropus*. In addition, most average values for all other measurements, which were not significantly different between subspecies,

Table 1.   The number and property of specimen in this study

	H. i. argyropus	*H. i. inermis*	Total
Male	28 (Seoul National Univ.)	30 (ECNU: 3, SSTM: 6 and CAS: 21)	58
Female	17 (Seoul National Univ.)	20 (SSTM: 2, CAS: 18)	37
Total	45	50	95

ECNU: East China Normal University, Shanghai; SSTM: Shanghai Science & Technology Museum; CAS: Chinese Academy of Sciences, Beijing.
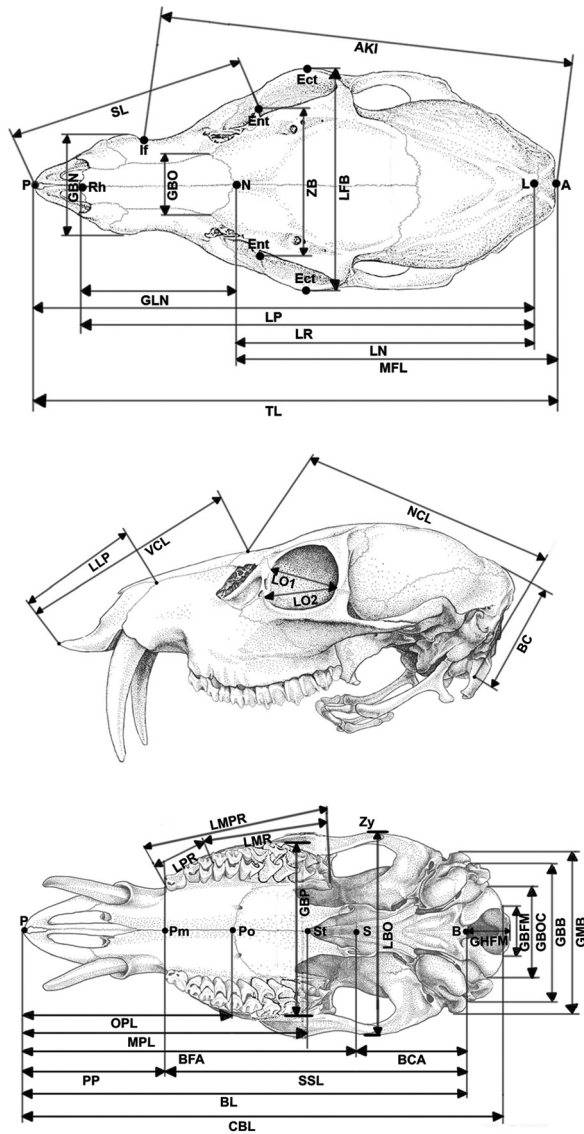

Fig. 2.   Craniofacial measurements. Abbreviations are given in Table 2. A: Akrokranion, B: Basion, Ect: Ectorbitale, Ent: Entorbitale, If: Infraorbitale, L: Lambda, N: Nasion, P: Prosthion, Pm: Premolare, Po: Palatinoorale, Rh: Rhinion, S: Synsphenion, St: Staphylion, and Zy: Zygion.

were larger in *H. i. argyropus* than *H. i. inermis* in females.

PCA: In the PCA of cranium measurements, the first (F1)

Table 2.   Measurements of crania

Abbreviation	Variable
TL	Total length
CBL	Condylobasal length
BL	Basal length
SSL	Short skull length
PP	Premolar − Prosthion
BCA	Basicranial axis
BFA	Basifacial axis
NCL	Neurocranium length
VCL	Viscerocranium length
MFL	Median frontal length
LN	Lambda − Nasion
LR	Lambda − Rhinion
LP	Lambda − Prosthion
AKI	Akrokranion − Infraorbitale of one side
GLN	Greatest length of the nasals
SL	Snout length
MPL	Median palatal length
OPL	Oral palatal length
LLP	Lateral length of the premaxilla
LMPR	Length of the molar and premolar row
LMR	Length of the molar row
LPR	Length of the premolar row
LO1	Length of the upper orbit
LO2	Length of the lower orbit
GMB	Greatest mastoid breadth
GBOC	Greatest breadth of the occipital condyles
GBB	Greatest breadth at the bases of the paraoccipital processes
GBFM	Greatest breadth of the foramen magnum
GHFM	Greatest height of the foramen magnum
LFB	Least frontal breadth
ZB	Zygomatic breadth
LBO	Least breadth between the orbits
GBO	Greatest breadth across the orbits
GBN	Greatest breadth across the nasals
GBP	Greatest breadth across the premaxillae
BC	Basion − the highest point of the superior nuchal crest

