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수의학석사 학위논문

Craniodental geometric morphometry of the Eurasian otter (*Lutra lutra*)

유라시아 수달의 두개골 및 치아에 존재하는
기하학적 형태 계측

2016 년 8 월

서울대학교 대학원

수의학과 수의해부학 전공

Alice LAU Ching Ching

유라시아 수달의 두개골 및 치아에 존재하는 기하학적 형태 계측

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otter (*Lutra lutra*)

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이 논문을 수의학석사 학위논문으로 제출함

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Craniodental geometric morphometry of the Eurasian otter (*Lutra lutra*)

by Alice LAU Ching Ching

A THESIS SUBMITTED IN PARTIAL FULFILLMENT
OF THE REQUIREMENT FOR THE DEGREE OF
MASTER

in

Veterinary Anatomy

Department of Veterinary Medicine, Graduate School
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A Thesis for the Degree of Master of Veterinary Anatomy

**Craniodental geometric morphometry of
the Eurasian otter (*Lutra lutra*)**

Advised by Professor Junpei KIMURA

By Alice LAU Ching Ching

The Dissertation Submits to the Faculty of the Graduate School
of Seoul National University in Partial Fulfillment of the
Requirements for the Degree of Master of Veterinary Anatomy

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Department of Veterinary Medicine
The Graduate School
Seoul National University

Abstract

Craniodental geometric morphometry of the Eurasian otter (*Lutra lutra*)

Eurasian otter (*Lutra lutra*) has been designated as near threatened species by IUCN red list and its population is decreasing since 2004. It's existence in the habitat is an important indicator of healthy wetlands or waterways. Many studies were done focusing on their ecology and population, however, morphological study is limited. Our morphological study of Eurasian otter is the first study by using geometric morphometrics. Geometric morphometrics is a method used to compare the morphological variations in 2D or 3D images. Different from the traditional linear method, this method ensures the description of the shape difference with no effect from the size difference. We identified the presence of sexual dimorphism by using the skull specimens of Korean otter. We also compared the craniodental morphology of Eurasian otter from Korea, Japan and Taiwan. For our first objective, we confirmed that sexual dimorphism exists in Korean otter with the size dimorphism appears to be greater than the shape dimorphism. Based on this result, we did not separate male and female skull specimen in our second objective. For the geographical comparison study, we concluded that morphological variations exist between these three populations.

KEY WORDS: craniodental morphology, Eurasian otter, geographical comparison, geometric morphometrics, sexual dimorphism

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국문초록

유라시아 수달(*Lutra lutra*)의 두개골 및 치아에 존재하는 기하학적 형태 계측

유라시아 수달은 2004년부터 야생의 개체군이 지속적으로 감소하게 되면서 IUCN의 red list에서 위협근접 종으로 지정되었다. 또한 야생에서 수달의 존재는 그 서식지가 건강한 습지나 하천으로 예상할 수 있는 중요한 환경 지표종으로 생태계에서 수달의 역할은 매우 중요하다. 국내에서 이루어진 대부분의 유라시아 수달에 관련한 연구들은 생태학 및 개체군에 관한 연구로, 형태학적 연구는 미비한 상태이다. 본 연구는 유라시아 수달의 두개골 및 치아에 존재하는 기하학적 형태를 계측한 것으로, 국내에서는 첫 시도이다. 수달의 두개골 및 치아에 존재하는 기하학적 형태는 그 크기와 모양의 변형을 평면(2D) 또는 공간(3D)으로 확인하여 계측하였다. 이 방법은 기존의 선형 측정과 달리 크기와 형태를 구분할 수 있다. 본 연구의 목적은 유라시아 수달의 두개골 및 치아에 존재하는 기하학적 형태를 계측함으로써 수컷과 암컷의 성적 이형성을 확인하고, 일본, 한국, 대만에 서식하는 유라시아 수달의 지리적 변이를 비교함에 있다. 한국 유라시아 수달의 성적 이형성에 대한 분석 결과, 크기 이형성이 모양 이형성보다 큰 차이를 보이는 것으로 나타났다. 또한 한국과 일본, 대만에 서식하는 유라시아 수달의 두개골에 대한 지리적 변이를 비교 분석한 결과, 형태의 차이가 큰 것으로 나타났다.

기준어: 두개골 및 치아, 유라시아 수달, 지리적 비교, 기하학적 형태 계측 학적, 성적 이형 성

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Chapter 1. Sexual dimorphism of the Eurasian otter (*Lutra lutra*) in South Korea: Craniodental geometric morphometry

Abstract

Sexual dimorphism of the craniodental morphology of the Eurasian otter in South Korea was studied with geometric morphometrics. 29 adult skulls (15 males and 14 females) were used. Images of the dorsal and ventral view of the cranium and right lateral view of the mandible were taken and then digitized, and measurements were taken on the right side. Results showed that size difference between males and females was significant. Correlations between the size and shape variations have not been observed in this study. The bivariate plots with centroid size showed size dimorphism between males and females with some overlapping. Most relative warp (RW) scores were not significantly different between males and females. We observed only RW2 for dorsal and ventral view of the skull, and only RW1 for mandible was significantly different between the sexes. Shape dimorphisms were revealed at the postorbital constriction, temporal-mandibular joint, coronoid process, mandibular condyle and angular process of the skull. Based on our study, sexual dimorphism exists in Eurasian otter from the South Korean population in terms of both the size and shape. Furthermore, the degree of size dimorphism is greater than shape dimorphism.

KEY WORDS: geometric morphometrics, sexual dimorphism, skull morphology

Introduction

Eurasian otter (*Lutra lutra*) is one of the 13 otter species in the world, and it is the species with the widest distribution (Kruuk, 2006). Various authors recognize 7 to 10 subspecies as a result of their different habitats across the Palaearctic from Ireland to Japan. This highly solitary and territorial species because of its endangered status was also designated in 1982 as a National Natural Monument in South Korea (Kim *et al.*, 2011). Their status and food habits were studied by using scats which revealed that the species can be found widely in the coastal region and some major river systems in the South Korea (Ando *et al.*, 1985).

The mustelid family is well known for its clear sexual size dimorphism, in which males are much bigger and heavier than the females (Ewer, 1985; Kruuk, 2006; Wiig, 1986). One of the earliest study (Wiig, 1986) compared sexual dimorphism between minks, badgers, and Eurasian otters.

Another study (Lynch *et al.*, 1996) also compared the skulls of Eurasian otters from five populations based on craniometrics variation without the mandible, and they found significance in sexual dimorphisms amongst males and females. In this study, we used geometric morphometrics which is the different method from previous studies. This method is now commonly used by biologist to study the form in either two or three dimensions by using a set of landmarks (Zelditch *et al.*, 2012). As size has always been the major consideration when describing the morphological variations, this method however, enables to define the shape separated from size with mathematical implication (Zelditch *et al.*, 2012). In our study using geometric morphometrics, we identified sexual dimorphisms among males and females of Eurasian otter in the South Korean population in terms of size or shape.

Materials and methods

A total of 29 with 15 male and 14 female skulls and mandibles were used for this study. 18 of the specimens are the collection in Department of Anatomy and Cell Biology, College of Veterinary Medicine, Seoul National University, and the other 11 specimens are the collection in Korean Otter Research Center. Only adult skull specimens were selected for this research to prevent the variations as a result from different stages of age. Adult features are determined by the complete suture closure of the cranium and fully erupted molar teeth.

