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Quantitative analysis of the skull in the Japanese wolf (*Canis lupus hodophilax*) using CT

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ABSTRACT. In this study using computed tomography (CT), the volumes of the internal cranial cavities, such as the braincase, frontal sinus and tympanic cavity, and the ratio of the volume of each cavity to the skull volume in Japanese wolves were quantified, and CT images of the frontal sinus were observed. The results were then compared with those of other wolf subspecies, including Akita, a dog breed, to clarify the characteristics of the internal cranial cavities in Japanese wolves. The present study revealed that the Japanese wolf had a relatively larger braincase volume and a relatively smaller frontal sinus volume than the wolf ssp. (a group of wild wolf subspecies except the Japanese wolf) and Akita. Moreover, the relative and absolute tympanic cavity volumes of the Japanese wolf and Akita were significantly smaller than those of the wolf ssp. In the CT image or macroscopic observations, the frontal sinuses of the wolf ssp. and Akita were relatively well developed to the caudal and dorsal directions, respectively, compared with that of the Japanese wolf, and the tympanic cavity of the wolf ssp. was more largely swelled ventrally and medially than that of other groups.

KEYWORDS: braincase, CT, frontal sinus, Japanese wolf, tympanic cavity

The gray wolf or wolf (*Canis lupus* Linnaeus, 1758), which is the largest canids, inhabits the Eurasian and North American continents and the North Pole [32, 36]. Dogs (*C. l. familiaris*), classified as subspecies of wolves, were domesticated from wild wolves by humans 15,000 years ago [41, 46]; however, the place of domestication remains controversial. A study on the genetic diversity with mitochondrial DNA (mtDNA) information suggested a Southeast Asian origin for domestication [41], whereas the single nucleotide polymorphism (SNP) genotyping array analysis suggested a simultaneous origin in several areas worldwide [53].

The Japanese wolf (*C. l. hodophilax*) is one of the subspecies of gray wolves and inhabited Honshu, Shikoku and Kyushu islands. Japanese wolves have a distinct mtDNA haplotype and are able to be genetically distinguished from other gray wolves [20, 22–24, 34, 35]. A recent phylogenetic study using the nuclear DNA (nDNA) extracted from nine Japanese wolves suggested that Japanese wolves are genetically separated from other wolves, including ancient wolves [14]. It is also possible to distinguish Japanese wolves from other subspecies by common morphological characters. For example, most Japanese wolves have a rostral alar foramen separated by a bony septum and a horizontal plate of the palatine bone with an anterior notch (Fig. 1) [15, 17, 18, 37–39, 51]. Regarding skull size, that of Japanese wolves is smaller than that of most other subspecies [17].

In the Meiji era (1868–1912), infectious diseases, such as rabies, introduced from overseas spread among Japanese wolves. In addition, with the development of the livestock industry in Japan, the extermination of Japanese wolves was recommended as they attacked livestock [10, 15, 54]. As a result, the population of the Japanese wolf decreased and a male wolf caught at Higashi Yoshino

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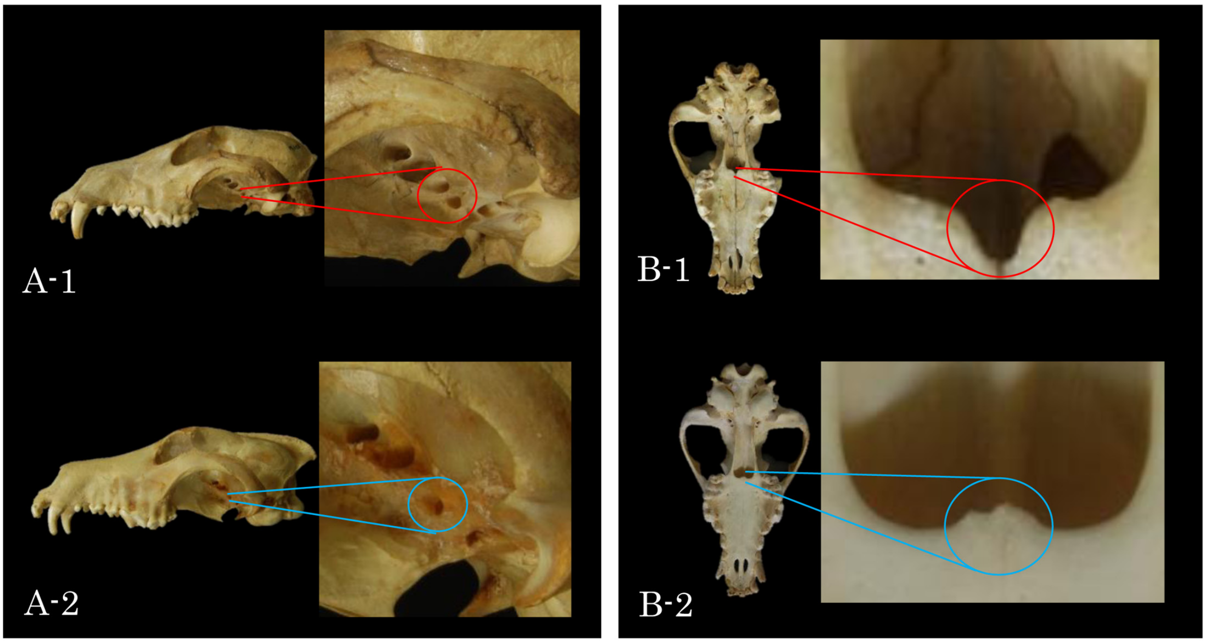

Fig. 1. Characteristics of the skull of Japanese wolves different from the other subspecies of gray wolves. **A**, Left lateral view of skull; **B**, Ventral view of skull; 1, Japanese wolf (M1185): 2, continental wild gray wolf (M31475). Japanese wolves have two rostral alar foramens (**A-1**), whereas the other wolves, including domestic dogs, have only one foramen (**A-2**). The mid-caudal end of the horizontal plate of palatine bone is indented rostrally in Japanese wolves (**B-1**), whereas that of the other wolves protrudes caudally or flat (**B-2**).

village in Nara prefecture on January 23, 1905 (38th year of Meiji) became the last Japanese wolf; therefore, this subspecies is believed to be extinct [10, 15, 44, 54].