Numbers correspond measurements shown in Fig. 2. The measurements were based on Driesch (1976).

and second (F2) components explained 37.90% and 10.53% of the total variation in males (Table 5), and 32.86% and 14.43% of the variation in females (Table 6). The reliability of this analysis as tested by standardized Cronbach's alpha was 0.94 in males and 0.92 in females. Therefore, the reliability of the results was accepted as fairly high. The first

Table 3.　Mean (in millimeters) and Standard Deviation (S.D) of measurements

| Measurements | Male | | | | Female | | | |
| | argyropus | | inermis | | argyropus | | inermis | |
	Mean	S.D	Mean	S.D	Mean	S.D	Mean	S.D
Geometric Mean	56.15		55.85		57.10		56.12	
TL	168.33	4.87	169.22	3.96	173.26	3.56	172.54	4.78
CBL	158.24	5.19	158.59	3.67	163.13	3.31	162.25	4.79
BL	147.79	4.99	148.62	3.50	152.87	3.41	152.07	4.52
SSL	94.36	2.34	94.29	2.43	97.15	1.96	96.37	3.39
PP	53.39	3.07	54.24	1.92	55.67	2.65	55.44	2.35
BCA	36.64	1.62	36.99	2.11	37.82	1.93	**39.91**	3.68
BFA	113.31	3.81	113.81	3.60	117.11	3.32	114.16	4.35
NCL	93.21	3.33	93.78	3.41	94.58	1.99	93.18	3.74
VCL	81.26	3.56	81.29	3.06	84.79	3.39	84.05	3.17
MFL	93.80	3.23	94.54	3.20	95.10	2.17	94.38	3.99
LN	83.25	3.09	83.32	3.35	83.68	2.25	83.57	4.09
LR	135.56	3.86	133.34	4.76	138.02	3.84	135.93	3.80
LP	160.67	4.80	160.73	4.31	164.73	3.95	164.22	4.77
AKI	117.23	3.08	117.29	3.22	120.14	1.87	119.44	4.01
GLN	**52.91**	3.50	50.93	3.93	54.99	3.37	53.10	3.39
SL	80.67	2.82	81.13	2.63	84.15	2.95	83.48	2.96
MPL	95.61	4.38	97.40	3.54	100.18	3.36	99.56	2.97
OPL	72.40	3.46	73.20	2.31	75.38	2.92	74.47	2.71
LLP	46.72	3.06	46.69	3.00	49.04	3.16	48.34	2.64
LMPR	**50.17**	2.22	48.84	1.96	**49.91**	2.40	48.32	2.29
LMR	27.98	1.11	27.90	1.35	27.87	1.46	27.45	1.47
LPR	**23.77**	1.57	22.97	1.04	23.82	1.54	22.89	1.25
LO1	25.44	1.04	25.51	0.79	26.05	0.86	25.86	1.08
LO2	25.24	1.22	24.99	0.93	25.26	1.00	25.13	0.95
GMB	47.28	1.62	47.39	1.93	47.45	1.99	46.93	1.78
GBOC	29.21	2.41	28.21	1.21	**29.28**	0.87	28.13	1.15
GBB	**41.87**	1.27	40.81	1.57	**42.25**	1.67	39.89	1.60
GBFM	14.39	0.83	13.86	0.83	14.06	0.95	14.08	1.18
GHFM	14.87	0.82	14.64	0.94	14.59	1.30	14.97	1.16
LFB	**71.97**	2.66	70.12	2.66	**73.32**	2.16	69.22	3.06
ZB	39.25	1.91	38.22	2.21	40.60	3.02	39.39	2.01
LBO	71.20	2.62	71.11	2.64	71.94	2.45	71.53	3.13
GBO	16.29	1.74	16.24	2.35	**16.76**	1.54	15.29	1.52
GBN	29.59	2.45	**31.28**	2.12	26.80	2.56	25.93	1.86
GBP	51.43	2.18	51.69	1.72	52.70	2.01	51.44	2.08
BC	**41.72**	1.54	40.92	1.48	**42.07**	1.30	40.82	1.59

Bold: significant difference between Korea and China.