Two-dimensional images were taken with a digital camera with a scale included. Images were taken from the dorsal and ventral view of the cranium, as well as the right lateral view of the mandible. All images taken were digitized by using tpsDig2 (Rohlf, 2010a), followed by the relative warp analysis by using tpsRelw (Rohlf, 2010b). Landmarks (Table 1) in our study were adopted from some of the landmarks used in the study about evolution of sexual dimorphism in mustelids (Berdnikovs, 2005) by using geometric morphometrics. Relative warp analysis is a method developed by Bookstein used to describe the shape variation with the shape deformations among the specimens (Bookstein 1991; Rohlf, 1993). Similar to principal component scores of principal component analysis, this method generated shape scores which are termed relative warp scores in the analysis. In geometric morphometrics, relative warp analysis describes shape variations as in Kendall's definition that location, scale and rotational effects are removed that only the differences of shape will be remained (Zelditch *et al.*, 2012). Kendall also showed that a set of geometric scales could be configured and it is called the centroid size (Zelditch *et al.*, 2012). Distances of all the

landmarks to a centroid will be computed and that making the shape independent from the size. Together with the relative warp analysis, thin-plate spline was used to visualize the deformation of the shape along the significant RW axis by using tpsRelw (Rohlf, 2010b). The data obtained from the analysis were applied in PAST (Hammer *et al.*, 2001) for subsequent statistical analysis. Mann Whitney U-test and MANOVA were performed for comparisons between male and female skull specimens for both size and shape variations. Pearson correlation test (Pearson's r/ two-tailed t test for significance) was also performed in two ways: relative warp scores with the centroid size using all specimens and then each sex separately.

Table 1. Overview of landmarks used.

No.	Dorsal view of cranium	No.	Ventral view of cranium	No.	Right lateral mandible
1	Anterior end of the incisive	1	Intradental superior	1	Coronoid process
2	Nasale	2	Posterior end of palatine	2	Mandibular condyle
3	Lateral end of the aperture	3	Basion	3	Angular process
4	Maxillary outline at the level of nasion	4	Occipital condyle	4	Masseteric fossa
5	Anterior end of the orbital	5	Mastoid process	5	Anterior of canine
6	Mesial end of the orbital	6	Mandibular fossa (left)	6	Molar cusp (on top of hypoconid)
7	Zygomatic process of frontal	7	Mandibular fossa (right)	7	Molar cusp (on top of protoconid)
8	Frontal process of zygomatic	8	Molar and premolar juncture (mesiolingual, first molar)	8	Molar cusp (on top of paraconid)
9	Postorbital constriction	9	Molar and premolar juncture (buccal)		
10	Posterior zygomatic arch	10	Molar and premolar juncture (distolingual, fourth premolar)		
11	Maximum breadth of cranial vault	11	Second premolar and carnassial juncture (buccal)		
12	External occipital protuberance	12	Second premolar and carnassial juncture (lingual)		
		13	Upper canine (posterior)		
		14	Upper canine (anterior)		

Results

The results of Mann-Whitney U-test, Pearson correlation test and MANOVA of dorsal and ventral view of the skull, as well as the mandible are shown in Table 2. In Pearson correlation test, no pair was significant for the comparisons of all the specimens, as well as each sex. Fig. 1 shows the bivariate plots of the centroid size with the RWs that are significantly different (U-test) between male and female of Eurasian otter. The deformation of the shapes is illustrated with thin-plate spline in Fig. 2. The centroid size was significantly different between sexes ($p=0.001$, U-test). In the bivariate plots (Fig. 1 a, b and c), males and females were separated by the size with some overlapping occurred. RW2 of the dorsal view of the skull was significantly different between the sexes which explained 14.54% (Table 2) of the total variation. It indicates the males show narrower postorbital constriction, as well as larger temporal fenestra, in comparison with the females (Fig. 2 a). Postorbital process of frontal bone, postorbital process of zygomatic bone and maxillary outline for males display broader facial cranium and snout than the females (Fig. 2 a). RW2 of ventral view of the skull was also significantly different ($p=0.028$; U-test) (Table 2), which explained 13.65% of the total deformation whose difference was observed at the temporal mandibular joint region as well as landmarks on the palatal region (Fig. 2 b). In the mandible, variation was explained mostly by RW1, accounted 28.33%, and was significantly different between sexes ($p=0.021$; U-test) whose difference was observed at coronoid process, mandibular condyle and angular process, respectively (Fig. 2 c). However, the MANOVA results using 90% cumulative relative warps revealed that only ventral view of the skull is significantly different between sexes ($p=0.013$; MANOVA) (Table 2).

Table 2. Results for Mann Whitney U-test and Pearson correlation test for dorsal view, ventral view of skull and right lateral mandible.

(a)

Dorsal view of skull	Singular values (%)	Result of U-test (p=)	Linear r/p value (RW/Centroid size)	Linear r/p value (Sex/Centroid size)
RW1	32.96	0.948	0.411/ 0.027	F: 0.520/0.057 M: 0.465/0.080
RW2	14.54	0.047	0.431/ 0.020	F: -0.126/0.668 M: 0.399/0.141
RW3	11.47	0.913	0.342/0.069	F: 0.540/ 0.046 M: 0.270/0.331
RW4	9.09	0.132	0.206/0.284	F: -0.142/0.628 M: -0.101/0.721

(b)

Ventral view of skull	Singular values (%)	Result of U-test (p=)	Linear r/p value (RW/Centroid size)	Linear r/p value (Sex/Centroid size)
RW1	19.51	0.647	0.211/0.272	F: -0.011/0.970 M: 0.303/0.273
RW2	13.65	0.028	0.317/0.094	F: -0.015/0.960 M: 0.153/0.587
RW3	13.17	0.326	0.243/0.203	F: -0.019/0.949 M: 0.304/0.271
RW4	9.20	0.055	0.089/0.645	F: 0.253/0.383 M: -0.547/ 0.035

(c)

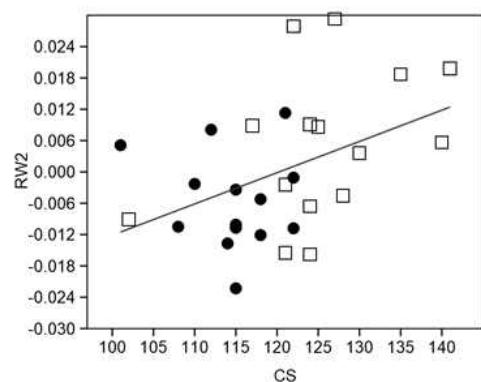
Right lateral mandible	Singular values (%)	Result of U-test (p=)	Linear r/p value (RW/Centroid size)	Linear r/p value (Sex/Centroid size)
RW1	28.33	0.021	0.429/ 0.023	F: -0.086/0.780 M: 0.416/0.123
RW2	25.92	0.174	0.315/0.102	F: 0.057/0.853 M: 0.230/0.410
RW3	11.54	0.963	0.305/0.115	F: 0.538/0.058 M: 0.270/0.331
RW4	10.44	0.818	0.099/0.615	F: 0.268/0.376 M: -0.060/0.832

Table 3. Results for MANOVA with 90% cumulative relative warps for dorsal view, ventral view of skull and right lateral mandible.

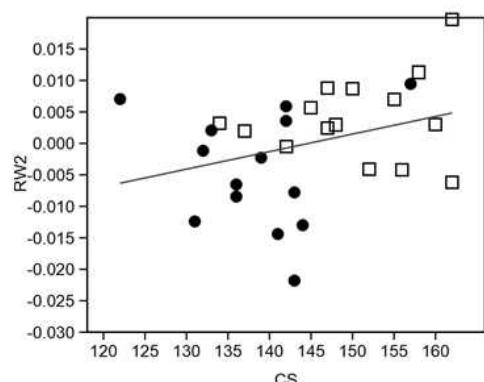
MANOVA (90% cumulative RWs)	
Dorsal view of skull	p=0.701
	Wilks' lambda: 0.751
Ventral view of skull	p= 0.013
	Wilks' lambda: 0.317
Right lateral mandible	p= 0.186
	Wilks' lambda: 0.638

Figure 1. Bivariate plots of centroid size (axis X) with RW which shows differences between the males and females (axis Y) at (a) dorsal view, (b) ventral view of skull and (c) right lateral mandible. Solid circles indicate females; open squares indicate males.

