

# Ancient DNA analysis of brown bear skulls from a ritual rock shelter site of the Ainu culture at Bihue, central Hokkaido, Japan

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**Abstract** Ancient DNA, sampled from ten brown bear (*Ursus arctos*) skulls excavated from a rock shelter site of the Ainu culture at Bihue, central Hokkaido, Japan, was analyzed. This ritual site (*iwaya* or *siratcise*), located in a mountainous area near the western coast of Lake Shikotsu, is considered to have been used for bear-sending ceremonies by the Ainu people at least until about 70 years ago. We identified four mitochondrial DNA (mtDNA) haplotypes at Bihue, all of which were shared by the modern brown bears of Hokkaido. A previous study showed that three mtDNA lineages (groups A, B, and C) of modern brown bears are allopatrically distributed: group A in north-central Hokkaido, group B in eastern Hokkaido, and group C in southern Hokkaido. The ritual site of Bihue is located in the group C area. While one haplotype of group C was recovered from four of the Bihue skulls, three haplotypes of group A were recovered from six skulls. No haplotype of group B was found. The distribution of the four identified haplotypes at Bihue overlaps extensively with the area of the Ainu community called *Shumukuru*. These results indicate that, at the Bihue ritual site, the Ainu people probably performed bear-sending ceremonies that included skulls of bears that lived far from the Bihue area. This suggests that, earlier in the Ainu culture period (after the 17th century), the Bihue ritual site could have been used by multiple villages within an Ainu community, such as the *Shumukuru*, and/or that bear-hunting areas may have expanded from Bihue. This might have led to the promotion of cultural communication and unity among local villages via bear-sending ceremonies.

**Key words:** ancient DNA, brown bear, mitochondrial DNA, bear-sending ceremony, Ainu culture

## Introduction

In the Ainu culture (17th century–present) of Hokkaido, northern Japan, people believe that the brown bear (*Ursus arctos*) is God of the mountains. The bear-sending ceremony is considered to be one of the most important activities in the Ainu culture. Two types of bear-sending ceremonies are known: one is the *opunire*, which concerns adult bears that were hunted locally in the mountains; the other is the *iyomante* which concerns juvenile bears that were kept by the people for several months or longer (reviewed by Utagawa, 1989; Fitzhugh and Dubreuil, 1999). The origins and history of the bear-sending ceremonies before the Ainu cultural period have not been fully clarified, because of the lack of old manuscripts, paintings, or sufficient archaeological data that date to before the 17th century.

From archaeological investigations of the Otafuku-iwa cave site, Rausu town, on the Shiretoko peninsula of eastern Hokkaido, Nishimoto and Sato (1991) and Sato (1993) suggested that the bear-sending ceremony or its prototype had begun by the end of the 13th century (i.e. within the Satsu-

mon culture period of Hokkaido). Meanwhile Ikeda (2000) pointed out that it is difficult to precisely determine when the bear-sending ceremony was established because of lack of information from the 400–500 year interval between the 13th and 17th centuries. Recently, Masuda et al. (2001) analyzed ancient DNA of brown bear remains excavated from an archaeological site of the Okhotsk culture (5th–11th centuries) on the Rebun island, northern Hokkaido. In conjunction with archaeological and morphological data reported by Ohyi et al. (1980), they demonstrated that a form of sending ceremony using juvenile bears (possibly a prototype of the *iyomante*) probably existed by the end of the Okhotsk cultural period. Masuda et al. (2001) compared the ancient mitochondrial DNA (mtDNA) haplotypes of the Rebun archaeological bears with those of modern brown bears of Hokkaido, which enabled them to trace the probable origins of the Rebun bears. This was based on the premise that the geographical distribution of the three mtDNA lineages (groups A, B, and C) of modern brown bears had formed after the last glacial period and not mixed so far (Matsuhashi et al., 1999, 2001).

On the other hand, some ritual sites, called *iwaya* or *siratcise*, known from the mountainous forests around Lake Shikotsu, southern Hokkaido, are considered to be locations of the *opunire* ceremony (Chitose Board of Education, 1984; Uwaya, 1984; Amano 2003; Sato, 2005). At these sites,

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located under large rocky walls of welded tuff, bone remains of the brown bear and other mammals, including the raccoon dog and red fox, and some ritualistic materials, such as *inaw* (pieces of special wood), were found (Chitose Board of Education, 1984; Uwaya, 1984; Amano 2003; Sato, 2005). The Bihue ritual site is one of the rock shelter sites located in the mountainous area near the western coast of Lake Shikotsu (Chitose Board of Education, 1984). This site is considered to have been used for the *opunire* by the Ainu people at least until about 70 years ago. Sex, age at death, and season of death of the bear remains were reported from examination of skull morphology and canine cementum layers (Chitose Board of Education, 1984).