In Japan, as taxidermy and preservation skills were underdeveloped before the extinction of Japanese wolves, there are few skin and skeletal specimens of Japanese wolves available. Among morphological studies on the skull of Japanese wolves, the external morphology was actively examined because of easy access to the target parts of the skull, whereas studies on the internal structure are difficult without destroying the skull. Recently, non-destructive analysis of the internal cranial structures in many species, including extinct species, has become possible by the development and widespread use of computed tomography (CT) and related software. There is an increasing number of reports on the volume and morphology of the cranial cavity, which houses the brain, the frontal sinus, which is a part of the paranasal sinuses, and the tympanic cavity, which forms a part of the middle ear [2, 3, 7, 9, 13, 28, 47]. In the examination of the internal cranial structure of several mammals, the classification of fossil species and the functions of the internal morphology have been reported [27, 31, 33, 43, 48, 50]. In this manner, non-destructive analyses of the internal cranial structure using CT have provided new findings in many animals.

In the gray wolf, comparative morphological studies of internal cranial structures have been reported with several other species [6, 30], and between wild populations and domestic dogs [45]. On the other hand, there are only two non-destructive studies of the internal cranial structure of the Japanese wolf [11, 12]. However, these studies did not quantitatively or three-dimensionally examine the internal structures of the skull. The internal cranial cavities, such as the braincase, frontal sinus and tympanic cavity are clear areas surrounded by bones, and it is important to examine these internal cranial structures with external one for a comprehensive understanding of the skull. The aim of this study was to clarify the characteristics of these internal cranial cavities in Japanese wolves by examining the structures non-destructively, quantitatively and three-dimensionally, and then comparing the results with those of other extant gray wolves.

MATERIALS AND METHODS

Specimens

Specimens used in this study are shown in Tables 1–3. In this study, the specimens with the mtDNA haplotype of the Japanese wolf by genetic analysis [22] and the external cranial characteristics of the Japanese wolf were defined as Japanese wolf, and other specimens of wild wolves were defined as wolf ssp. To compare wild wolves with domestic dogs, the skulls of the Akita breed, a native Japanese dog that resembles Japanese wolves in cranial size [11, 56], were used and defined as Akita. In this study, a total of 24 samples of wolves including dogs (4 Japanese wolf; 16 wolf ssp.; 4 Akita) were analyzed. As most samples had no information of sex, the analyses were performed without consideration of sex. Although there was no information of age for all samples, we estimated that all wolves were adults because of the complete closure of cranial sutures.

Table 1. Specimens used in this study

ID	Subspecies	Group name	SEX	Facilities of preservation
M1185	*Canis lupus hodophilax*	Japanese wolf	UKN	National Museum of Nature and Science, Tokyo
M26696	*Canis lupus hodophilax*	Japanese wolf	UKN	National Museum of Nature and Science, Tokyo
Shimane	*Canis lupus hodophilax*	Japanese wolf	UKN	Private
Nara	*Canis lupus hodophilax*	Japanese wolf	UKN	Ooyodo-cho Town Official Board of Education
M17278	*Canis lupus* ssp.	Wolf ssp.	Male	National Museum of Nature and Science, Tokyo
M18304	*Canis lupus* ssp.	Wolf ssp.	Male	National Museum of Nature and Science, Tokyo
M31474	*Canis lupus* ssp.	Wolf ssp.	Male	National Museum of Nature and Science, Tokyo
M31475	*Canis lupus* ssp.	Wolf ssp.	Male	National Museum of Nature and Science, Tokyo
M31476	*Canis lupus* ssp.	Wolf ssp.	UKN	National Museum of Nature and Science, Tokyo
M25791	*Canis lupus* ssp.	Wolf ssp.	UKN	National Museum of Nature and Science, Tokyo
M25792	*Canis lupus* ssp.	Wolf ssp.	UKN	National Museum of Nature and Science, Tokyo
M25793	*Canis lupus* ssp.	Wolf ssp.	UKN	National Museum of Nature and Science, Tokyo
M25794	*Canis lupus* ssp.	Wolf ssp.	UKN	National Museum of Nature and Science, Tokyo
M25795	*Canis lupus* ssp.	Wolf ssp.	UKN	National Museum of Nature and Science, Tokyo
M25796	*Canis lupus* ssp.	Wolf ssp.	UKN	National Museum of Nature and Science, Tokyo
M43287	*Canis lupus* ssp.	Wolf ssp.	Female	National Museum of Nature and Science, Tokyo
KPM-NFM 2014	*Canis lupus* ssp.	Wolf ssp.	UKN	Kanagawa Prefectural Museum of Natural Science
KPM-NFM 2015	*Canis lupus* ssp.	Wolf ssp.	UKN	Kanagawa Prefectural Museum of Natural Science
KPM-NFM 2303	*Canis lupus* ssp.	Wolf ssp.	Male	Kanagawa Prefectural Museum of Natural Science
KPM-NFM 2913	*Canis lupus* ssp.	Wolf ssp.	UKN	Kanagawa Prefectural Museum of Natural Science
M3879	*Canis lupus familiaris*	Akita	Male	National Museum of Nature and Science, Tokyo
M12921	*Canis lupus familiaris*	Akita	Male	National Museum of Nature and Science, Tokyo
M18303	*Canis lupus familiaris*	Akita	Male	National Museum of Nature and Science, Tokyo
M19301	*Canis lupus familiaris*	Akita	Male	National Museum of Nature and Science, Tokyo

ID, specimen number; UKN, unknown. Shimane and Nara are shown as prefecture names where these specimens are preserved because there are no specimen numbers.

Table 2. Descriptions of cranial measurement points

Measurements *	Abbreviations
Total length: Akrokranion−Prosthion	TL
Basal length: Basion−Synsphenion	BL
Zygomatic beradth: Zygion−Zygion	ZB
Skull height. The two pointers of the slide gauge are placed basally on the basis of the skull (on the basioccipital) and dorsally on the highest elevation of the sagittal crest.	SH

*Skulls were measured by the methods of von den Driesch [52].

Table 3. Linear measurements of each skull with a caliper

ID	Group name	Mearurment points			
		TL	BL	ZB	SH
M1185	Japanese wolf	204.3	187.2	NA	64.0
M26696	Japanese wolf	202.5	176.7	116.9	62.5
Shimane*	Japanese wolf	226.9	197.2	128.0	NA
Nara	Japanese wolf	212.5	188.4	116.9	63.7
M17278	Wolf ssp.	221.2	193.2	120.6	66.8
M18304	Wolf ssp.	251.2	226.7	133.7	76.3
M31474	Wolf ssp.	245.8	217.0	134.0	71.3
M31475	Wolf ssp.	247.6	214.4	124.9	72.1
M31476	Wolf ssp.	254.7	221.4	136.4	75.0
M25791	Wolf ssp.	260.3	227.2	141.6	74.3
M25792	Wolf ssp.	246.7	220.3	130.1	71.2
M25793	Wolf ssp.	246.5	NA	135.9	NA
M25794	Wolf ssp.	229.1	210.9	117.8	58.3
M25795	Wolf ssp.	234.4	206.2	131.0	63.4
M25796	Wolf ssp.	258.2	225.9	129.5	72.2
M43287	Wolf ssp.	239.4	212.1	134.0	62.6
KPM-NF1 2014	Wolf ssp.	266.6	235.9	143.0	76.2
KPM-NF1 2015	Wolf ssp.	271.6	235.2	146.0	78.0
KPM-NF1 2303	Wolf ssp.	245.9	217.7	127.3	73.9
KPM-NF1 2913	Wolf ssp.	246.3	221.2	133.8	79.6
M3879	Akita	224.3	197.2	133.1	71.9
M12921	Akita	223.2	200.6	125.0	70.7
M18303	Akita	214.0	193.8	120.6	67.3
M19301	Akita	201.9	184.3	116.3	62.8