Table 4.　Pairwise morphological distance matrix

Male	argyropus	inermis	Female	argyropus	inermis
argyropus	0.972		argyropus	0.973	
inermis	0.971	0.972	inermis	0.970	0.971

8 components which account for more than 1 eigenvalue explained 80.35% of total variance for males (Table 5). For the PC1 of males, values of thirteen components (TL, CBL, BL, SSL, PP, BFA, VCL, LP, AKI, SL, MPL, OPL and LLP) were significant. For females, the first 8 components which account for more than 1 eigenvalue explained 82.90% of total variance for females (Table 6). For the PC1 of females,

values of thirteen components (TL, CBL, BL, PP, BFA, VCL, LR, LP, AKI, SL, MPL, OPL and LLP) were significant. In the scatter plots, individuals of 2 subspecies overlapped each other (Fig. 3). Factor loading values of males and females were not significantly different ($P>0.05$).

DA: The result of DA could not discriminate populations significantly for males and females ($P=0.359$ for males and $P=0.487$ for females). From DA, 70.69% of males and 75.69% of females were correctly classified into their original population. Figure 4 is a bar plot of the DA between the two populations.

Morphological distance and cluster analysis: The within-population and inter-population morphological similarities computed by the Euclidean method were estimated for both

Table 5. Principal components of males which account for more than 1 of eigenvalue from PCA

Variable	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8
TL	**0.769**	**0.535**	0.174	−0.017	0.170	0.033	0.170	−0.012
CBL	**0.841**	0.377	0.191	−0.094	0.073	−0.035	−0.005	−0.114
BL	**0.844**	0.455	0.168	−0.065	0.063	0.042	0.046	0.041
SSL	**0.560**	**0.673**	0.209	0.223	0.027	0.002	−0.058	−0.017
PP	**0.880**	0.135	0.073	−0.300	0.097	0.089	0.139	0.066
BCA	0.113	**0.653**	0.005	−0.340	0.311	0.202	−0.284	0.147
BFA	**0.899**	0.202	0.155	0.115	−0.081	−0.013	0.203	−0.070
NCL	0.052	**0.641**	0.029	0.123	−0.053	0.398	0.156	0.284
VCL	**0.838**	−0.095	0.034	0.022	0.445	−0.035	0.059	−0.056
MFL	0.199	**0.877**	0.142	0.034	−0.173	0.093	0.222	0.028
LN	0.197	**0.846**	0.174	0.102	−0.118	−0.026	0.256	−0.048
LR	0.459	0.414	0.170	0.085	**0.592**	0.020	0.253	−0.231
LP	**0.759**	0.499	0.159	0.077	0.223	−0.030	0.206	−0.077
AKI	**0.518**	**0.683**	0.274	0.038	0.172	−0.042	0.205	−0.130
GLN	0.410	−0.223	0.029	0.014	**0.767**	0.022	0.102	−0.218
SL	**0.863**	0.191	0.121	−0.021	0.199	0.122	−0.036	0.002
MPL	**0.909**	0.001	-0.068	−0.027	−0.041	−0.025	0.100	0.219
OPL	**0.850**	0.089	0.119	−0.040	0.047	0.126	0.091	0.247
LLP	**0.534**	−0.059	0.134	0.008	0.405	0.089	−0.017	0.090
LMPR	−0.020	0.040	0.037	**0.953**	0.053	−0.032	−0.083	−0.111
LMR	0.067	0.149	0.170	**0.823**	−0.055	−0.246	0.061	−0.014
LPR	−0.201	−0.068	−0.197	**0.847**	0.021	0.220	−0.131	−0.052
LO1	0.133	0.319	0.230	−0.130	0.157	0.097	**0.735**	0.241
LO2	0.328	0.171	0.196	−0.099	−0.019	0.089	**0.758**	−0.011
GMB	0.198	**0.545**	**0.510**	−0.097	−0.129	0.106	−0.035	−0.036
GBOC	0.395	0.111	0.193	0.023	0.020	**0.576**	−0.311	0.223
GBB	0.179	0.043	**0.622**	−0.113	0.093	0.460	−0.126	−0.091
GBFM	0.007	0.089	0.086	−0.061	−0.002	**0.838**	0.205	−0.084
GHFM	−0.204	0.068	0.184	−0.033	0.112	0.447	0.078	−0.481
LFB	0.117	0.208	**0.819**	−0.032	0.189	0.104	0.246	−0.065
ZB	−0.018	0.227	0.553	0.137	0.180	0.304	0.064	0.141
LBO	0.262	0.093	**0.678**	0.039	0.205	−0.167	0.376	0.264
GBO	0.089	−0.005	0.356	−0.028	**0.677**	0.031	−0.035	0.210
GBN	0.043	0.059	0.226	−0.261	0.036	0.014	0.155	**0.704**
GBP	0.275	0.171	**0.646**	0.159	0.052	−0.057	0.350	0.392
BC	0.338	0.330	0.457	−0.081	0.027	0.427	−0.095	−0.220
Eigenvalue	13.645	3.790	2.970	2.531	2.256	1.479	1.229	1.024
Proportion	37.903	10.527	8.251	7.030	6.268	4.109	3.415	2.844
Cumulative	37.903	48.430	56.681	63.711	69.979	74.088	77.504	80.347

