

ORIGINAL ARTICLE

Morphological and genetic analysis of Vietnamese *Sus scrofa* bones for evidence of pig domestication

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ABSTRACT

In the present study, we used morphological and genetic analyzes to distinguish bones of domestic boars from those of wild boars. We analyzed 65 *Sus* bones (cranium, mandible and teeth) stored in three research institutes in Vietnam and in a village in Vietnam. Based on comparison of bucco-lingual measurements of mandibular parts, the 58 specimens were morphologically classified into two size groups: a large bone group and a small bone group. Analysis of 572-bp mitochondrial DNA (mtDNA) sequences indicated that the large bones had genetic links to wild boar lineage including Ryukyu, Taiwan and Korean wild boars, and that the small bone group was closely related to East Asian domestic pigs. The phylogenetic analysis and parsimonious networks constructed among mtDNA haplotypes belonging to Ryukyu wild boar lineage showed that the Ryukyu wild boar is closely related to the Vietnamese wild boars, and uniquely miniaturized on their islands after the Ryukyu archipelago became isolated from the Asian continent.

Key words: bone, domestication, mtDNA, Vietnam, wild boar.

INTRODUCTION

Wild boars (*Sus scrofa*) inhabit wide areas of Asia, Europe and north-western Africa. At least 16 wild boar species are known to exist, and they comprise local populations that are well-adapted to regional environments (Epstein 1984; Ruvinsky & Rothschild 1998). Different types of domestic pigs are thought to have been independently domesticated from different wild boar species in Asia and Europe (Watanabe *et al.* 1985; Giuffra *et al.* 2000). In Asia, the domestication of pigs is thought to have occurred using local wild boar species, and is thought to have occurred in China and Vietnam between 5000 and 9000 years ago (Xu 1950). During the 18th and early 19th cen-

turies, Asian domestic pigs were used as a genetic resource for improvement of European pig breeds (Jones 1998).

There are two wild boar subspecies in Japan: Japanese wild boars (*S. s. leucomystax*), on the three main Japanese islands of Honshu, Shikoku and Kyushu; and Ryukyu wild boars (*S. s. riukiuanus*), on the Ryukyu archipelago (the islands of Amami-Oshima,

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Kakeroma, Tokuno-shima, Okinawa, Ishigaki and Iriomote). The Ryukyu wild boar is smaller than the Japanese wild boar (Endo *et al.* 1998, 2000), and there are documented genetic differences between the two subspecies (Watanabe *et al.* 1985; Kurosawa & Tanaka 1988; Kurosawa *et al.* 1984; Watanabe *et al.* 1999). For many years, there has been controversy regarding the origin and ancestry of the Ryukyu wild boar. In a recent study using mitochondrial DNA (mtDNA) analysis, Hongo *et al.* (2002) found that large-sized skeletons stored in two Vietnamese research institutes have genetic links to Ryukyu wild boars. Their findings indicate that the genetic origin of Ryukyu wild boars is in Vietnam, rather than Taiwan or China.

We conducted the present study to confirm the genetic relationship between Ryukyu wild boars and Vietnamese wild boars. Using 65 *Sus* bones stored at three research institutes in Vietnam, we performed morphological measurements, and performed mtDNA analysis of bone powder. Here, we use the findings of those analyzes to characterize the phylogenetic relationships among Ryukyu wild boars, East Asian domestic pigs, Vietnamese wild boars, and Vietnamese domestic pigs. Also, we describe the characteristic morphological and genetic evidence of domestication distinguishing domestic pigs from wild boars.

MATERIALS AND METHODS

Bone samples and morphological measurement

We used the following 65 *Sus* bone samples for morphological and genetic analyzes: two craniums and two mandibles from the Institute of Archaeology in Hanoi; 14 mandibles from the Zoological Museum in Hanoi; 26 mandibles from Hanoi Agricultural University; and 13 teeth and eight mandibles from a village in Hoe Binh province (Table 1). The specimens from Hanoi Agricultural University were purchased on January 7, 1997, in Ba Vi Village, Ba Vi County, Ha Tay Province, near Hanoi, by a group of Japanese and Vietnamese researchers (Yamamoto *et al.* 1998). Although the exact origin of these bones is not known, they appear to have been taken from recently hunted or slaughtered animals (Hongo *et al.* 2002). The specimens from the Institute of Archaeology and the Zoological Museum in Hanoi were collected from various localities in northern Vietnam, and the dates on which those animals were hunted or slaughtered are not known. The 21 specimens from Hoe Binh province (13 teeth and eight mandibles) were collected in a small village in 2003, and the exact dates and locations at which those animals were hunted or slaughtered are not known. Because many of the craniums and mandibles were broken, the following dimensions were measured with digital calipers