(a)



(b)



(c)

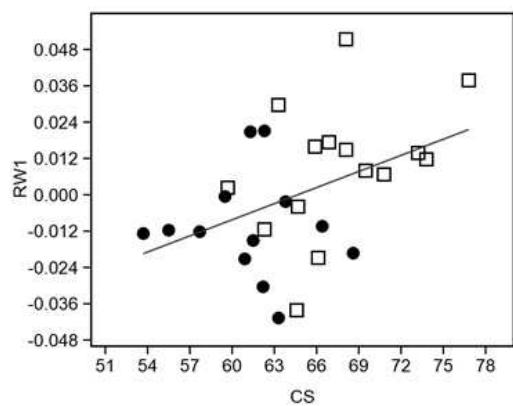
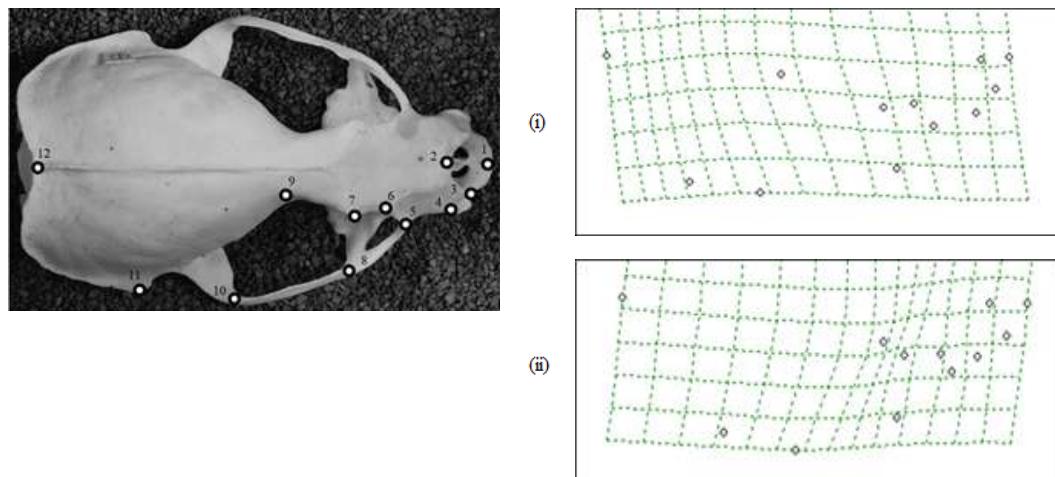
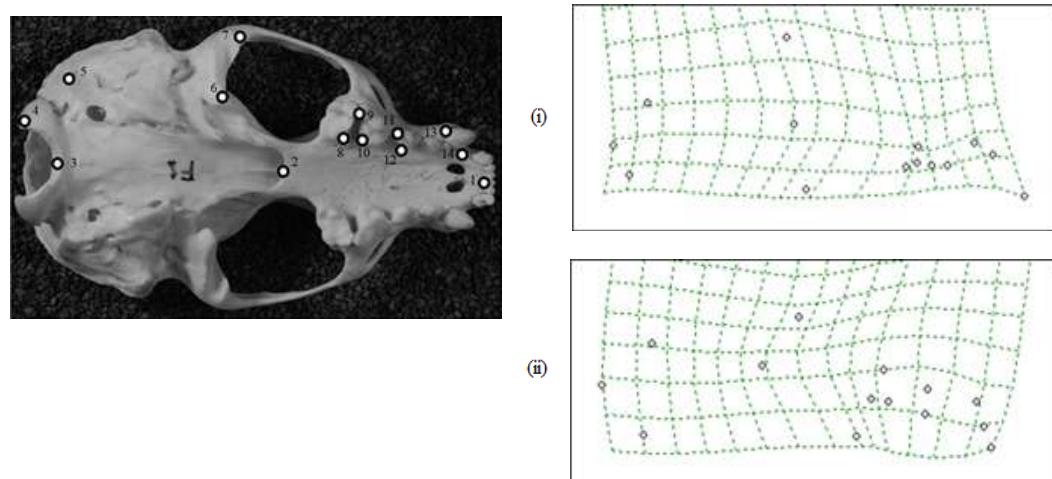


Figure 2. Thin-plate spline showing shape deformation for (a) dorsal view with RW1, (b) ventral view with RW2 of the skull and (c) right lateral mandible with RW1 on deformation grid with (i) from 0.00 to 0.10 and (ii) from 0.00 to -0.10. Landmark description as in Table 1.

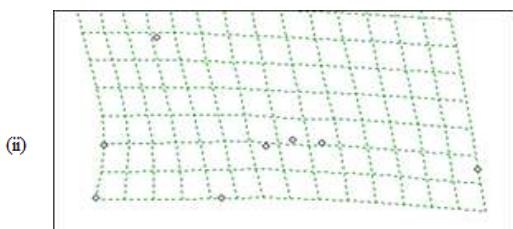
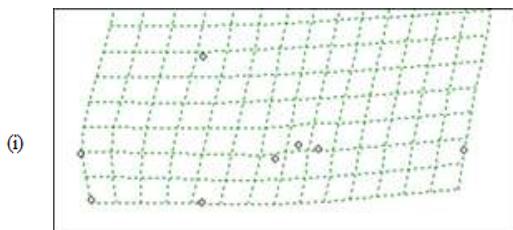
(a)



(b)



(c)



Discussion and conclusion

Sexual dimorphism between minks, badgers and Eurasian otters using skull measurements was studied and the study had found sexual size dimorphism in all three species (Wiig, 1986). In another study with the linear measurement by comparing the five populations of Eurasian otter also revealed that most measurements for males were bigger than females, except for postorbital constriction (Lynch *et al.*, 1996). These findings are consistent with our result where from our centroid size comparison, male skull specimens were larger than females in general (Fig. 1). Both of the previous studies with linear measurement had supported that sexual dimorphism exists in Eurasian otter (Lynch *et al.*, 1996; Wiig, 1986). In the study by comparing the five populations of Eurasian otter, shape dimorphism was found to be greater than the size dimorphism (Lynch *et al.*, 1996). In their study, the first principal component is considered as the size factor, while the rest of the principal component as the shape factor. They performed subsequent canonical variate analyses on all principal components (size-in) and those other than the first component (size-out) and found that the latter analysis well-discriminates sexual dimorphism (Lynch *et al.*, 1996). However, our study shows that size dimorphism appeared to be greater than shape dimorphism, which is shown in our bivariate plots where males and females were separated by the centroid size (Fig. 1 a, b and c) with minimum overlapping. Another evidence that shape dimorphism is low in Korean Eurasian otter from our study is that most relative warps and MANOVA results were insignificant (Table 2). The different results between our study results and previous study (Lynch *et al.*, 1996) could be caused by the difference in the methodology. An example of

these different results can be seen in the sexual dimorphism study (Natori *et al.*, 2003) and shape variation study (Asahara, 2013, 2014) on Japanese raccoon dog skulls. The former study by using linear measurement found sexual dimorphism (Natori *et al.*, 2003), while the latter with geometric morphometrics did not find that in shape (Asahara, 2013, 2014). Among the geographical comparisons in the former study, two of the populations exhibited low degree of sexual dimorphism, but male Japanese raccoon dogs had a longer cranium than the female individuals (Natori *et al.*, 2003). However, the latter two studies did not find any sexual dimorphism, using geometric morphometrics, in shape between populations of Japanese raccoon dogs (Asahara, 2013, 2014).