ID, specimen number; NA, not analyzed. Measurement points are shown by abbreviations. The relationship between measurement points and abbreviations is described in Table 2. *Shimane was covered with dry soft tissue, so this specimen was measured on the medical workstation for image processing (ziocube, Tokyo, Japan).

CT scanning

All specimens were scanned by a CT scanner (Aquilion LD, Canon, Tokyo, Japan). Scanning conditions were 120 kV, 150 mA and 0.5-mm slice thickness, and scanning data were preserved in DICOM format. A private specimen of the Japanese wolf (ID, Shimane) was covered with dry soft tissue and scanned under different scanning conditions (135 kV and 250 mA). Moreover, KPM-NF1 2913 had a metallic bolt in the occipital bone and was scanned at 135 kV and 300 mA.

Measurement of volume

Using the medical workstation for image processing (Virtual Place Fujin, AZE, Tokyo, Japan), the braincase, bilateral frontal sinus and tympanic cavity were three-dimensionally reconstructed from DICOM data (Fig. 2), and each volume was measured. In the frontal sinus and tympanic cavity, both sides were summed and used for statistical analysis. To compare the ratio of the volume of each cavity to the skull volume, the volume of each skull was measured except tooth volume. M1185 was missing a part of the right zygomatic arch; therefore, the volume of the left half of the skull was doubled. Similarly, M25794 and KPM-NF 2014 had a damaged

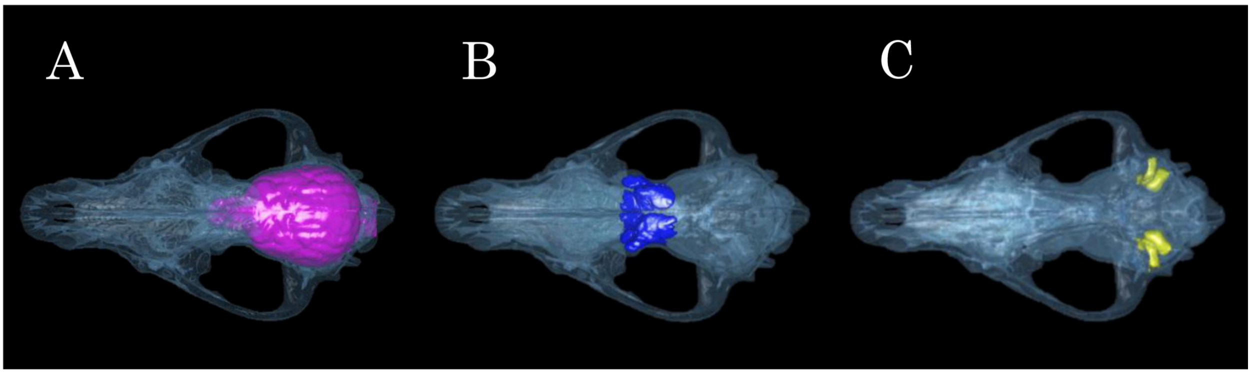

Fig. 2. CT images of the braincase (**A**), frontal sinus (**B**) and tympanic cavity (**C**) within the skull. The skull of the Japanese wolf (Nara) is used in this figure. A and B, dorsal view; C, ventral view.

Table 4. The volume of each cavity and the ratio of each cavity volume to the skull volume

ID	Group name	Skull	Braincase		Frontal sinus				Tympanic cavity			
		Volume (mL)	Volume (mL)	Ratio (%)	Volume (L) (mL)	Volume (R) (mL)	Volume (L+R) (mL)	Ratio (L+R) (%)	Volume (L) (mL)	Volume (R) (mL)	Volume (L+R) (mL)	Ratio (L+R) (%)
M1185	Japanese wolf	343.1	122.4	35.7	4.8	4.7	9.5	2.8	1.97	2.29	4.26	1.24
M26696	Japanese wolf	NA	100.5	NA	4.1	6.0	10.1	NA	2.35	2.33	4.68	NA
Shimane	Japanese wolf	457.3	139.0	30.4	6.0	7.0	13.0	2.8	2.69	2.82	5.51	1.20
Nara	Japanese wolf	407.0	130.4	32.0	5.0	4.5	9.5	2.3	2.27	2.39	4.66	1.14
M17278	Wolf ssp.	425.8	130.3	30.6	13.1	13.7	26.8	6.3	4.54	4.47	9.01	2.12
M18304	Wolf ssp.	623.3	164.4	26.4	19.1	19.0	38.2	6.1	4.92	5.80	10.72	1.72
M31474	Wolf ssp.	433.7	134.1	30.9	7.7	7.2	14.8	3.4	4.40	4.46	8.86	2.04
M31475	Wolf ssp.	471.6	136.4	28.9	14.7	14.6	29.3	6.2	4.76	4.49	9.25	1.96
M31476	Wolf ssp.	633.2	159.2	25.1	12.7	12.8	25.5	4.0	6.59	6.59	13.18	2.08
M25791	Wolf ssp.	479.5	144.2	30.1	13.1	12.6	25.7	5.4	5.15	5.03	10.18	2.12
M25792	Wolf ssp.	571.1	149.1	26.1	11.6	11.1	22.7	4.0	6.14	5.94	12.08	2.12
M25793	Wolf ssp.	589.1	164.7	28.0	6.4	6.6	13.0	2.2	6.12	6.46	12.58	2.14
M25794	Wolf ssp.	583.4	143.1	24.5	17.6	19.1	36.7	6.3	6.52	6.52	13.04	2.24
M25795	Wolf ssp.	555.9	148.0	26.6	12.0	14.9	26.9	4.8	5.33	5.54	10.87	1.96
M25796	Wolf ssp.	NA	157.0	NA	10.2	10.1	20.3	NA	4.84	4.73	9.57	NA
M43287	Wolf ssp.	505.7	132.3	26.2	9.1	9.0	18.1	3.6	4.02	3.95	7.97	1.58
KPM-NFM 2014	Wolf ssp.	723.6	163.7	22.6	15.7	14.6	30.2	4.2	5.54	5.54	11.08	1.53
KPM-NFM 2015	Wolf ssp.	740.8	175.5	23.7	11.8	14.0	25.8	3.5	4.99	5.17	10.16	1.37
KPM-NFM 2303	Wolf ssp.	542.6	149.4	27.5	13.6	13.4	27.0	5.0	7.43	6.57	14.00	2.58
KPM-NFM 2913	Wolf ssp.	641.0	154.6	24.1	16.7	17.0	33.7	5.3	5.35	5.75	11.10	1.73
M3879	Akita	501.1	109.4	21.8	19.2	19.6	38.8	7.7	3.11	3.43	6.54	1.31
M12921	Akita	530.6	99.5	18.8	15.4	16.5	31.9	6.0	2.89	2.91	5.80	1.09
M18303	Akita	390.3	106.8	27.4	13.1	14.4	27.4	7.0	2.10	2.44	4.54	1.16
M19301	Akita	335.7	96.4	28.7	6.8	7.4	14.2	4.2	2.59	2.48	5.07	1.51