Bold: absolute >0.5.

sexes (Table 4). For males, the intra-population morphological similarity was 0.972 for both populations, and the inter-population distance was 0.971. For females, the intra-population similarity was 0.973 for the Korean population and 0.971 for the Chinese population. The inter-population similarity was 0.970. Figures 5 and 6 show the results of cluster analysis using the UPGMA and NJ methods, respectively, as well as the cladogram topology. Both methods showed that specimens from each population had mixed topologies in two cladograms; the morphological distance (=similarity, Y-axis) was <1% in the UPGMA cladogram.

DISCUSSION

Morphological differences between H. i. inermis and H. i. argyropus: The present study investigated the 2 subspecies of water deer (*H. inermis*) distributed in Korea and China. The major goal of this research was to morphologically test the conventional subspecific classification of this species. The results of a Student's *t*-test and PCA suggested that these 2 subspecies are not well-differentiated, meaning that individuals of the 2 populations share common morphological traits. DA results also indicated that some individuals share characteristics of both populations, suggesting that not all individuals can be assigned to their original population based on morphometrics. The results of cluster analysis using 2

Table 6. Principal components of females which account for more than 1 of eigenvalue from PCA

Variable	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8
TL	**0.865**	0.402	0.113	0.008	0.098	0.046	0.119	0.114
CBL	**0.842**	0.339	0.074	–0.112	0.182	–0.181	0.064	0.125
BL	**0.845**	0.317	0.057	–0.161	0.144	–0.192	0.069	0.196
SSL	0.497	0.468	–0.063	0.186	0.233	–0.336	0.187	0.460
PP	**0.808**	0.011	0.173	–0.415	–0.010	0.084	-0.129	–0.178
BCA	0.168	0.066	0.196	–0.174	–0.166	–0.108	**0.854**	–0.001
BFA	**0.627**	0.272	–0.071	0.008	0.269	–0.081	**–0.572**	0.197
NCL	0.217	**0.778**	0.068	0.254	0.303	–0.205	–0.065	–0.060
VCL	**0.889**	–0.325	–0.021	–0.036	0.091	–0.015	0.046	0.168
MFL	0.235	**0.861**	0.140	0.254	0.049	0.091	0.076	–0.009
LN	0.333	**0.834**	0.104	0.268	–0.098	0.095	0.051	–0.108
LR	**0.712**	0.289	0.257	0.261	0.152	0.097	0.011	0.330
LP	**0.904**	0.332	0.079	0.063	0.028	0.059	0.116	0.019
AKI	**0.586**	**0.613**	0.181	0.131	0.124	–0.043	0.184	0.240
GLN	0.442	**–0.511**	0.145	0.106	0.228	0.013	–0.065	**0.524**
SL	**0.903**	0.036	–0.190	–0.089	0.092	–0.122	–0.024	–0.144
MPL	**0.823**	0.078	0.157	–0.070	–0.194	0.092	–0.086	0.102
OPL	**0.823**	0.084	0.129	–0.238	–0.057	–0.047	–0.209	–0.231
LLP	**0.635**	–0.040	0.233	–0.069	0.095	0.196	0.170	0.114
LMPR	–0.125	0.178	–0.034	**0.939**	0.108	–0.036	–0.136	0.050
LMR	–0.287	0.119	–0.129	**0.868**	0.102	–0.014	0.069	0.208
LPR	–0.104	0.079	0.094	**0.884**	0.109	0.071	–0.104	–0.119
LO1	0.080	**0.724**	0.292	–0.191	–0.140	–0.055	–0.204	0.150
LO2	0.401	0.064	0.357	0.090	–0.063	0.147	–0.146	**0.501**
GMB	0.179	0.070	**0.786**	0.126	0.017	0.015	0.112	–0.179
GBOC	0.056	–0.026	0.014	0.136	**0.795**	0.237	–0.223	–0.036
GBB	0.122	0.033	**0.511**	0.011	**0.634**	–0.006	0.019	0.050
GBFM	–0.186	0.008	0.089	–0.213	0.286	**0.789**	0.034	0.226
GHFM	0.145	0.010	–0.115	0.205	0.081	**0.790**	–0.073	–0.177
LFB	0.187	0.243	**0.810**	–0.019	0.077	–0.054	–0.108	–0.001
ZB	–0.005	0.378	0.323	0.053	0.354	**–0.538**	0.104	–0.142
LBO	–0.134	0.171	**0.786**	–0.272	0.018	–0.095	0.227	0.206
GBO	0.160	–0.157	**0.730**	–0.001	0.341	0.143	0.067	0.075
GBN	0.025	**0.544**	0.499	–0.144	0.221	–0.312	–0.034	0.058
GBP	0.033	0.203	**0.861**	0.024	0.070	–0.122	0.041	0.143
BC	0.190	0.176	0.327	0.282	**0.691**	–0.033	–0.057	0.134
Eigenvalue	11.830	5.195	3.892	3.141	1.827	1.674	1.199	1.085
Proportion	32.862	14.430	10.811	8.724	5.076	4.651	3.331	3.013
Cumulative	32.862	47.293	58.104	66.828	71.904	76.555	79.886	82.898