The relationship between sexual size dimorphism of feeding habits and breeding system of the Eurasian otter was discussed in another study (Moors, 1980). In this study, the researcher hypothesized that intersexual competition for food is present and that small size females are related with less daily maintenance energy required making them more efficient for breeding (Moors, 1980). Rensch's rule about the relation of sexual size dimorphism with body size was also discussed with geometric morphometrics, and noted that larger body size tends to yield greater sexual size dimorphism (Hood, 2000). As in general, the body size and mass differences between the sexes with regional variation are observed in Eurasian otter which matched the findings and discussion in both studies (Hood, 2000; Moors, 1980). Therefore, although the behavioral and feeding ecology are not documented for South Korean population, the above hypotheses from previous studies might be related to our finding of greater size dimorphism than shape dimorphism. However, the theories were claimed to be insufficient to explain the sexual dimorphism observed among the moderately dimorphic species in mustelid family by some researcher (Moors, 1980; Wiig, 1986).

Correlation between size and shape is not directly related with sexual dimorphism. The shape variations indicate that males have narrower postorbital constriction, as well as larger temporal fenestra, in comparison with females. One craniometric study had also pointed out the same finding (Wiig, 1986). However, there was another much more earlier study than that which did not find any difference in the postorbital constriction between the sexes (Hysing-Dahl, 1959). Narrow postorbital constriction in comparison with the skull size is related with the distribution of temporalis muscle, which furtherly affects the biting force (Wiig, 1986). Males had a narrower postorbital constriction in our study. Therefore, males may have greater biting force compared to the females. Furthermore, from the ventral view of the skull, as well as the mandible of Eurasian otter in our study, temporal mandibular joint, coronoid process, mandibular condyle and angular process were dimorphic [Fig. 2 c]. All these features are correlated with the mechanism of jaw closing and biting force that have been discussed in previous studies (Biknevicius and Valkenburgh, 1996; Ewer, 1985). Their studies mentioned that mustelids have well-developed temporalis muscle, as well as well-developed temporal mandibular joint that contributes to the greater biting force during prey hunting (Biknevicius and Valkenburgh, 1996). The differences in biting force could be closely related to the adaptations to the ecology and then contributed to the evolutions in order of Carnivora (Christiansen *et al.*, 2007). Body mass and dietary factors were included in biting force estimation in their study, and they found a positive relation between prey sizes with the biting force (Christiansen *et al.*, 2007). The morphological differences in the skull also allowed for the separation of diets (Lynch *et al.*, 1996). Thus, our findings showed that dietary difference either due

to the adaptation or in conjunction with different body mass and size for the prey selection probably exists among both sexes.

In summary, sexual dimorphism exists in the South Korean Eurasian otter population with size dimorphism being greater than shape dimorphism. Shape dimorphisms in the skulls were observed to a lesser degree and may be related to their feeding habits.

Chapter 2. Craniodental Morphological Comparison of Eurasian otter (*Lutra lutra*) in East Asia: a morphometric and geographic analysis

Abstract

Craniodental morphology of the Eurasian otter (*Lutra lutra*) in the Korean Peninsula, Japanese islands, and Kinmen Island (Taiwan) was studied using geometric morphometrics to identify the skull variations between the populations. Forty adult skulls were examined (29 specimens from the Korean peninsula, six from Shikoku, Honshu, and Hokkaido of Japan, and five from Kinmen Island). Images of the dorsal and ventral view of the skull and the right lateral view of the mandible were analyzed. Specimens from the Korean Peninsula were larger than those from the Japanese islands and Kinmen Island. However, no correlation was observed between the shape variations in the three populations and the centroid size of the skull. The Mann–Whitney *U*-test showed that relative warps (RWs) RW1, RW2, and RW4 of the dorsal view and RW2 of the ventral view of the skull differed significantly between the populations. Some craniodental differences between the populations were seen in the dorsal and ventral view of the skull, mostly at the snout and parietal region. The MANOVA test revealed significant differences between the specimens from the Japanese islands and Korean Peninsula and between the specimens from the Korean Peninsula and

Kinmen Island. RWs plots showed an overlap of all three populations. In conclusion, the comparisons of the three examined populations revealed significant differences in their craniodental morphology.

KEY WORDS: craniodental variations, geometric morphometrics, Japan, Korea, Taiwan

Introduction

The Eurasian otter (*L. lutra*) is distributed across the Palaearctic from Ireland to Japan. Despite the fact that it has the widest distribution among otter species, it has been listed as near threatened on the red list of the International Union for Conservation of Nature (IUCN). Since the year 2004, its population has been continuously decreasing (Kruuk, 2006; www.iucnredlist.org). Seven subspecies are recognized by IUCN: *L. l. lutra*, *L. l. nair*, *L. l. monticola*, *L. l. kutab*, *L. l. aurobrunnea*, *L. l. barang*, and *L. l. chinensis*. *L. l. lutra* is the subspecies found in Korea and *L. l. chinensis*, in Taiwan (www.iucnredlist.org). There is some controversy surrounding the classification of the Japanese otter as it might be classified as a subspecies of *L. lutra* or a distinct species (Endo *et al.*, 2000; Hung *et al.*, 2004; Suzuki *et al.*, 1996; Waku *et al.*, 2016). The IUCN will not recognize it as a distinct species until an additional review clarifies its taxonomic status (www.iucnredlist.org).

Imaizumi and Yoshiyuki (1989) have concluded, on the basis of their morphological study, that the Japanese river otter *L. nippon* is different from *L. lutra*. The *L. l. whitleyi* specimens from Hokkaido have been reported as subspecies of *L. lutra*. Some molecular genetic studies were carried out by different researchers to identify its taxonomic status. Suzuki *et al.* (1996) have reported that the Japanese river otter should be categorized as a species distinct from *L. nippon*. Geographically, *L. nippon* is the species of otter found in Honshu, Kyushu, and Shikoku. Endo *et al.* (2000) have studied skull specimens of the Japanese river otter from Shikoku Island and have revealed that their skull morphology is different from that of the Chinese population. Recently, Waku *et*

al. (2006) have evaluated the phylogenetic status of the Japanese river otter using two specimens from Honshu and Shikoku. The specimens have different divergence times; the individual from Honshu might be a member of *L. lutra* and the specimen from Shikoku, a distinct species. Both species have been reported to be extinct in the Japanese islands (Ando, 2008; Conroy *et al.*, 1998). The Red List of the Ministry of the Environment of Japan cited the Japanese river otter as extinct in 2012, after the last sighting of the animal in 1979 (Ohdachi *et al.*, 2015).

The Eurasian otter is designated a Korean National Natural Monument, and the species is considered endangered in Korea (Kim *et al.*, 2011). Ando *et al.* (1985) have studied the status of the Eurasian otter in Southern Korea and have reported sightings of these animals in the coastal areas and some main river systems. However, a general survey has concluded that the population is declining in Korea (Conroy *et al.*, 1998). A phylogeographic study has suggested that the Korean subspecies is separate from the European *L. l. lutra* and is closer to the Far-eastern Asian species. However, further studies, including specimens from the Amur region and Northeast China, are necessary to confirm its taxonomic status (Koh *et al.*, 2004).

The species is also found in Taiwan (Smith and Xie, 2009) and it has been categorized as endangered by the Wildlife Conservation Act of Taiwan (Lee and Lee, 2015). Foster-Turley *et al.* (1991) have reported no recent status update for the species in Taiwan. Some studies have shown that the Kinmen Island (Fig. 1) is the only area in Taiwan with a stable population of Eurasian otters (Hung *et al.*, 2004; Lee and Lee, 2015). However, this population is now declining (Hung *et al.*, 2004; Lee and Lee, 2015).