In the present study, we analyzed mtDNA of brown bear skulls excavated from the Bihue ritual site, and then compared the geographical distribution of the identified mtDNA haplotypes with that of modern brown bears. If the probable geographical origins of the bear skulls of the Bihue ritual site are clarified, it would be possible to gain insight into the relationships between community activities of the Ainu people as represented by past bear-hunting areas and locations of ritual sites.

## Materials and Methods

### Skull remains and ancient DNA extraction

The skull remains of the brown bears were archaeologically excavated from the ritual site of Bihue in Hokkaido. They are currently preserved at the Archaeological Research Center of the Chitose Board of Education (Chitose Board of Education, 1984). Of the 13 skull remains of the Bihue ritual site, ten were well-preserved (Figure 1), enabling individual recognition and sexual identification (Chitose Board of Education, 1984). These were used in the present study. The profiles of all specimens examined are shown in Table 1.

Remarkable sexual dimorphism is known in the adult skull morphology of the brown bears of Hokkaido (Yoneda and Abe, 1976; Ohdachi et al., 1992). For determination of sex of the Bihue specimens, widths at the canine roots and other parts of the skull were measured (Chitose Board of Education, 1984). Meanwhile, it is known that the Ainu people make a hole on the left side of the brain case of males and

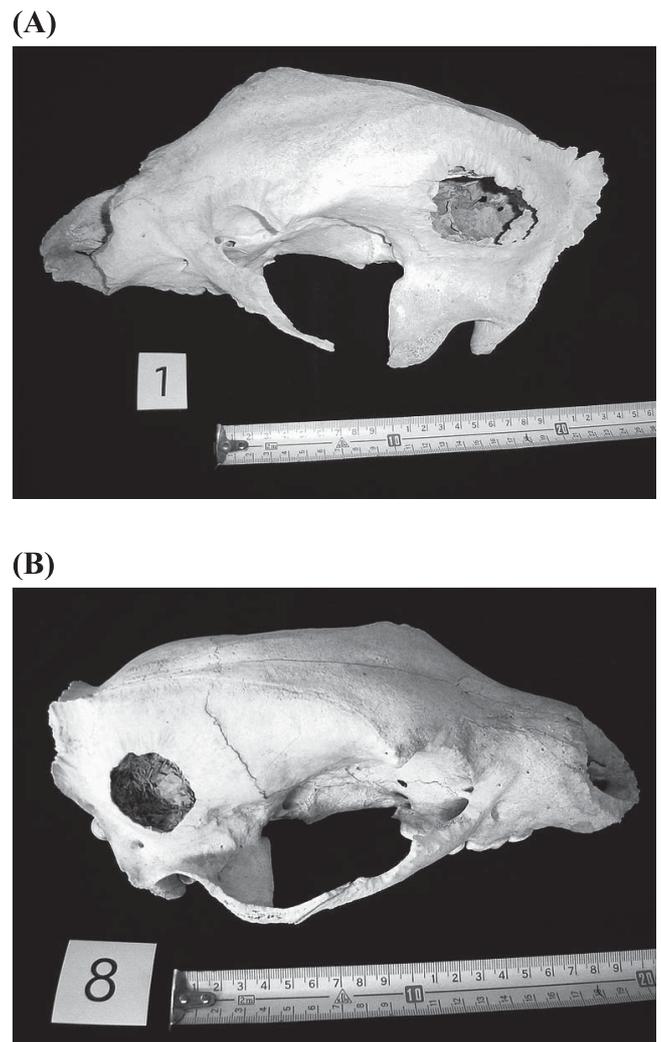


Figure 1. (A) Specimen No. 1 of a brown bear skull which is a 22- to 23-year-old male with a hole on the left side of the brain case. (B) Specimen No. 8 of a brown bear skull which is a 10- to 11-year-old female with a hole on the right side of the brain case. This female skull had *inawkike* in the brain case. Specimen numbers and ages from Chitose Board of Education (1984).