ID, specimen number; NA, not analyzed. L, left side; R, right side.

tympanic cavity, the volume of the skull was calculated by doubling the intact side [25, 40], and for the broken tympanic cavity, the same value of the intact cavity was used (Table 4). M26696 and M25796 were omitted from the analysis of the volume ratio of each cavity to the skull volume because of breakage of the occipital part across both sides.

Statistical analysis

The median and mean volumes of the braincase, frontal sinus and tympanic cavity, and the volume ratio of each cavity to the skull volume were calculated in each group. To compare the values among three groups, the Steel-Dwass method was performed with R version 4.0.3 (significant difference $P<0.05$).

RESULTS

Volume of the skull

The volume of each cavity and the ratio of each cavity volume to the skull volume in individuals are shown in Table 4. Regarding the skull volume, the medians (means ± standard error, SE) of the Japanese wolf, wolf ssp. and Akita were 407.0 mL (402.5 ± 33.0 mL), 571.1 mL (568.0 ± 24.6 mL) and 445.7 mL (439.4 ± 45.9 mL), respectively. The wolf ssp. had the largest skull volume among the three groups, followed by the Akita and the Japanese wolf (Fig. 3). In the statistical analysis, the skull volume of the Japanese wolf was significantly smaller than that of the wolf ssp. ($P<0.05$; Fig. 3). The skull volume of the Akita tended to be smaller than that of the wolf ssp. ($P=0.090$), although there was no significant difference between the Akita and wolf ssp.

Braincase

The medians (means) of the braincase volume of the Japanese wolf, wolf ssp. and Akita were 126.4 mL (123.1 ± 8.3 mL), 149.3 mL (150.4 ± 3.3 mL) and 103.2 mL (103.0 ± 3.0 mL), respectively. Based on the statistical analysis, the braincase volume of the Japanese wolf and Akita was significantly smaller than that of the wolf ssp. ($P<0.05$; Fig. 4A). The medians (means) of the braincase volume to the skull volume in the Japanese wolf, wolf ssp. and Akita were 32.0% (32.7 ± 1.6%), 26.4% (26.8 ± 0.7%) and 24.6% (24.2 ± 2.3%), respectively. The statistical analysis revealed that the ratio to the braincase volume in the Japanese wolf was significantly larger than that in the wolf ssp. ($P<0.05$). The ratio in the Japanese wolf tended to be larger than that in the Akita ($P=0.086$), although there was no significant difference between the Japanese wolf and Akita (Fig. 4B). There was no significant difference between the median and mean braincase volume and volume ratio.

Frontal sinus

The medians (means) of the frontal sinus volume in the Japanese wolf, wolf ssp. and Akita were 9.8 mL (10.5 ± 0.8 mL), 26.3 mL (25.9 ± 1.8 mL) and 29.7 mL (28.1 ± 5.8 mL), respectively. According to the statistical analysis, the volume of the frontal sinus in the Japanese wolf was significantly smaller than that in the wolf ssp. ($P<0.05$). The volume in the Japanese wolf tended to be smaller than that in the Akita ($P=0.055$), although there was no significant difference between the Japanese wolf and Akita (Fig. 5A). The medians (means) of the frontal sinus volume ratio to the skull volume in the Japanese wolf, wolf ssp. and Akita were 2.8% (2.6 ± 0.5%), 4.8% (4.6 ± 0.3%) and 6.5% (6.2 ± 0.8%), respectively. The statistical analysis revealed that the frontal sinus volume ratio did not significantly differ among the three groups. However, the ratio in the Japanese wolf was tended to be smaller than that in the other groups (wolf ssp., $P=0.054$; Akita, $P=0.086$). There was no significant difference between the median and mean braincase volume and volume ratio.

In the morphological observation, in the Akita, the stop, which is a depression (slope) made by an altered angle between the nasal and frontal bones [5], was the deepest among the three groups (Fig. 6A). The frontal bone of the Akita was dorsally directed and well

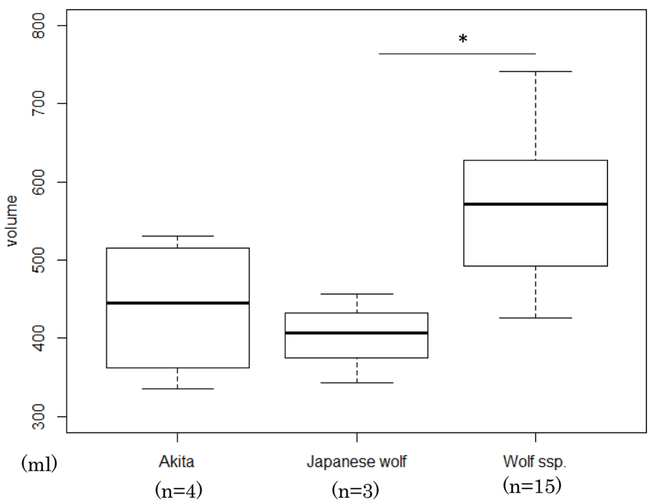

Fig. 3. The representative values of the skull volume in three groups of gray wolves. Asterisk indicates a significant difference (*$P<0.05$, Steel-Dwass test).