Bold: absolute >0.5.

different algorithms, NJ and UPGMA, showed that specimens of the two populations had highly mixed topologies in the cladograms.

Previous research has confirmed that the 2 subspecies have a close genetic distance, with an average genetic distance of 2.1% in the control region and 1.3% in cytochrome *b* [17]. The topology of a phylogenetic NJ tree from the control region and cytochrome *b* showed that *H. i. inermis* and *H. i. argyropus* blended within a major clade. In the present study, it was found that the morphological distance between the populations was also very close. The inter-population similarity was almost the same as the intra-population similarity. These facts suggest that the 2 populations cannot be clearly distinguished genetically nor morphologically.

Bergmann's rule posits that body size is negatively correlated with temperature among closely related species in mammals and birds [4, 15]. This pattern has been pointed out to be obvious, especially within species [19, 20] and has been regarded as one of the major factors producing within-species geographic variation [10, 25]. However, our results show that water deer do not follow Bergmann's rule. TL, which represents skull size, was not significantly different between *H. i. inermis* and *H. i. argyropus* (Table 3). The Zhoushan Islands, the habitat of the Chinese water deer in central China (Fig. 1A), are located around 30°N latitude, and Korean water deer are distributed around 35–38°N latitude. Similar to the present results, others have demonstrated that this rule is not always applicable [3, 7, 22, 24, 27, 29]. Al-