and used as size markers for the morphological analysis: the occlusal length and greatest breadth of the mandibular third molar (M3), the bucco-lingual crown breadth of the third and fourth premolars (P3W, P4W), and the trigonid and talomid breadth of the first and second molars of the mandible (M1M, M2M, M1D and M2D (Table 1), using the measurement codes of Kusatman (1991). Those measurements were compared with corresponding measurements for a standard population, using the logarithmic ratio technique (Simpson 1941). The standard population used in the present study comprised 22 modern Japanese wild boars from Kanagawa Prefecture (Anezaki 2007).

DNA extraction

DNA was extracted from all 65 *Sus* specimens. Bone powder (0.2–0.5 g) was collected using an electric drill, and was decalcified using 0.5 mol/L ethylenediaminetetraacetate (EDTA). The bone powder was mixed with 5 mL of 0.5 mol/L EDTA containing proteinase K (300 µg/mL) and N-lauroylsarcosine (0.5%) (Watanabe *et al.* 2001). The sample was extracted twice with phenol and once with chloroform to remove the protein. The supernatant was concentrated with a Centricon 30 microconcentrator (Amicon, Beverly, MA, USA), and was washed with distilled water. The DNA samples extracted from the bones were directly used as PCR templates.

PCR and direct sequencing of mtDNA

To construct the mtDNA control 572-bp region, we independently amplified three mtDNA control regions (A, 258 bp; B, 305 bp; and C, 229 bp) by PCR using three primer sets (Watanabe *et al.* 2001). The PCR products were purified using a QIAquick PCR Purification Kit (Qiagen, Valencia, CA, USA), as described elsewhere (Ishiguro & Nishimura 2005). We directly sequenced the PCR products using the corresponding primers and a BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA). The 572-bp nucleotide sequence was formed by connecting the three DNA fragments that were amplified using the A, B and C primer sets.

DNA analysis

Phylogenetic analysis was performed using the sequences obtained from the 65 present specimens and 78 haplotypes of wild boars and domestic pigs obtained in previous studies (Hongo *et al.* 2002; Ishiguro & Nishimura 2005). Multiple sequence alignment was performed using GENETYX-MAC software (Software Development Co., Tokyo, Japan). Genetic distance was calculated using the two-parameter method, and a phylogenetic tree was constructed using the neighbor-joining method (Saitou & Nei 1987) and MEGA4 program (<http://www.megasoftware.net>) with bootstrap values generated by 500 replications. Using the split decomposition method (Dopazo *et al.* 1993), we performed parsimonious network analysis with the new haplotypes obtained in the present study and haplotypes obtained in previous studies (Hongo *et al.* 2002; Watanabe *et al.* 2002).