Table 1. Specimen list and mtDNA haplotypes of brown bear skulls excavated from the Bihue ritual site

Specimen No. <sup>1</sup>	Sex	Side of hole	Age (years old) <sup>2</sup>	Season of death <sup>2</sup>	mtDNA type <sup>3</sup>	mtDNA group <sup>3,4</sup>
No. 4	Male	Left	2	May	HB14	C
No. 2	Male	Left	12–13	March–April	HB04	A
No. 1	Male	Left	22–23	May–June	HB14	C
No. 3	Male	Left	22–24	May–June	HB05	A
No. 6	Female	Right	2	March–April	HB14	C
No. 8	Female	Right	10–11	March–April	HB05	A
No. 10	Female	Right	12	March	HB02	A
No. 5	Female	Right	12–13	May–June	HB05	A
No. 9	Female	Right	14–15	May–June	HB05	A
No. 7	Female	Right	16–17	April–May	HB14	C

<sup>1</sup> Chitose Board of Education (1984).

<sup>2</sup> Estimated from canine cementum (Chitose Board of Education, 1984).

<sup>3</sup> Present study.

<sup>4</sup> A, group A from north-central Hokkaido; C, group C from southern Hokkaido.

on the right side of females when performing bear-sending ceremonies. As shown in Table 1 and Figure 1, each of the Bihue skulls had a hole on either the left or the right side, and the sex indicated by the hole position was in agreement with that determined by morphological measurement (Chitose Board of Education, 1984).

The methods used to extract ancient DNA were almost the same as those of Masuda et al. (2001). Small parts of the skulls were sampled and powdered with an electric drill. The DNA extracts, obtained from about 0.2–0.3 g of powder, were concentrated into about 50–100 µl of TE buffer using VivaSpin 6 (VivaScience). An aliquot (1–5 µl) of the DNA extract was subjected to subsequent polymerase chain reaction (PCR). To eliminate contamination of external DNA, disposable plastic gloves and apparatus were used throughout the experiments. We confirmed that negative controls of the reaction mixtures generated no PCR products.

### PCR amplification and sequencing

Following the method of Masuda et al. (2001), three fragments of the mtDNA control region (partially overlapping with each other) were amplified by PCR per specimen. After the first PCR, using one of three sets of primers (forward/reverse: UR6/UR3, mtF/mtR, and UR4/UR7), nested and semi-nested PCRs were performed using inner-side primers (UR6in, UR3in, mtFin, mtRin, UR4in and UR7in). These primers were designated in Masuda et al. (2001), except for mtF and mtR reported by Hänni et al. (1994). The PCR products were purified using the centrifugal dialysis kit QIAquick (Qiagen). The inner-side primers 5'-labeled with the Texas red were used for the cycle PCR using the Thermo Sequenase pre-mixed cycle sequencing kit (Amersham). The combination of the three fragments yielded about 360 base-pairs (bp) per specimen.

### Sequence analysis

Sequence alignment was done using the computer software GeneWorks (Intelligenetics). Insertions or deletions (indels) were compensated by observation. Parsimonious networks among haplotypes were drafted by hand. The 17 haplotypes, HB01–17 (Accession Nos. AB013040, AB013045–47, AB013050, AB013052–59, AB013061–63, AB013065), of the mtDNA control region of modern brown bears of Hokkaido were taken from Matsuhashi et al. (1999) and Masuda et al. (2001).

## Results and Discussion

From all ten skulls examined, fragments of the mtDNA control region, which is about 360 bp long, were successfully sequenced. These sequences were classified into four haplotypes (HB02, 04, 05, and 14), all of which are commonly known in the modern brown bears of Hokkaido (Matsuhashi et al., 1999). Of the ten Bihue skulls, four were of haplotype HB14, another four were of haplotype HB05, and one each were of haplotypes HB02 and HB04 (Table 1). No new haplotype was identified from the Bihue skulls.

The parsimonious networks (Figure 2) show that mtDNA haplotypes of the brown bears of Hokkaido are classified into three groups, A, B, and C, which are allopatrically dis-

tributed: group A occupies north-central Hokkaido; group B occupies eastern Hokkaido; and group C occupies southern Hokkaido (Figure 3) (Matsuhashi et al., 1999). Three of the four mtDNA haplotypes identified from the Bihue ritual site belong to group A (six individuals of HB02, 04, or 05), and one haplotype belongs to group C (four individuals of HB14). There were no haplotypes attributed to group B (Table 1).