We undertook a comparison of the skulls of the Eurasian otters from East Asian regions (the Japanese islands, Korean Peninsula, and Kinmen Island) (Fig. 1) because of the critical status and the limited morphological data on this species. Our aim was to identify the differences between the subspecies or species in terms of size and shape. Besides the molecular phylogenetic clarification (Hung *et al.*, 2004; Imaizumi and Yoshiyuki, 1989; Koh *et al.*, 2004; Suzuki *et al.*, 1996; Waku *et al.*, 2016), the morphological definition might also contribute to better understanding of *Lutra* in East Asia. We used the same methods as in our previous sexual dimorphism study, geometric morphometrics (Bookstein, 1991; Zelditch *et al.*, 2012). The method allows independent comparisons of shape and size (Bookstein, 1991; Lau *et al.*, 2016; Zelditch *et al.*, 2012).

Materials and methods

We examined the specimens collected in the Japanese islands (Honshu and Shikoku), Korean Peninsula, and Kinmen Island, Taiwan (Fig. 1). One specimen from Hokkaido, deposited in the Kyoto University Museum, was also examined. As its taxonomic position is controversial (Imaizumi and Yoshiyuki, 1989), we did not include this specimen in the Japanese island group in our further analysis. The number of the specimens and their sex are shown in Table 1. The details of the museums where we obtained the specimens are shown in Table 2. We selected only the adult skulls with complete suture closure and fully erupted molar teeth. Even though sexual dimorphism is a distinct characteristic in mustelids (Kruuk, 2006), in this study, we analyzed the skulls without separating the males and females. The results of our previous study of sexual dimorphism in Korean otter population have shown a low level of sexual dimorphism in terms of shape (Lau *et al.*, 2016). Furthermore, in the current study, we had a similar number of male and female specimens of each species or subspecies.

For all the specimens, we obtained two-dimensional images of the dorsal and ventral view of the cranium, as well as the right lateral view of the mandible, with a scale included. We then digitized all the images using the tpsDig2 program (Rohlf, 2010a), assigning landmarks to the structures to be compared. Landmarks are homologous points that can be found easily in all the images (Bookstein, 1991; Zelditch *et al.*, 2012). We used the same set of landmarks as in our previous study of sexual dimorphism in the Korean otter (Lau *et al.*, 2016). We compared only the right sides of our specimens, assuming that they were symmetrical. Centroid size is the measure of size used in

geometric morphometrics, independent from the shape (Bookstein, 1991; Zelditch *et al.*, 2012). The landmark coordinates created with tpsDig2 program (Rohlf, 2010a) were then used for the RWs analysis using tpsRelw program (Rohlf, 2010b). In geometric morphometrics, the RW analysis explains shape variations independently from the centroid size (Zelditch *et al.*, 2012). The method is similar to the principal component analysis but, instead of principal component scores, it generates the RW scores (Bookstein, 1991; Zelditch *et al.*, 2012). The RW scores were used in the statistical analysis. Shape deformations along the significant RW axis were visualized using tpsRelw program (Rohlf, 2010b) and presented using thin-plate splines (Bookstein, 1991; Zelditch *et al.*, 2012).

The data obtained from the RW analysis were entered into the PAST program (Hammer *et al.*, 2001) for statistical analysis. Mann–Whitney *U*-test, Pearson’s correlation test (Pearson’s r/two-tailed t-test of significance), and MANOVA (multivariate analysis of variance) were performed. The Mann–Whitney *U*-test was conducted to compare the centroid sizes within the populations. Then, we compared the RW scores in different populations to identify the shape variations. We used the Pearson’s test to analyze the correlation between RW scores and centroid size: first, using all specimens and then for each population separately. MANOVA was conducted with 90% cumulative of RW scores. All the significant data were further adjusted using the Holm–Bonferroni correction. Box plots were used to visualize the centroid size differences between the populations.

Figure 1. Geographical distribution of the otter populations from Japanese islands, Korean peninsula and Kinmen Island, Taiwan.

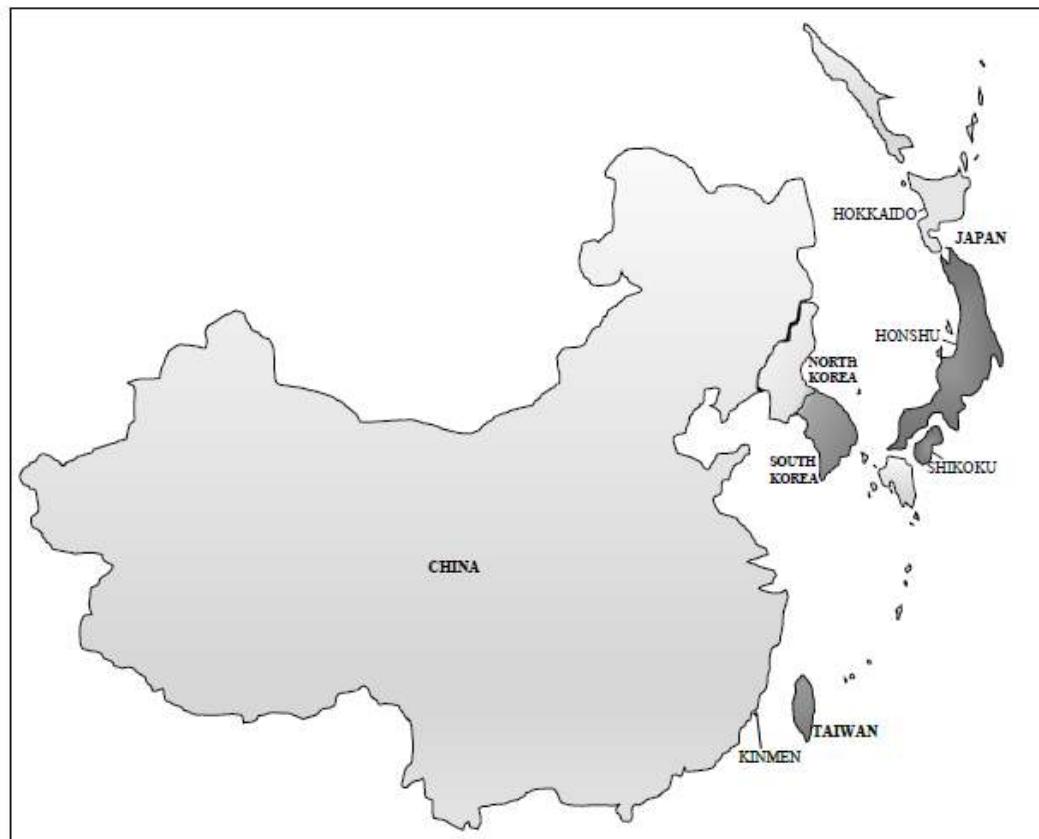


Table 1. Number of specimens for each sex of each country that were used in this study for both dorsal and ventral view of cranium and mandible.

Country		Sex			
		Male	Female	Unknown	Total
Cranium: Dorsal view	Japan	2	2	1	5
	Korea	16	13		29
	Taiwan	3	2		5
	Total	21	18	1	40
Cranium: Ventral view	Japan	2	2	1	5
	Korea	16	13		29
	Taiwan	1	1	1	3
	Total	19	17	2	38
Mandible	Japan	2	2	1	5
	Korea	15	13		28
	Taiwan	2	1	1	4
	Total	19	17	2	38

Table 2. Number of specimens based on the country and institution.

Country and institution		Number of specimens
Japan	National Museum of Nature and Science, Tokyo	4
	Ehime Tobe Zoo	1
		Total: 5
Hokkaido, Japan*	The Kyoto University Museum	Total: 1
Korea	Laboratory of Veterinary Anatomy and Cell Biology in Seoul National University	18
	Korean Otter Research Centre	11
		Total: 29
Taiwan	National Taiwan University	1
	Taipei Zoo	1
	National Museum of Natural Science, Taichung	1
	Kinmen National Park and Kinmen County Government, Kinmen Island	3
		Total: 6

*A specimen from Hokkaido is not used for the statistical analyses.

Results

The results of the Mann–Whitney *U*-test and Pearson’s correlation test are shown in Tables 3 and 4. Table 5 shows the MANOVA results. Fig. 3 shows the box plots for the three populations with their centroid sizes. RW plots are shown in Fig 4. Shape deformations along the significant RW axis of the dorsal and ventral view of the skull, as well as the right lateral mandible view, are shown in Fig. 5.