Table 1 Vietnamese pig bone samples

| Sample No. | Institution† | Original No. | Sample | Morphological measurement (mm) | | | | | | | Length of M3 | Size‡ | Accession No. or reference | | | | |
|------------|--------------------|--------------|----------|--------------------------------|-------|-------|-------|-------|-------|---------------|--------------|-------|----------------------------|--|--|--|-------------------------------|
| | | | | P3 | P4 | M1M | M1D | M2M | M2D | Breadth of M3 | | | | | | | |
| 1 | L.A.H. | AI-2 | Cranium | | | | | | | | | | | | | | |
| 2 | L.A.H. | AI-15 | Cranium | 9.3 | 12 | 13.15 | 13.84 | 18.17 | 18.04 | 18.21 | | | | | | | AB326936 |
| 3 | L.A.H. | AI-17 | Mandible | 7.04 | 9.92 | 11.27 | 11.77 | 15.14 | 14.84 | | | | | | | | Hongo <i>et al.</i> (2002) |
| 4 | L.A.H. | AI-19 | Mandible | | | | | | | | | | | | | | AB326937 |
| 5 | Village (Hoa Binh) | HBW1 | Tooth | 8.32 | 11.38 | 10.72 | 11.73 | 16.04 | 16.53 | 20.79 | 45.98 | | | | | | Hongo <i>et al.</i> (2002) |
| 6 | Village (Hoa Binh) | HBW2 | Tooth | 5.09 | 7.41 | 8.46 | 9 | 10.91 | 11.47 | 12.82 | 27.15 | | | | | | Hongo <i>et al.</i> (2002) |
| 7 | Village (Hoa Binh) | HBW3 | Tooth | 8.02 | 11.17 | 12.9 | 12.85 | 17.71 | 17.51 | 13.12 | | | | | | | Hongo <i>et al.</i> (2002) |
| 8 | Village (Hoa Binh) | HBW4 | Tooth | 5.09 | 7.41 | 8.46 | 9 | 10.91 | 11.47 | 12.82 | 27.15 | | | | | | Hongo <i>et al.</i> (2002) |
| 9 | Village (Hoa Binh) | HBW5 | Tooth | 8.61 | 11.08 | 12.89 | 13.59 | 16.7 | 16.92 | 21.13 | 41.1 | | | | | | Ishiguro and Nishimura (2005) |
| 10 | Village (Hoa Binh) | HBW6 | Tooth | 8.96 | 12.92 | 12.89 | 14.65 | 18.1 | 19.24 | 22.93 | 43.02 | | | | | | Hongo <i>et al.</i> (2002) |
| 11 | Village (Hoa Binh) | HBW7 | Tooth | 7.64 | 10.63 | 12.04 | 13.07 | 16.74 | 17.28 | 19.64 | 42.61 | | | | | | Hongo <i>et al.</i> (2002) |
| 12 | Village (Hoa Binh) | HBW8 | Tooth | 8.03 | 12.22 | 12.12 | 13.42 | 17.83 | 17.32 | 22.06 | 44.58 | | | | | | Hongo <i>et al.</i> (2002) |
| 13 | Village (Hoa Binh) | HBW9 | Tooth | 7.21 | 11.25 | 11.51 | 12.42 | 17 | 17.36 | 20.4 | 40.02 | | | | | | Hongo <i>et al.</i> (2002) |
| 14 | Village (Hoa Binh) | HBW10 | Tooth | 7.7 | 11.57 | 12.48 | 12.48 | 17 | 17.82 | 21.19 | 46.25 | | | | | | Hongo <i>et al.</i> (2002) |
| 15 | Village (Hoa Binh) | HBW11 | Tooth | 7.69 | 10.35 | 11.15 | 12.07 | 16.57 | 16.36 | 19.57 | 46.87 | | | | | | Hongo <i>et al.</i> (2002) |
| 16 | Village (Hoa Binh) | HBW12 | Tooth | 7.58 | 10.58 | 11.48 | 12.05 | 16.3 | 16.86 | 19.55 | 39.72 | | | | | | Hongo <i>et al.</i> (2002) |
| 17 | Village (Hoa Binh) | HBW15 | Tooth | | | | | 11.21 | 11.59 | 13.6 | 31.05 | | | | | | Hongo <i>et al.</i> (2002) |
| 18 | Village (Hoa Binh) | MP3 | Mandible | 6.36 | 8.64 | 9.05 | 9.75 | 12.43 | 12.42 | 14 | | | | | | | Ishiguro and Nishimura (2005) |
| 19 | Village (Hoa Binh) | MP4 | Mandible | | | | | | | | | | | | | | Ishiguro and Nishimura (2005) |
| 20 | Village (Hoa Binh) | MP5 | Mandible | | | 8.36 | 8.78 | | | | | | | | | | Hongo <i>et al.</i> (2002) |
| 21 | Village (Hoa Binh) | MP6 | Mandible | | | 8.12 | 8.61 | | | | | | | | | | Hongo <i>et al.</i> (2002) |
| 22 | Village (Hoa Binh) | MP7 | Mandible | | | 8.81 | 9.15 | | | | | | | | | | Ishiguro and Nishimura (2005) |
| 23 | Village (Hoa Binh) | MP8 | Mandible | | | 8.32 | 8.68 | | | | | | | | | | Ishiguro and Nishimura (2005) |
| 24 | Village (Hoa Binh) | MP9 | Mandible | | | 8.59 | 8.97 | | | | | | | | | | Ishiguro and Nishimura (2005) |
| 25 | Village (Hoa Binh) | MP10 | Mandible | | | 9.08 | 9.57 | | | | | | | | | | Ishiguro and Nishimura (2005) |
| 26 | Z.M. | 899 | Mandible | | | | | | | | | | | | | | Ishiguro and Nishimura (2005) |
| 27 | Z.M. | 682 | Mandible | | 10.38 | 11.04 | 12.08 | 16.07 | 16.39 | | | | | | | | AB326938 |
| 28 | Z.M. | 593 | Mandible | | | 12.02 | 12.56 | 16.53 | 16.58 | | | | | | | | AB326939 |