Fig. 4. Frequency distribution of Discriminant analysis by males (up) and females (down). Black: the Korean population and grey: the Chinese population. *P*-value=0.359 for males and 0.487 for females.
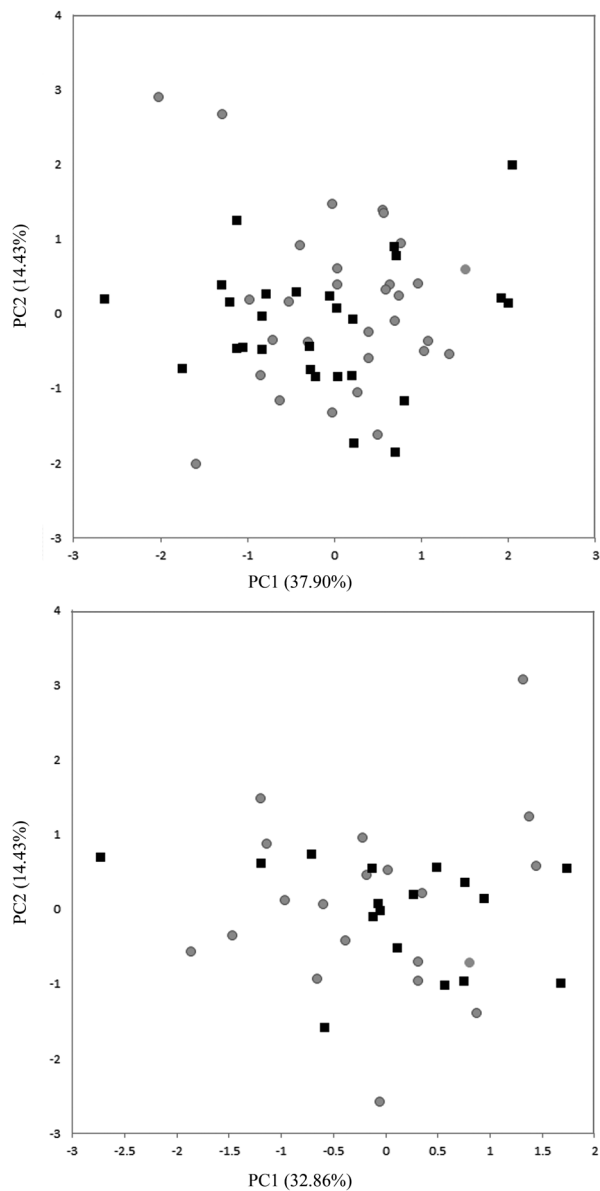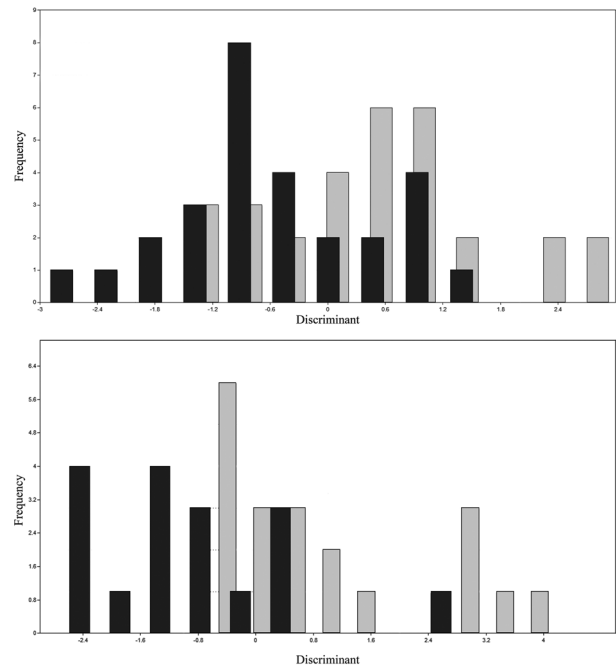

Fig. 3. Two-dimensional scatter plots of the first and second principal component scores of males (up) and females (down). Black: the Korean population and grey: the Chinese population.

though the reason for the lack of clear morphocline in water deer is yet unclear, the establishment of current distribution of this species was perhaps a relatively recent event, producing the observed genetic and morphological homogeneity.

Necessity to reconsider the subspecies classification of water deer: Subspecies of water deer were initially designated by Swinhoe [26] and Heude [13]. Although the concept of subspecies has varied, it is generally defined as members of a polytypic species, not simply as a "slightly different" local population [21]. Results of PCA indicate that the 2 populations show more than 75% of overlap and reject the subspecies classification under the "75% rule" [2]. Phylogenetic

analysis studying the mtDNA proposed that the subspecific classification may not be valid and indicated the need for examination of this issue from a morphological perspective [17]. Our results demonstrate that there is no clear difference in craniodental morphology between the 2 populations, lending further support to the reconsideration of the subspecific classification of water deer. The differences between the Chinese and Korean populations are thus not exceptional, other than their pelage color [28]. However, the pelage color variation has not been studied quantitatively and remains to be evaluated [18].