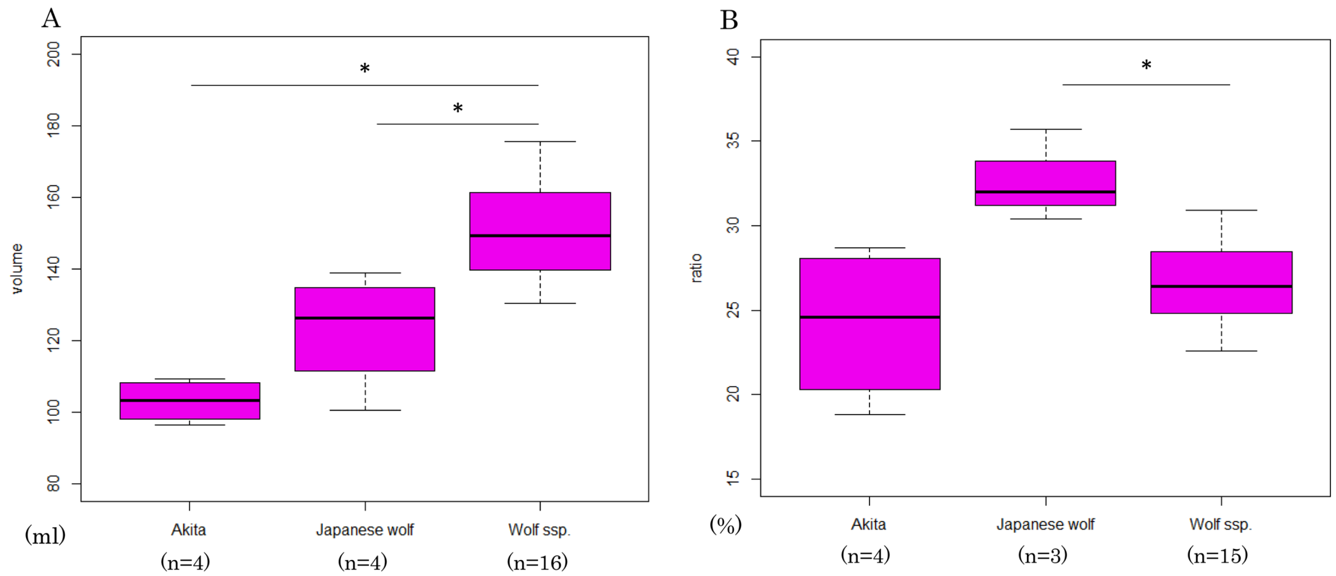

Fig. 4. The representative values of the braincase volume (**A**) and the ratio of the braincase volume to the skull volume (**B**) in three groups of gray wolves. Asterisks indicate a significant difference (*P<0.05, Steel-Dwass test).

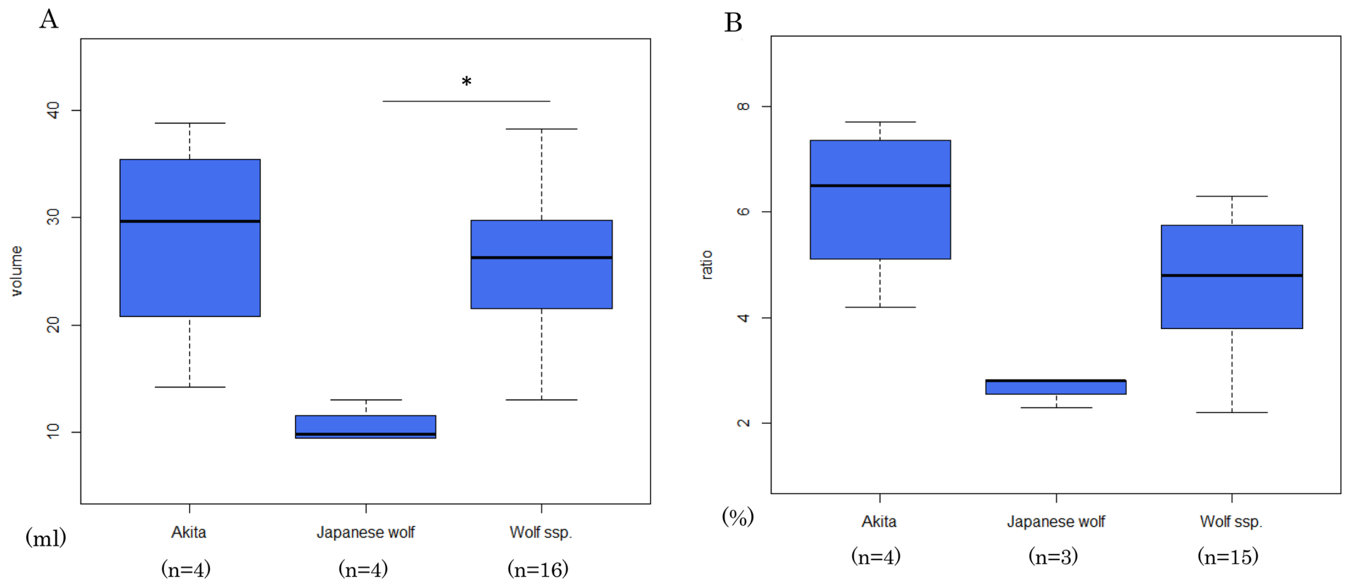

Fig. 5. The representative values of the frontal sinus volume (**A**) and the ratio of the frontal sinus volume to the skull volume (**B**) in three groups of gray wolves. Asterisks indicate a significant difference (*P<0.05, Steel-Dwass test).

developed with a developed stop, thus the frontal sinus was also well developed dorsally with the development of the frontal bone (Fig. 6A and 6C). The frontal sinus of the Japanese wolf did not extend posteriorly to the skull compared with the wolf ssp. and Akita (Figs. 2B and 6B).

Tympanic cavity

The medians (means) of the tympanic cavity in the Japanese wolf, wolf ssp. and Akita were 4.7 mL (4.8 ± 0.3 mL), 10.8 mL (10.9 ± 0.4 mL) and 5.4 mL (5.5 ± 0.4 mL), respectively. Based on the statistical analysis, the volumes of the tympanic cavity in the Japanese wolf and Akita were significantly smaller than that in the wolf ssp. (P<0.01; Fig. 7A). The medians (means) of the tympanic cavity volume ratio to the skull volume in the Japanese wolf, wolf ssp. and Akita were 1.2% (1.2 ± 0.0%), 2.0% (2.0 ± 0.1%) and 1.2% (1.3 ± 0.1%), respectively. The statistical analysis demonstrated that the ratio of the tympanic cavity volume did not significantly differ between the Japanese wolf and Akita, but the ratio in the wolf ssp. was significantly larger than that in the other two groups (P<0.05; Fig. 7B). There was no significant difference between the median and mean braincase volume and volume ratio.