As shown in Figure 3, the Bihue ritual site is located within the area currently occupied by HB14 of group C. Haplotype HB05 of group A occupies an area east of the Bihue ritual site as well as around the Ishikari lowland. While HB02 occurs in the region further east of the HB05 area, its distribution is widespread in north-central Hokkaido. Haplotype HB04 is specific to the Hidaka mountains. No overlap among distribution areas of the four mtDNA haplotypes was reported (Matsuhashi et al., 1999), although a more precise survey of mtDNA haplotypes of modern brown bears around Lake Shikotsu would be desirable.

Thus, the four mtDNA haplotypes identified from the ten skulls are distributed in modern brown bears, both within and outside the Bihue area, but relatively close to Bihue (Figure 3). Because female brown bears have much smaller territories than do the males, only one haplotype is usually found from modern female bears of the same sampling locality (Matsuhashi et al., 1999). However, the present study revealed that the female skulls of the Bihue ritual site had three different mtDNA haplotypes (HB02, HB05, and HB14). This shows that the female skulls of the Bihue ritual

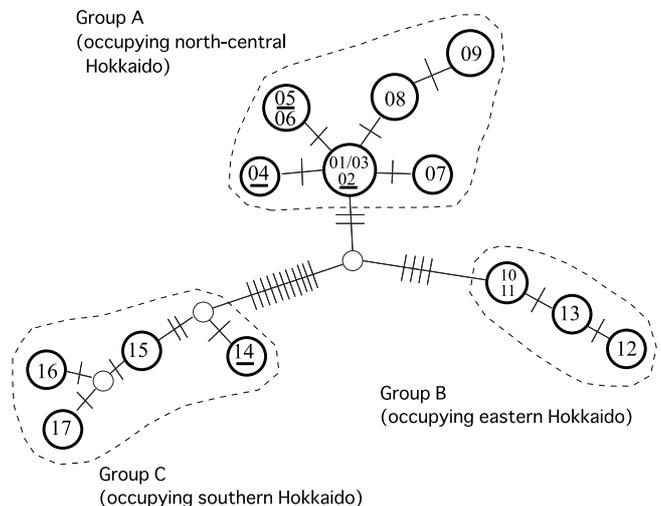


Figure 2. Parsimonious network of haplotypes of the mtDNA control region (about 360 bp). Sequences were cited from Matsuhashi et al. (1999) and Masuda et al. (2001). Circles with numbers indicate observed haplotypes and open circles show predicted haplotypes. Each slash on the branches indicates a single nucleotide substitution. Three haplotype groups A, B, and C were defined and are shown by the broken lines. Since HB01 and HB03 shared an identical sequence of the 360 bp region, they are shown together as a common haplotype HB01/03. Haplotypes HB01/03 and HB02 become identical after eliminating indels and are figured as the same operational taxonomic unit (OTU). Similarly HB05 and HB06 are figured as a single OTU after eliminating indels. The four underlined haplotypes (HB02, 04, 05, and 14) were identified from the skulls of the Bihue ritual site.