Although the Chinese water deer was originally distributed widely throughout China, these animals have gradually become rarer, and their distribution has been fragmented over the past century due to poaching for traditional medicine and habitat destruction by industrialization [12, 30, 32, 33]. Today, the Chinese water deer is classified as a vulnerable species by the International Union for Conservation of Nature (IUCN) [12]. In contrast to the situation in China, Korean water deer are distributed throughout the Korean peninsula, where its numbers are both stable and abundant [31]. If the population of Chinese water deer continues to decrease, plans for restoration will become more urgent. Given the homogeneity of Chinese and Korean water deer demonstrated by molecular evidence [17] and morphological evidence (this study), the introduction of Korean water deer into the Chinese population might be the most logical, and ultimately successful, restoration strategy.
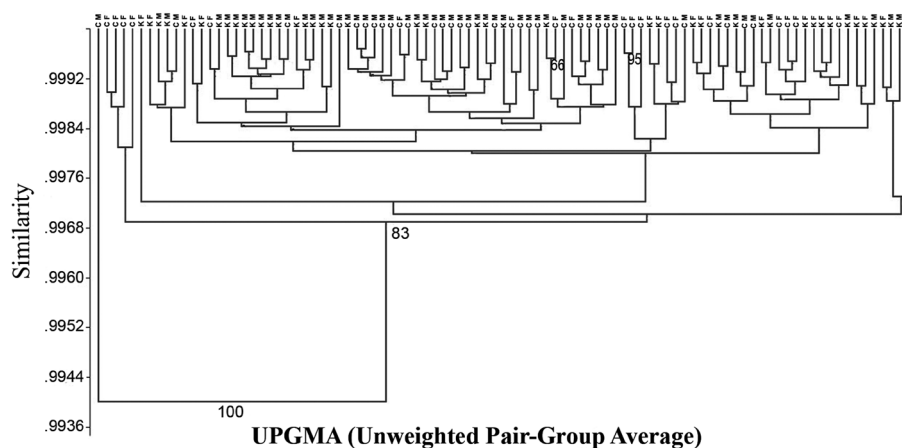
Fig. 5.   UPGMA clustering diagram of the Korean population and Chinese population. (C: Chinese
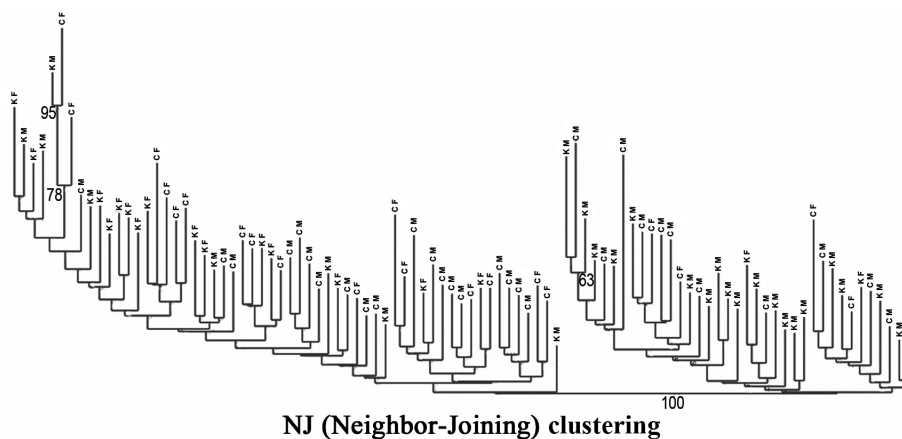population; K: Korean population; M: male; F: female).

Fig. 6.   NJ clustering diagram of the Korean population and Chinese population. (C: Chinese popula-
tion; K: Korean population; M: male; F: female).

REFERENCES

 1. Allen, J. 1940. Mammals of China and Mongolia. The American Museum of Natural History, New York.
 2. Amadon, D. 1949. The seventy-five percent rule for subspecies. *Condor* **51**: 250–258. [CrossRef]
 3. Ashton, K. G., Tracy, M. C. and Queiroz, A. D. 2000. Is Bergmann's rule valid for mammals? *Am. Nat.* **156**: 390–415. [CrossRef]
 4. Bergmann, C. 1847. Uber die Verhaltnisse der Warmekonomie der Thiere zu ihrer Grosse. *Gottinger Studien* **3**: 595–708.
 5. Butzler, W. 1990. Grzimek's encyclopedia of mammals. McGraw-Hill Publishing, New York.
 6. Corbet, G. B. 1978. The mammals of the Palaearctic region: a taxonomic review. British Museum of National History and Cornell University Press, London.
 7. Dayan, T., Simberloff, D., Tchernov, E. and Yom-Tov, Y. 1991. Calibrating the paleothermometer: climate, communities, and the evolution of size. *Paleobiology* **17**: 189–199.
 8. Dixon, W. J. 1987. BMDP Statistical Software. University of California Press, Los Angeles.
 9. von den Driesch, A. 1976. A guide to the measurement of animal bones from archaeological sites. Harvard University Press, Cambridge.
10. Futuyma, D. J. 2009. Evolution. 2nd ed. Sinauer Associates Inc., Sunderland.
11. Hammer, Ø., Harper, D. A. T. and Ryan, P. D. 2001. PAST: Paleontological Statistics Software Package for Education and Data Analysis. *Palaeontol. Electronica* **4**: 9 Available at http://palaeo-electronica.org/2001_1/past/issue1_01.htm.
12. Harris, R. B. and Duckworth, J. W. 2008. *Hydropotes inermis. In*: IUCN 2008. 2008 IUCN Red List of Threatened Species.