| 29 | Z.M. | 750 | Mandible | | 10.17 | 11.92 | 12.9 | 15.67 | 15.86 | 18.45 | 36.86 | | | | | | AB326940 |
| 30 | Z.M. | 586 | Mandible | | 10.35 | 11.92 | 12.9 | 15.92 | 15.96 | | | | | | | | AB326941 |
| 31 | Z.M. | 591 | Mandible | | 10.46 | 12.41 | 12.44 | 16.91 | 16.97 | 17.45 | | | | | | | AB326942 |
| 32 | Z.M. | 586 | Mandible | | 10.35 | 12.05 | 12.9 | 15.92 | 15.96 | 17.45 | | | | | | | AB326943 |
| 33 | Z.M. | 592 | Mandible | | | | | | | | | | | | | | AB326944 |
| 34 | Z.M. | 752 | Mandible | | 11.57 | 12.21 | 13.23 | 17.08 | 17.36 | | | | | | | | AB326938 |
| 35 | Z.M. | M1015 | Mandible | | | 12.52 | 12.61 | 17.84 | 17.54 | | | | | | | | AB326941 |
| 36 | Z.M. | M1006 | Mandible | | | 11.34 | 12.12 | 15.98 | 16.69 | | | | | | | | AB326945 |
| 37 | Z.M. | 717 | Mandible | | | 11.49 | 12.09 | 15.04 | 15.97 | 18.36 | | | | | | | AB326938 |
| 38 | Z.M. | RJT40(PN05) | Mandible | 7.19 | 10.54 | | | | | | | | | | | | Hongo <i>et al.</i> (2002) |
| 39 | Z.M. | RJT37 | Mandible | | | | | 17.06 | 17.25 | | | | | | | | Hongo <i>et al.</i> (2002) |
| 40 | H.A.U. | AU1 | Mandible | 8.06 | 11.29 | 13.15 | 13.18 | | | | | | | | | | Hongo <i>et al.</i> (2002) |
| 41 | H.A.U. | AU7 | Mandible | | | 11.49 | 12.91 | | | | | | | | | | Hongo <i>et al.</i> (2002) |
| 42 | H.A.U. | AU5 | Mandible | | | 11.93 | 12.68 | 16.62 | 16.88 | 20.37 | 43.23 | | | | | | Hongo <i>et al.</i> (2002) |
| 43 | H.A.U. | AU16 | Mandible | 9.29 | 12.38 | 8.65 | 9.36 | 11.68 | 11.71 | | | | | | | | AB326946 |
| 44 | H.A.U. | AU17 | Mandible | 6.63 | 8.34 | 7.82 | 8.41 | 10.69 | 11.38 | 12.71 | 27.1 | | | | | | Ishiguro and Nishimura (2005) |
| 45 | H.A.U. | AU19 | Mandible | 4.74 | 6.83 | 7.82 | 8.59 | 13.04 | 13.22 | 14.95 | 28.8 | | | | | | Ishiguro and Nishimura (2005) |
| 46 | H.A.U. | AU28 | Mandible | 5.66 | 7.91 | 8.59 | 9.64 | 16.99 | 17.04 | | | | | | | | AB326947 |
| 47 | H.A.U. | AU30 | Mandible | | 10.51 | 12.22 | 12.51 | 16.99 | 17.04 | | | | | | | | AB326947 |
| 48 | H.A.U. | AU32 | Mandible | | 7.18 | 8.77 | 10 | 11.7 | 12.4 | | | | | | | | AB326948 |
| 49 | H.A.U. | AU35 | Mandible | | | 9.16 | 10.09 | | | | | | | | | | AB326949 |
| 50 | H.A.U. | AU36 | Mandible | 6.21 | 8.25 | 7.79 | 8.65 | 12.3 | 12.8 | 13.09 | 20.18 | | | | | | AB326950 |
| 51 | H.A.U. | AU37 | Mandible | | | 9.68 | 10.52 | 11.01 | 11.5 | 12.98 | 27.3 | | | | | | Ishiguro and Nishimura (2005) |
| 52 | H.A.U. | AU40 | Mandible | 6.01 | 7.1 | 8.1 | 9.16 | 10.99 | 11.66 | 13.39 | 26.23 | | | | | | Ishiguro and Nishimura (2005) |
| 53 | H.A.U. | AU42 | Mandible | 5.34 | 7.4 | 7.94 | 8.8 | 10.79 | 11.27 | | | | | | | | Ishiguro and Nishimura (2005) |
| 54 | H.A.U. | AU43 | Mandible | 6.2 | 7.92 | 8.63 | 9.46 | 11.2 | 11.57 | 13.21 | | | | | | | Ishiguro and Nishimura (2005) |
| 55 | H.A.U. | AU45 | Mandible | 5.63 | 7.3 | 8.79 | 9.65 | 11.5 | 12.17 | | | | | | | | AB326948 |
| 56 | H.A.U. | AU47 | Mandible | 5.63 | 7.3 | 8.79 | 9.65 | 11.5 | 12.17 | | | | | | | | Ishiguro and Nishimura (2005) |
| 57 | H.A.U. | AU49 | Mandible | 8.35 | 11.81 | 12.55 | 13.23 | 16.99 | 17.48 | | | | | | | | Ishiguro and Nishimura (2005) |
| 58 | H.A.U. | AU50 | Mandible | | | 11.54 | 12.85 | | | | | | | | | | Hongo <i>et al.</i> (2002) |
| 59 | H.A.U. | AU52 | Mandible | | | 8.28 | 9.26 | 11 | 11.57 | | | | | | | | AB326951 |
| 60 | H.A.U. | AU53 | Mandible | 5.27 | 6.99 | 8.29 | 8.91 | 11.01 | 11.61 | | | | | | | | Ishiguro and Nishimura (2005) |
| 61 | H.A.U. | AU55 | Mandible | 8.02 | 11.15 | 11.69 | 12.47 | 15.75 | 16.82 | | | | | | | | Ishiguro and Nishimura (2005) |
| 62 | H.A.U. | AU57 | Mandible | 6.31 | 7.78 | 8.3 | 9.27 | 11.47 | 11.97 | 13.78 | | | | | | | Hongo <i>et al.</i> (2002) |
| 63 | H.A.U. | AU58 | Mandible | 5.99 | 7.99 | 8.13 | 9.24 | 10.89 | 11.68 | | | | | | | | AB326952 |
| 64 | H.A.U. | AU60 | Mandible | | | | | | | | | | | | | | Hongo <i>et al.</i> (2002) |
| 65 | H.A.U. | AU61 | Mandible | | | | | | | | | | | | | | AB326948 |