The comparison of centroid sizes of the populations using the Mann–Whitney *U*-test and Holm–Bonferroni correction revealed that they were not significantly different (Table 3). However, the box plots showed that specimens from the Korean Peninsula were larger than those from the Japanese islands and Kinmen Island, Taiwan (Fig. 3). Specimens from Kinmen Island were also smaller than those in the Japanese and Korean populations (Fig. 3).

Results of the Mann–Whitney *U*-test showed that RW1 analysis ($p = 0.015$ for Korean and Japanese pair; *U*-test), RW2 ($p = 0.011$ for Korean and Taiwanese pair; *U*-test), and RW4 ($p = 0.005$ for Korean and Japanese pair; *U*-test) (Table 4) for the dorsal view of the skull detected significant differences between the populations. RW1 to RW4 explained 72.98% of the total variations in the dorsal views of the skull. Among the RW plots (Fig. 4 a), the first axis (RW1) best explained the variations between these three populations. The shape variation was observed at the snout region and the parietal bone (Fig. 5 a). We noted that as the RW1 increased, the nasal aperture narrowed, as shown in the deformation grid (Fig. 5 a. i.) The distance between the anterior end and mesial end of the orbital

region appeared to be smaller, too (Fig. 5 a. i.). Furthermore, the distance between the postorbital constriction and the zygomatic process of frontal bone increased, as did the maximum breadth of the cranial vault and the external occipital protuberance (Fig. 5 a. ii.). In the RW plot, the specimens from Kinmen Island overlapped with the specimens from the Korean peninsula (Fig. 4 a). Overall, the individuals from the Japanese islands had a broader nasal aperture than the specimens from the Korean Peninsula. The Korean specimens had much narrower postorbital constriction and smaller nasal aperture and orbit. However, the Korean group showed the larger distance between the maximum width of the cranial vault and the external occipital protuberance, which indicated that the parietal bone in the Korean group was larger than in the other two populations. The specimens from the Japanese islands and Kinmen Island had a smaller distance between these two landmarks than the Korean individuals (Fig. 5 a).

The ventral view of the skull was significantly different in the Japanese and Korean populations in the RW2 ($p = 0.007$; *U*-test) (Table 4). RW2 explained 14.08% of the total deformation. The RW plots (Fig. 4 b) showed the differences for the mandibular fossa, basion, occipital condyle, and the mastoid process. Changes were also observed at the premolar and molar junctures (Fig. 5 b). There was no correlation between the centroid size and the shape variations (Table 4) either in the comparisons of all the specimens or in each population. Thus, the variations observed in the RWs were not a result of size differences.

MANOVA results revealed that, in the dorsal view of the skull, the skull shape was significantly different in the specimens from the Korean peninsula in comparison with the Japanese ($p = 0.000$; MANOVA) and Kinmen Island specimens ($p = 0.014$; MANOVA) (Table 5). Other pair comparisons (such as

between the Japanese islands and Kinmen Island) did not show significant differences for either the dorsal or the ventral view of the skull. For the mandible, no significant differences were shown in either the Mann–Whitney *U*-test (Table 4) or MANOVA results (Table 5) for any of the pairs.

In the RW plots (Fig. 4 a, b, and c), all populations overlap for dorsal and ventral views of the skull, as they do for the right lateral mandible view. One specimen from Hokkaido that had been included in the RW plots plotted closer to the specimens from the Japanese islands.

Table 3. Results for Mann-Whitney U test with the centroid size between the specimens (dorsal view of the skull) from Japanese islands, Korean peninsula and Kinmen Island, Taiwan with Holm-Bonferroni correction (Significance with $p<0.05$, $p<0.025$ for the second smallest p value, $p<0.017$ for the smallest p value). Numbers in bold and italic indicate significance.

	Japanese islands	Korean peninsula	Kinmen Island, Taiwan
Japanese islands	0.381	0.293	
Korean peninsula	0.381	0.024	
Kinmen Island, Taiwan	0.293	0.024	

Table 4. Results for Mann-Whitney U test and Pearson correlation test for specimens from Japanese islands, Korean peninsula and Kinmen Island, Taiwan (Indicated in short as Japan, Korea and Taiwan in the table). Results with Holm-Bonferroni correction (Significance with p<0.05, p<0.025 for the second smallest p value, p<0.017 for the smallest p value). Numbers in bold and italic indicate significance.

Relative warps (RW)	Result of U-test (p=)			Singular values (%)	Linear r/p value (RW/centroid size)	Linear r/p value (Country/ centroid size)			
	Japan	Korea	Taiwan						
Cranium: Dorsal view	RW1	Japan	0.015	0.249	33.18	0.383			
		Korea	0.015	0.496		0.307			
		Taiwan	0.249	0.496		0.427			
	RW2	Japan	0.025	0.835	15.62	0.183			
		Korea	0.025	0.011		1.15E-05			
		Taiwan	0.835	0.011		0.952			
	RW3	Japan	0.466	0.676	14.27	0.486			
		Korea	0.466	0.734		0.375			
		Taiwan	0.676	0.734		0.508			
	RW4	Japan	0.005	0.296	9.37	0.834			
		Korea	0.005	0.080		0.416			
		Taiwan	0.296	0.080		0.410			

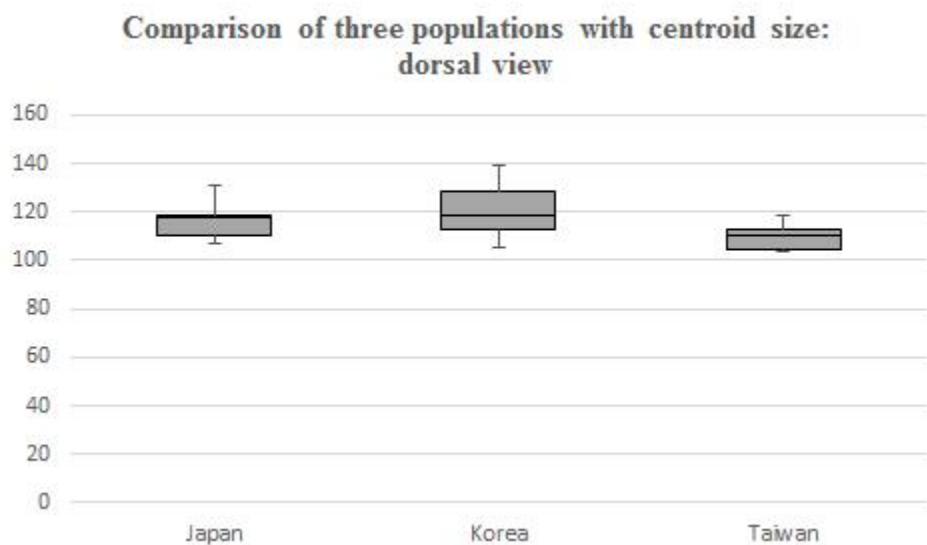
		Japan	Korea	Taiwan	
Cranium: Ventral view	RW1	Japan	0.466	0.766	
		Korea	0.466	0.245	21.30
		Taiwan	0.766	0.245	
	RW2	Japan	0.007	0.037	
		Korea	0.007	0.245	14.42
		Taiwan	0.037	0.245	
	RW3	Japan	0.072	0.037	
		Korea	0.072	0.039	12.11
		Taiwan	0.037	0.039	
Mandible	RW4	Japan	0.065	0.371	
		Korea	0.065	0.796	8.91
		Taiwan	0.371	0.796	
	RW1	Japan	0.940	0.178	
		Korea	0.940	0.073	26.62
		Taiwan	0.178	0.073	
	RW2	Japan	0.269	0.623	
		Korea	0.269	0.628	20.02
		Taiwan	0.623	0.628	
Mandible	RW3	Japan	0.042	0.111	
		Korea	0.042	0.104	17.19
		Taiwan	0.111	0.104	
	RW4	Japan	0.219	0.178	
		Korea	0.219	0.442	9.34
		Taiwan	0.178	0.442	

Table 5. MANOVA results with 90% cumulative relative warps for all the three views with Holm-Bonferroni correction (Significance with $p < 0.05$, $p < 0.025$ for the second smallest p value, $p < 0.017$ for the smallest p value). Numbers in bold and italic indicate significance.