Furthermore, the tympanic bulla of the wolf ssp. swelled more ventrally than that of the Japanese wolf and Akita (Figs. 1 and 8).

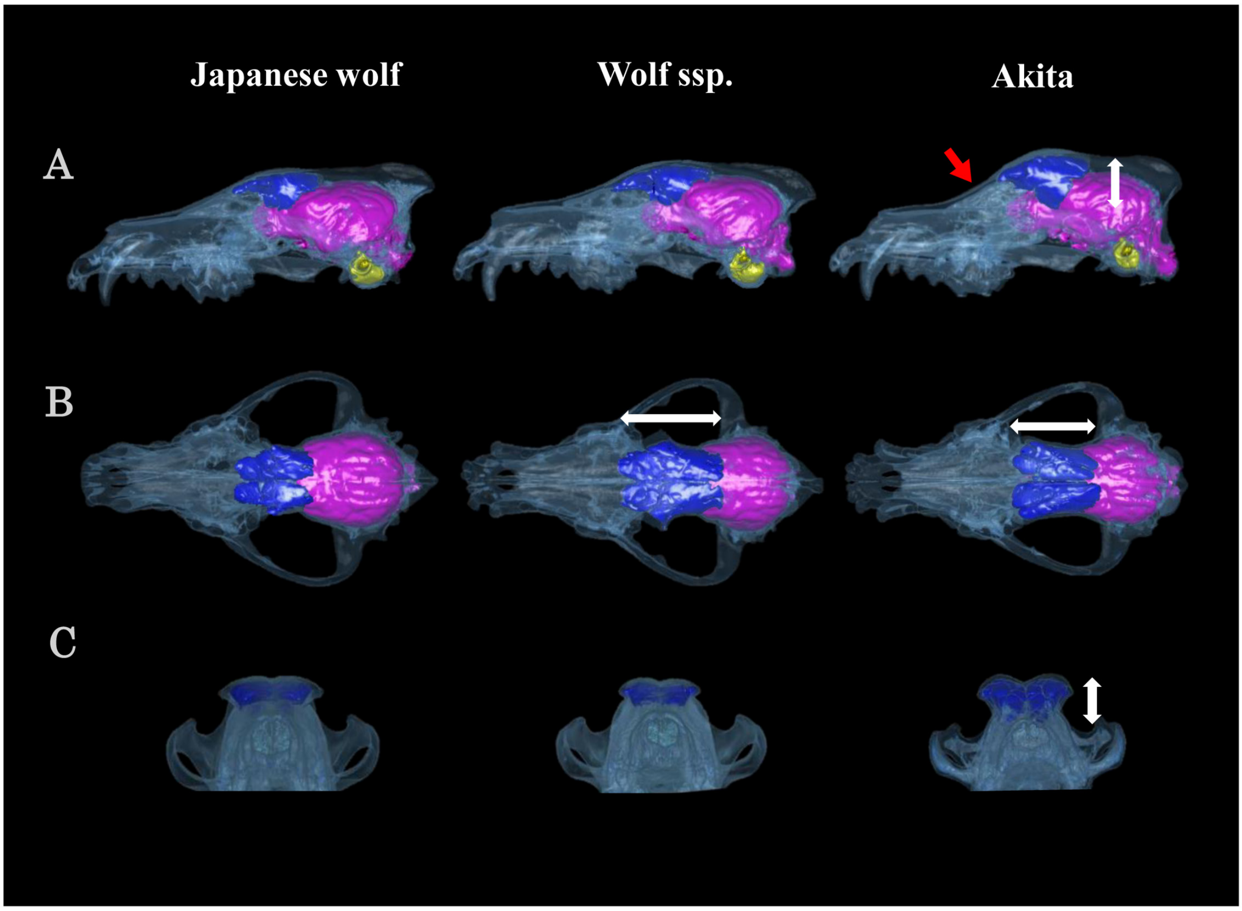

Fig. 6. Comparison of the morphology of frontal sinuses. Left lateral (**A**; frontal sinus, braincase and tympanic cavity are superposed.), dorsal (**B**; frontal sinus and braincase are superposed.) and rostral (**C**; only frontal sinus) views of CT images of the frontal sinus within the skull in the Japanese wolf (M26696), Wolf ssp. (M31475) and Akita (M18303). White arrows indicate significant differences macroscopically from the Japanese wolf. A red arrow shows the stop. The size is matched by condylobasal length.

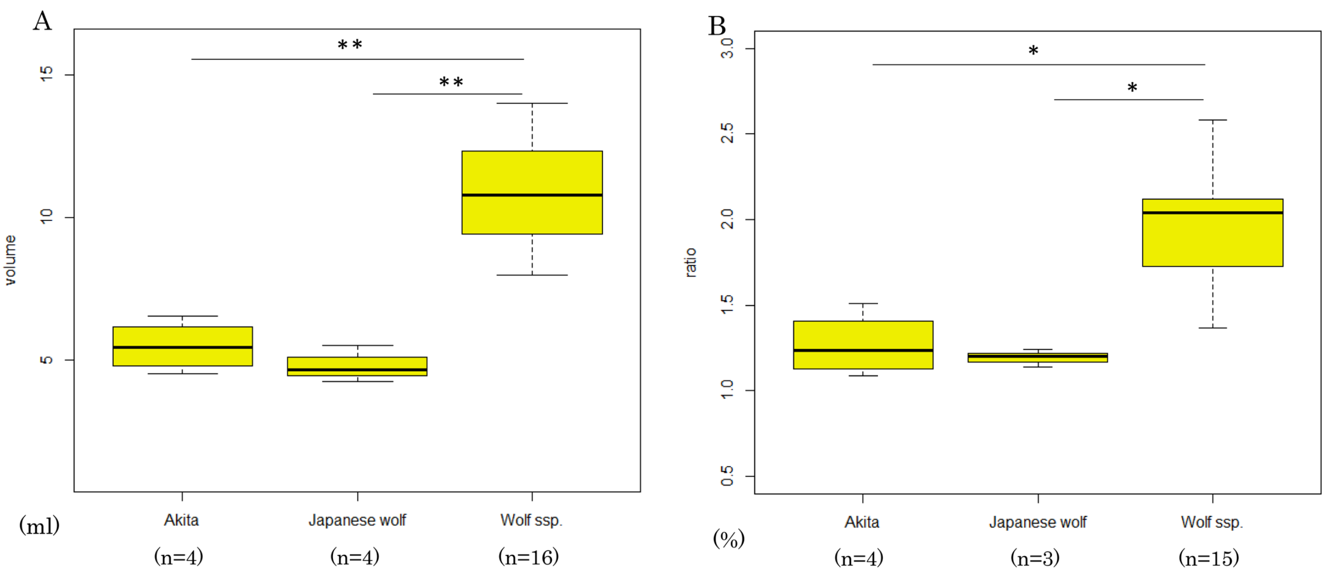

Fig. 7. The representative values of the tympanic cavity volume (**A**) and the ratio of the tympanic cavity volume to the skull volume (**B**) in three groups of gray wolves. Asterisks indicate a significant difference (*P<0.05, **P<0.01, Steel-Dwass test).