Version 3.1. Available at http://www.iucnredlist.org.

13. Heude. 1884. Hydropotes inermis argyropus. *Comptes Rendus des Séances de l'Académie des Sciences* **98**: 1017–1018.

14. Hood, G. M. 2010. PopTools, Version 3.2.5. Pest Animal Control CRC, Canberra. Available at http://www.poptools.org.

15. James, F. C. 1970. Geographic size variation in birds and its relationship to climate. *Ecology* **51**: 365–390. [CrossRef]

16. Janis, C. M. 2005. Correlation of cranial and dental variables with body size in ungulates and macropodoids. pp. 255–300. *In*: Body Size in Mammalia Paleobiology: Estimation and Biological Implications (Damuth, J. and MacFadden, B. J. eds.), Cambridge University Press, New York.

17. Koh, H. S., Lee, B. K., Wang, J., Heo, S. W. and Jang, K. H. 2009. Two sympatric phylogroups of the Chinese water deer (*Hydropotes inermis*) identified by mitochondrial DNA control region and cytochrome *b* gene analyses. *Biochem. Genet.* **47**: 860–867. [Medline] [CrossRef]

18. Koyabu, D. B., Malaivijitnond, S. and Hamada, Y. 2008. Pelage color variation of *Macaca arctoides* and its evolutionary implications. *Int. J. Primatol.* **29**: 531–541. [CrossRef]

19. Mayr, E. 1956. Geographical character gradients and climatic adaptation. *Evolution* **10**: 105–108. [CrossRef]

20. Mayr, E. 1963. Animal species and evolution. Harvard University Press, Cambridge.

21. Mayr, E. 1982. Of what use are subspecies? *Auk* **99**: 593–595.

22. McNab, B. K. 1971. On the ecological significance of Bergmann's rule. *Ecology* **52**: 845–854. [CrossRef]

23. Meiri, S. and Dayan, T. 2003. On the validity of Bergmann's rule. *J. Biogeogr.* **30**: 331–351. [CrossRef]

24. Oishi, T., Uraguchi, K., Abramov, A. V. and Masuda, R. 2010. Geographical variations of the skull in the red fox *Vulpes vulpes* on the Japanese Islands: An exception to Bergmann's rule. *Zoolog. Sci.* **27**: 939–945. [Medline] [CrossRef]

25. Ridley, M. 1996. Evolution. 2nd ed. Blackwell Science, Cambridge.

26. Swinhoe, R. 1870. On a new deer from China (Plates VI. & VII.). In Proceedings of the general meetings for scientific business of the Zoological Society of London. Zoological Science of London, London.

27. Takeuchi, M. 1995. Morphological and ecological study of the red fox *Vulpes vulpes* in Tochigi, central Japan: a biological monograph on morphology, age structure, sex ratio, mortality, population density, diet, daily activity pattern, and home range use. Ph. D. Thesis, Kanagawa University, Kanagawa.

28. Tate, G.H.H. 1940. Mammals of eastern Asia. MacMillan Co., New York.

29. Uraguchi, K. 2009. *Vulpes vulpes* (Linnaeus, 1758). *In*: The wild mammals of Japan. Shoukadoh Book Sellers, Kyoto.

30. Wang, S. 1998. China Red Data Book of Endangered Animals (Mammal Volume). Science Press, Beijing.

31. Won, C. M. and Smith, K. G. 1999. History and current status of mammals of the Korean peninsula. *Mammal Rev.* **29**: 3–36. [CrossRef]

32. Xu, H. F., Zheng, X. Z. and Lu, H. J. 1998. Impact of human activities and habitat changes on distribution of Chinese water deer along the coast area in northern Jiangsu. *Acta Theriol. Sin.* **18**: 161–167 (in Chinese with English abstract).

33. Zhang, E. 1996. Behavioural ecology of the Chinese water deer at Whipsnade Wild Animal Park, England. Ph. D. Thesis, University of Cambridge, Cambridge.