	Country	Japan	Korea	Taiwan
Cranium: Dorsal view	Japan		<i>0.000</i>	
	Korea			<i>0.014</i>
	Taiwan			
Cranium: Ventral view	Japan		0.057	
	Korea			0.043
	Taiwan			
Mandible	Japan		0.034	0.757
	Korea			0.252
	Taiwan			

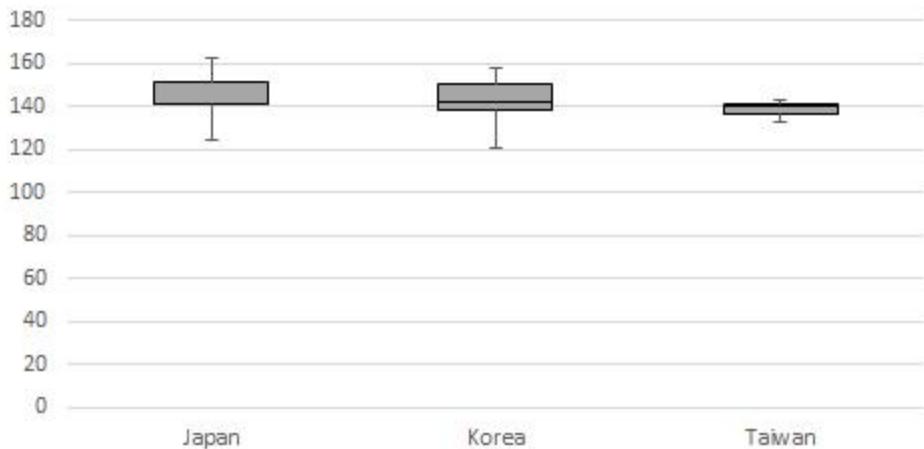
Figure 2. Box plots of each population (X axis) from Japanese islands, Korean peninsula and Kinmen Island, Taiwan (termed as Japan, Korea and Taiwan in chart) were compared for (a) dorsal view; (b) ventral view of the cranium and (c) mandible with the centroid size (Y axis). The third quartile is indicated by the first horizontal line, following by the median and the first quartile. The whiskers extend to the maximum value (on the top) and minimum value (on the bottom).

(a)



(b)

Comparison of three populations with centroid size:
ventral view



(c)

Comparison of three populations with centroid size:
mandible

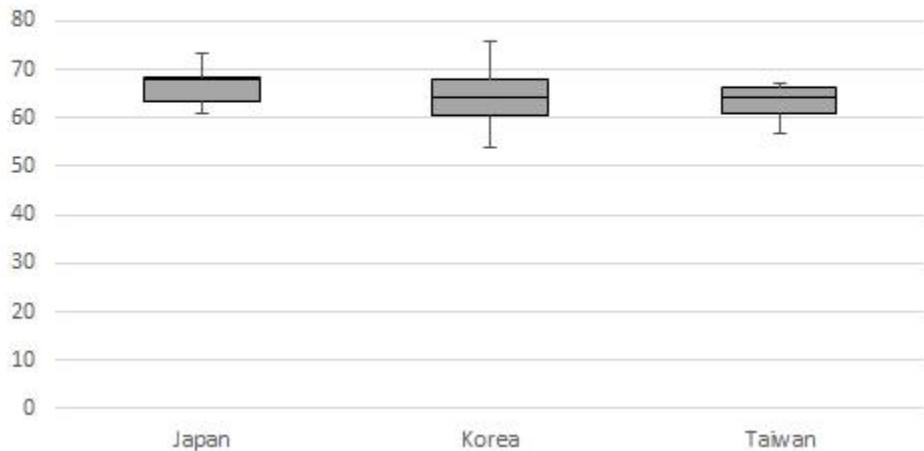
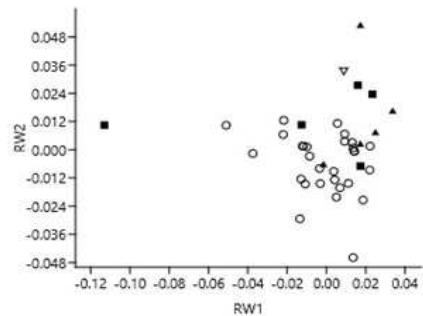
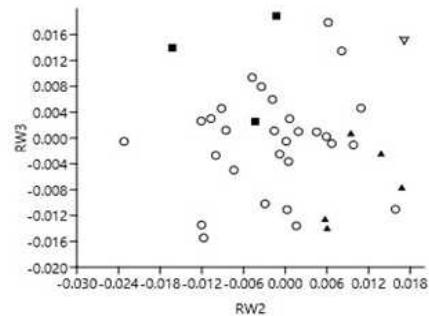


Figure 3. Relative warp (RW) plots of RWs (X axis and Y axis) to show the differences between the populations at (a) RW1 and RW2 of dorsal view, (b) RW2 and RW3 of ventral view of skull and (c) RW3 and RW4 of right lateral mandible. Triangles indicate the specimens from Japanese islands (filled triangles indicate Shikoku and Honshu, inverted open triangle indicates Hokkaido); open circles indicate Korean peninsula; filled squares indicate Kinmen Island, Taiwan.

(a)



(b)



(c)

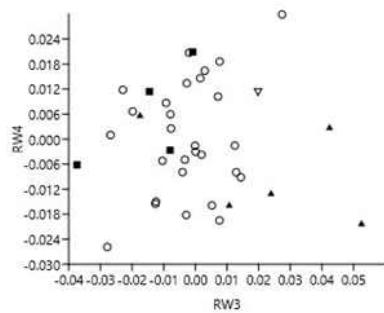
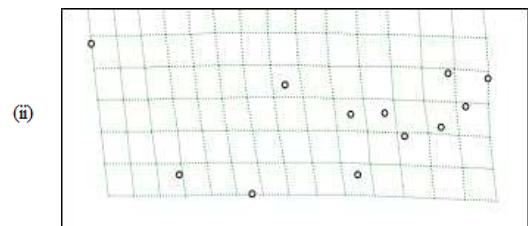
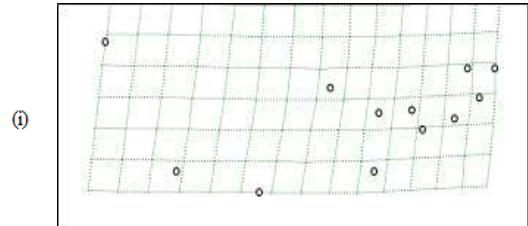
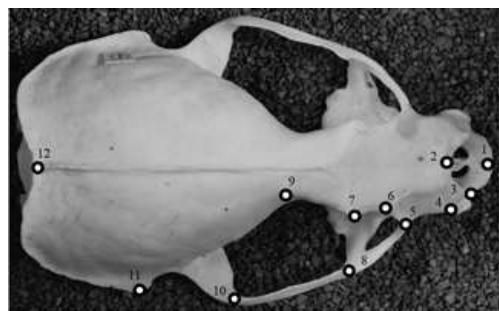
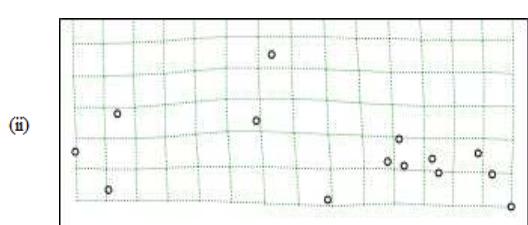
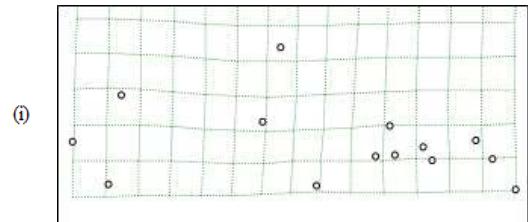
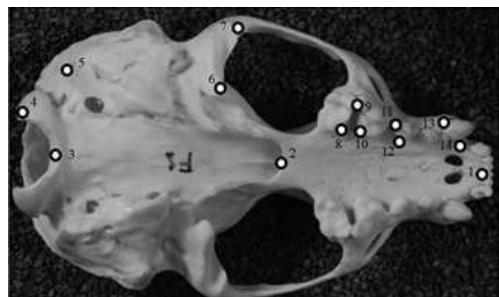