DISCUSSION

The volume of the braincase

The braincase is the space that contains the brain, and its shape and size reflect the shape and size of the brain in most mammals [26]. There are many studies of the braincase and its volume in gray wolves. The volume of the braincase was reported to be 115 mL to 175 mL in wild gray wolves [8, 28, 29, 31, 49]. In this study, the medians of the braincase volume of the Japanese wolf and wolf ssp. were 126.4 mL and 149.3 mL, respectively, and the braincase volume was significantly smaller in the Japanese wolf than in the wolf ssp. Compared with previous studies, Japanese wolves comprise a group with a small braincase volume among groups of wild gray wolves.

The braincase volume was examined in several dog breeds and reported as 52.5 mL (toy foxteria) to 166 mL (bull dog) [8, 29, 49]. There is a large difference in body size among dog breeds due to human selection; therefore, a broader range of braincase volumes was observed in the dog than in the wild wolf. The median braincase volume in the Akita (103.2 mL; range, 96.4–109.4 mL) examined in this study was within the range reported in the above studies. Although there was no significant difference between the Japanese wolf and Akita, the braincase volume of the Japanese wolf (126.4 mL) tended to be larger than that of the Akita (103.2 mL). Compared with other dogs, the braincase volume of the Japanese wolf is considered to be slightly larger than average between maximum and minimum (109.3 mL), although it is small among wild gray wolves.

The volume of the frontal sinus

The frontal sinus, which is one of the paranasal sinuses, is formed by the pneumatization of frontal bones. The frontal sinus is generally thought to play roles in protecting the cranium from mechanical loads and in increasing the attachment sites of the temporal muscles to the cranium without increasing the weight of the cranium [48, 50]. The frontal sinus of the gray wolf is more developed than that of other canids such as the red fox (*Vulpes vulpes*) and raccoon dog (*Nyctereutes procyonoides*) [6]. In gray wolves, the frontal sinus is located widely from the rostral side of the frontal bone to the coronal suture, the boundary between the frontal and parietal bones (Fig. 6B). Curtis and VanValkenburgh [6] reported that the average total frontal sinus volume of both sides was 16 mL (n=4) in wolves. In this study, however, the median frontal sinus volume in wolf ssp. was 26.3 mL (n=16), being larger than that reported by Curtis and VanValkenburgh [6]. In their study, two of the four wild wolves used in the analysis were Mexican wolves (*C. l. baileyi*), which are said to be the smallest subspecies of wolf that lived in North America. Therefore, this inclusion of small wolves may have caused the frontal sinus volume to be smaller than that of the wolf ssp.

The frontal sinus volume of the Japanese wolf (med. 9.8 mL) was significantly smaller than that of wolf ssp. Moreover, the volume of the Japanese wolf was smaller than that reported by Curtis and VanValkenburgh [6]. As the basal length of the cranium in Japanese wolves is shorter than that in Mexican wolves, the frontal sinus volume of Japanese wolves may have been smaller in absolute value [6, 17, 19].

The median of the frontal sinus volume of the Akita was 29.7 mL, and there was no significant difference between that of the Akita and wolf ssp. The basal cranial length of continental wild wolves is longer than that of Japanese wolves [17, 19], and the total skull length of the Akita is almost the same as that of Japanese wolves [11, 56]. Therefore, the similarity in frontal sinus volume between the Akita and wolf ssp. suggests that the relative frontal sinus volume of the Akita is larger than that of wild wolves, including Japanese wolves, compared with the cranial length.

Ratio of braincase and frontal sinus volumes to skull volume

The relative size of the braincase and frontal sinus varies in relation to the skull size. In wild canids, the braincase is relatively smaller in species with larger skulls, and the species with larger skulls form relatively larger frontal sinuses due to the development of the frontal sinus backwards [6]. Among the vombatiform marsupials, species with larger skulls have a relatively smaller braincase, resulting in relatively larger frontal sinuses. Conversely, species with smaller skulls have a relatively larger cranial cavity, resulting in a smaller posterior extension of the frontal sinus and relatively smaller frontal sinuses [47]. In this study, the ratio of the braincase volume to the skull volume in the Japanese wolf was significantly larger than that in wolf ssp., and the ratio of the frontal sinus volume to the skull volume in Japanese wolves tended to be smaller than that in wolf ssp. The relatively large cranial cavity and small frontal sinus of the Japanese wolf are likely to be features caused by differences in the skull volume between the Japanese wolf and wolf ssp. because the skull volume of the Japanese wolf was significantly smaller than that of wolf ssp. (Figs. 3 and 6).

On the other hand, the Akita, which has a cranial size similar to that of the Japanese wolf, had a smaller braincase volume and larger frontal sinus volume than the Japanese wolf. In general, domestic dogs have several skull features that distinguish them from wolves in wild populations. For example, the frontal sinus of dogs becomes larger than that of wild wolves because the frontal bone of dogs swells up in the dorsal direction [11]. It is known that the stop between the nasal and frontal bones exhibits a large slope with the swelling of the frontal sinus in the dorsal direction [5, 51] (Fig. 6A and 6C). In previous studies on the morphology of the skull in the Japanese wolf, the frontal bone did not largely protrude to the dorsal direction and the slope of the stop was gentle [17, 21, 37, 39, 44] (Fig. 6A). The two-dimensional and non-quantitative morphological observations of the frontal sinus in the Japanese wolf and Akita using CT revealed that the Akita develops the frontal sinus dorsally compared with the Japanese wolf [11, 12]. In our three-dimensional analysis, the same pattern was noted (Fig. 6A). In addition, the brain weight to body weight and the braincase volume are smaller in domestic dogs than in wild wolves [29, 49]. Therefore, due to domestication, the Akita developed a relatively small braincase and dorsalized formation of frontal bones with a developed stop, allowing the frontal sinus to extend caudally and dorsally, respectively (Fig. 6), causing the difference in the braincase and frontal sinus volume ratios to the skull between the Japanese wolf and Akita.