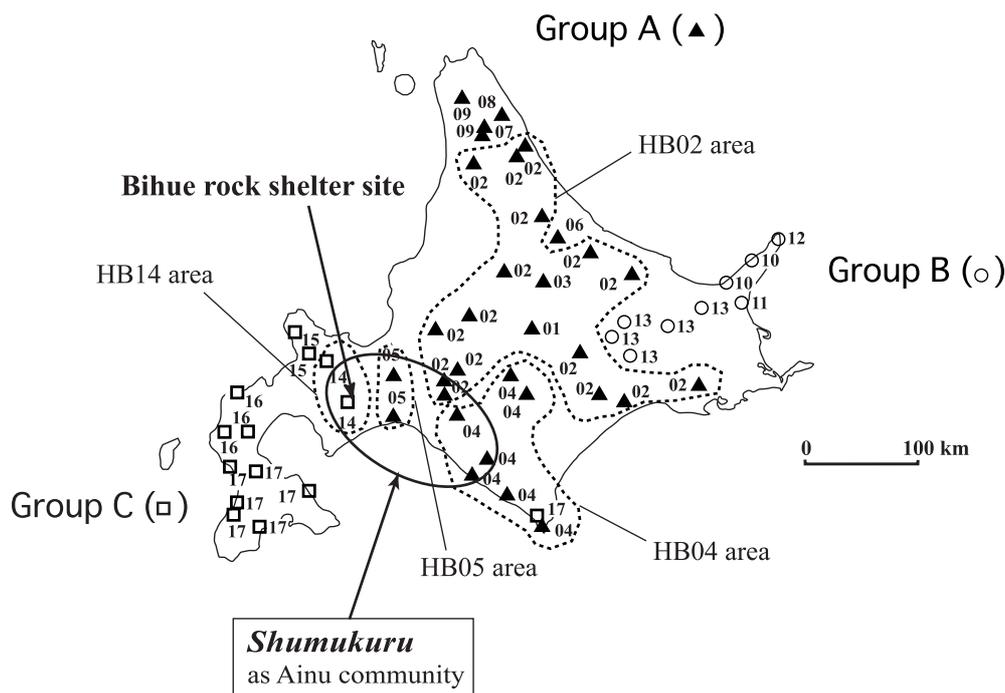


Figure 3. Location of the Bihue rock shelter site and geographical distribution of the mtDNA haplotypes of the modern brown bears of Hokkaido. Haplotypes of group A (closed triangles and numbers) are distributed in north-central Hokkaido, those of group B (open circles and numbers) in eastern Hokkaido, and those of group C (open squares and numbers) in southern Hokkaido (cited from Matsushashi et al., 1999). Each symbol and number corresponds to a single individual. Natural distributions of the four mtDNA haplotypes (HB02, 04, 05, and 14), identified from the ten Bihue skulls, are circumscribed by the broken lines. The Bihue rock shelter site is located within the HB14 area of group C. The Ainu community *Shumukuru* (Kaiho, 1974), shown by the circle, overlaps with portions of the distribution areas of haplotypes HB02, 04, and 14, and the entire area of HB05.

site must have originated from three or more areas.

It is noticed that some portions of the distribution areas of haplotypes HB02, HB04, and HB14 and the entire distribution area of haplotype HB05 overlap with the area of the Ainu community called *Shumukuru* (or *Sarunkuru*) which reaches from the Ishikari lowland and the surroundings of Lake Shikotsu to the western region of the Hidaka district (Figure 3) (Kaiho, 1974). The *Shumukuru* shows a range where the Ainu people shared common spiritual matters such as styles of grave posts; this community had formed by 1669 in the Edo period and continued until the Showa period (Kaiho, 1974). The results of the present study suggest that the Ainu people may have hunted brown bears within the *Shumukuru* region and brought the bear skulls or heads to the Bihue ritual site for the bear-sending ceremonies, i.e. this ritual site could have been used by the people of several villages located within an area of a community such as the *Shumukuru*. Some skulls including No. 8 (Figure 1B) had *inawkike* (fragments of *inaw*) in the brain case, which is usually put through the holes made when performing the bear-sending ceremony. Considering the above DNA data together with existence of the *inawkike*, some of the skulls were likely to have been initially handled and decorated with the *inawkike* at some local village, and then brought to the Bihue ritual site for an additional bear-sending ceremony.

Meanwhile, all of the Bihue skulls were of individuals older than two years, with no younger juveniles represented (Chitose Board of Education, 1984). However, there were

two individuals, estimated to be two-year-olds, which were not full adults. As it is known that only adult bears are used in the *opunire* (Utagawa, 1989), the morphologically based data, as well as the genetic data of the present study, suggest that the bear-sending ceremony performed at the Bihue site might have differed from the typical *opunire* in which the people used adult bears just after hunting them in the mountains.

As demonstrated by Masuda et al. (2001), analysis of ancient DNA provides a basis to estimate the geographical location of origin of the brown bear remains excavated from archaeological sites on islands, such as the Rebun island, where no natural bear populations have been known to be distributed. On the other hand, the Bihue ritual site is located within the present habitat of the brown bear. Therefore, in the near future, a further precise survey regarding the distribution of mtDNA haplotypes of modern brown bears of the Lake Shikotsu region, including the Bihue area, is required, in order to check on possible movement of mtDNA haplotypes, especially through male bears which have much wider home ranges than females.

At present, aside from the archaeological Bihue ritual site, at least four rock shelter sites of the Ainu culture period are known along the Izari river in the eastern coastal region of Lake Shikotsu, and the brown bear remains from these sites show some signs of ritual (Uwaya, 1984; Amano 2003; Sato, 2005). Ethnoarchaeological analyses of these sites and their remains would provide additional standards from which to

judge and interpret the archaeological evidence of bear-sending ceremonies dating to before the 17th century. Especially, genetic studies of the brown bear remains of these ritual sites could lead to further insights into the mode and cultural meanings of bear-sending ceremonies, such as regarding changes in bear-hunting areas and their cultural relationships with Ainu communities.

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