Figure 4. Thin-plate spline showing shape deformation for (a) dorsal view with RW1, (b) ventral view with RW2 of the skull and (c) right lateral mandible with RW1 on deformation grid with (a) (i) from 0.00 to 0.05 and (ii) from 0.00 to -0.05; (b) (i) from 0.00 to 0.03 and (ii) from 0.00 to -0.03 and (c) (i) from 0.00 to 0.05 and (ii) from 0.00 to -0.05.

(a)



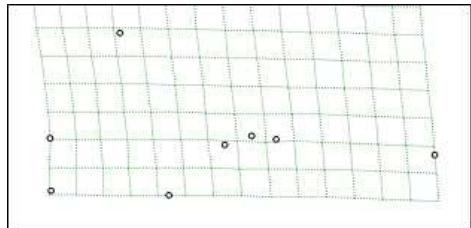
(b)



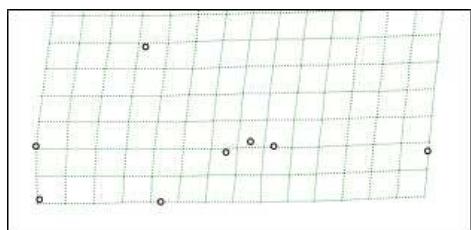
(c)



(i)



(ii)



Discussion and conclusion

A recent molecular phylogeographic study of the Japanese river otter by Waku *et al.* (2016) has concluded that their specimen from Kochi (Shikoku) has a distinct lineage in the *Lutra* clade, while a specimen from Kanagawa (Honshu) might be a subspecies of *L. lutra*. An earlier study by Suzuki *et al.* (1996) has also used the specimen from Kochi and a specimen from Ehime. The study conducted a cytochrome *b* gene analysis and detected a difference between the nucleotide sequence from the Japanese river otter and the Eurasian otters from Latvia and China. The authors have concluded that the Japanese river otter could be a distinct species rather than a subspecies of *L. lutra*. Imaizumi and Yoshiyuki (1989) have described the Japanese river otters from Honshu, Shikoku, and Kyushu as *L. nippon*, a distinct species. Our findings are compatible with their taxonomic studies as we could see significant differences between the specimens from the Japanese islands and Korean peninsula (Mann–Whitney *U*-test and MANOVA; Table 4 and Table 5). Furthermore, the deformation grids (Fig. 5 a, b) also revealed craniodental differences. These were mainly the different width of the nasal aperture, broader or narrower snout, and parietal bone size in the dorsal view of the skull. There were also differences at the mandibular fossa and premolar and molar juncture in the ventral view. Imaizumi and Yoshiyuki (1989) have reported that the Japanese river otter has a different shape of rhinarium and a larger nostril pad but smaller ala nasi. These findings are consistent with the craniodental changes that were observed at the nasal aperture and snout region of the skulls in our study. The divergence of the Japanese otters may be explained

by a migration via the land bridge around 1.27 Ma and the subsequent isolation of the species (Waku *et al.*, 2016).

As mentioned in the study of Koh *et al.* (2004), the subspecies found in the Korean peninsula is probably closer to the subspecies in the Amur and Northeast China regions than to the European otter. The subspecies in Kinmen Island and Taiwan is *L. l. chinensis* (www.iucnredlist.org). *L. l. chinensis* is considered separate from *L. lutra* in Korea and Europe according to the molecular phylogenetic tree of Park *et al.* (personal communication). In our study, the sample from Taiwan was small, making the comparisons harder. However, we found significant differences between the dorsal views of the skull in the Korean and Taiwan specimens (Table 4). We observed that the distance between the external occipital protuberance and other landmarks became shorter, which indicated a smaller parietal bone. Variations were also observed for the landmarks around the nasal region and orbital region. In comparison with the Korean population, specimens from Kinmen Island had a shorter snout region (Fig. 5 a). The craniodental differences between the animals from Kinmen Island and the Korean peninsula could be due to their different divergence. However, there was no significant difference between skulls from the Japanese islands and from Taiwan, which could be due to the paucity of available Taiwan specimens.

Hernandez-Romero *et al.* (2015) have analyzed geographical variation in *Lontra longicaudis* (neotropical otter) using geometric morphometrics. The study has included species distributed across South America and found craniodental changes that could be partly due to geographical separation and distance. The authors have shown that the sizes of the mandibles in the northern (Mexico) and southern (La Plata) populations were significantly different, and the differences

followed Bergmann's rule. We did not perform a regression analysis of the correlation between environmental factors (such as climate and temperature) and the skull size. However, the temperatures are lower in Korea than in Japan and lower in Japan than in Taiwan. This factor might contribute to the size difference shown in the box plots (Fig. 3). We observed that the specimens from the Korean Peninsula were generally the biggest, followed by the specimens from the Japanese islands and Kinmen Island. The differences were not significant (Mann-Whitney *U*-test) (Table 3) after Holm-Bonferroni correction. This might be due to the small sample sizes of the Japanese and Taiwanese populations.

Other factors, such as adaptation to different environments, could also have contributed to the significant changes in craniodental morphology. The diets and different feeding habits are two of the crucial factors that affect craniodental morphology and feeding performance. A recent study by Timm-Davis *et al.* (2015) has analyzed otter skull morphology, taking into account two different feeding habits. The results suggest that the divergences in skull morphology correspond to divergences in feeding modes. The authors have also concluded that the otters have divergent feeding habits. Similarly, Lynch and O' Sullivan (1993) have reported that the cranial morphology of Irish otters might be related to their diet and feeding habits. Brain size and masticatory muscles, particularly the temporalis muscle, might affect the shape of the skull. In our study, we observed variations in the size of the parietal bone (Fig. 5 a), which forms part of the brain case. These bones were smaller in the Japanese and Kinmen Island specimens than in the Korean animals. Furthermore, the width of the postorbital constriction is associated with the size of the temporalis muscle (1993). Our Korean specimens had narrower postorbital constriction (Fig. 5 a) and, thus, a larger temporalis muscle, which allows more powerful bites. Our previous study of sexual

dimorphism in Korean otters discussed the relationship between the temporalis muscle and biting force, which affects hunting (Lau *et al.*, 2016). We have also concluded that differences in biting force might be an evolutionary factor in Carnivora, associated with adaptation to different habitats (Lau *et al.*, 2016).

In summary, we observed the geographic variations in the size and shape of the craniodontal morphology elements in specimens from the Japanese islands, Korean Peninsula, and Kinmen Island. The shape variations observed in our study might be a result of divergences caused by geographical separation and adaptation to different environments. Some difficulties encountered in our study included a paucity of specimens in Japan and Taiwan and a lack of phylogenetic studies of the Korean and Taiwanese populations. Further integrative research, employing both molecular and morphological methods, should include specimens from mainland China and Taiwan.

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