Tympanic cavity volume and tympanic cavity volume ratio to skull volume

The tympanic cavity covers most of the middle ear from the ventral side of the skull and fills the inside with air [4, 55]. Gray wolves have a pair of swelled tympanic cavities on the ventral side of the skull. However, domestic dogs have smaller tympanic cavities than other wild subspecies, and this characteristic can distinguish dogs from wolves [5, 16]. It is therefore suggested that the significant differences in tympanic cavity volume and tympanic cavity volume to skull volume ratio between the wolf ssp. and Akita were caused by domestication.

On the other hand, in this study, the tympanic cavity volume of the Japanese wolf was significantly smaller than that of the wolf ssp. and as small as that of the Akita. The comparison of the tympanic cavity volume to the skull volume ratio between the Japanese wolf and wolf ssp. yielded the same result. Regarding the morphological characteristics of the tympanic cavity in the Japanese wolf, it has been reported that it is shorter in diameter and width than that in Korean wolves [44], and Japanese wolves have ventrally flat tympanic cavities compared with other wild wolves (Fig. 8) [1, 42, 51]. Our quantitative examination of the tympanic cavity volume and morphological observation in the Japanese wolves were consistent with previous studies [1, 15, 18, 19, 37, 39, 44, 51].

Regarding the tympanic bulla size, studies of crossbreeding between wild wolves and dogs reported that the size and swelling of the tympanic bulla follow the Mendelian laws of inheritance [16]. Regarding the degree of swelling of the tympanic bulla, the swelling of the tympanic bulla at F1 showed intermediate level between wild wolves and dogs, and in the swelling level at F2, the wild wolf, dog and intermediate types were observed. For the reason why the tympanic bulla is small in the Japanese wolf, the hybridization between wild wolves and dogs has been suggested [16]. However, a recent study that analyzed nDNA of nine Japanese wolves reported that ancient genomic introgression occurred from the ancestors of Japanese wolves to the East Eurasian lineage dogs, and Japanese wolves are genetically closest to dogs [14]. Therefore, the small size of the tympanic bulla in the Japanese wolf may not be due to hybridization with dogs but due to an ancestral trait. Thus, it is possible that the small size of the tympanic bulla in dogs was inherited from the ancestral population of Japanese wolves. However, the specific common morphological features of the Japanese wolf, such as the separated rostral alar foramen and the palatine bone with an anterior notch, are not inherited into dogs. Therefore, the ancestor of Japanese wolves may have acquired the morphological characteristics after diverging East Eurasian lineage dogs.

In conclusion, the present study revealed that the Japanese wolf has a relatively large cranial cavity and small frontal sinus compared with the wolf ssp. and Akita, and that the tympanic cavity volume ratio to the skull volume in the Japanese wolf and Akita was significantly smaller than that in the wolf ssp. In this study, we clarified the characteristics of the internal cranial structures in the Japanese wolf three-dimensionally and quantitatively.

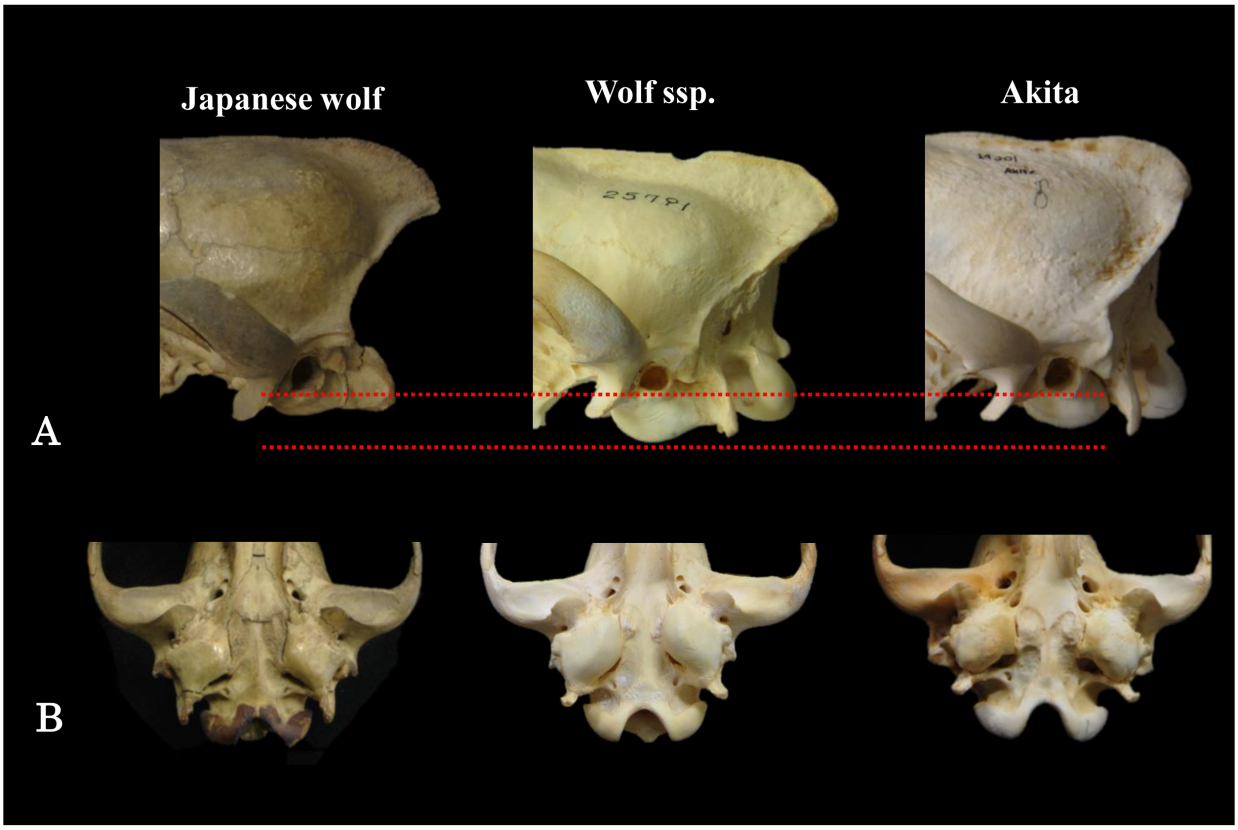

Fig. 8. Comparison of the morphology of the tympanic cavity in gray wolves. Left lateral (**A**) and ventral (**B**) views of the tympanic cavity in the Japanese wolf (Nara), Wolf ssp. (M25791) and Akita (M19301). The size of all specimens is matched by condylobasal length. The upper red dotted line runs through the ventral end of the external acoustic pore in all wolf groups, and a lower line is drawn on the ventral end of the tympanic cavity in Wolf ssp.

CONFLICT OF INTEREST. The authors have nothing to disclose.

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