†L.A.H.: Institute of Archaeology in Hanoi; Z.M.: Zoological Museum; H.A.U.: Hanoi Agricultural University. ‡Based on morphological measurements in Figure 1. L, Large-sized bone; S, small-sized bone. §ND: Not-determined.

RESULTS

Morphological analysis

As in a previous study by Hongo *et al.* (2002), the *Sus* bone samples were morphologically divided into two groups: a large-sized bone group and small-sized bone group. Among the large-sized bones, the occlusal length of mandibular third molars (M3) ranged from approximately 51.51 mm to 38.80 mm. Among the small-sized bones, the occlusal length of M3 ranged from approximately 31.05 mm to 20.18 mm. Only 20 of the 65 samples could be used for measurement of the occlusal length of M3 (Table 1). No morphological measurement was obtained from seven samples (AI-2, AI-19, MP4, 899, RJT37, AU60 and AU61). For precise comparison of the relative sizes of the *Sus* teeth, we used the logarithmic ratio technique to compare the

bucco-lingual measurements of mandibular P3, P4, M1M, M1D, M2M and M2D of 58 specimens (Fig. 1). Figure 1 shows the log ratio data of the measurements obtained for those six sites. Comparison of the logarithmic ratio with the standards clearly divided the specimens into two groups with the base line at 0.00 without any overlap. Among the 58 bones thus examined, 31 bones belonged to the large size group, and the 27 bones whose data fell below the base line belonged to the small size group.

mtDNA analysis

Table 1 shows the mtDNA haplotypes of the present 65 *Sus* bones. We identified 20 novel mtDNA haplotypes (Viet18 to Viet37) in the present study, and deposited them in the DDBJ/EMBL/GenBank database (accession nos. AB326933-AB326952). The Viet17 haplo-

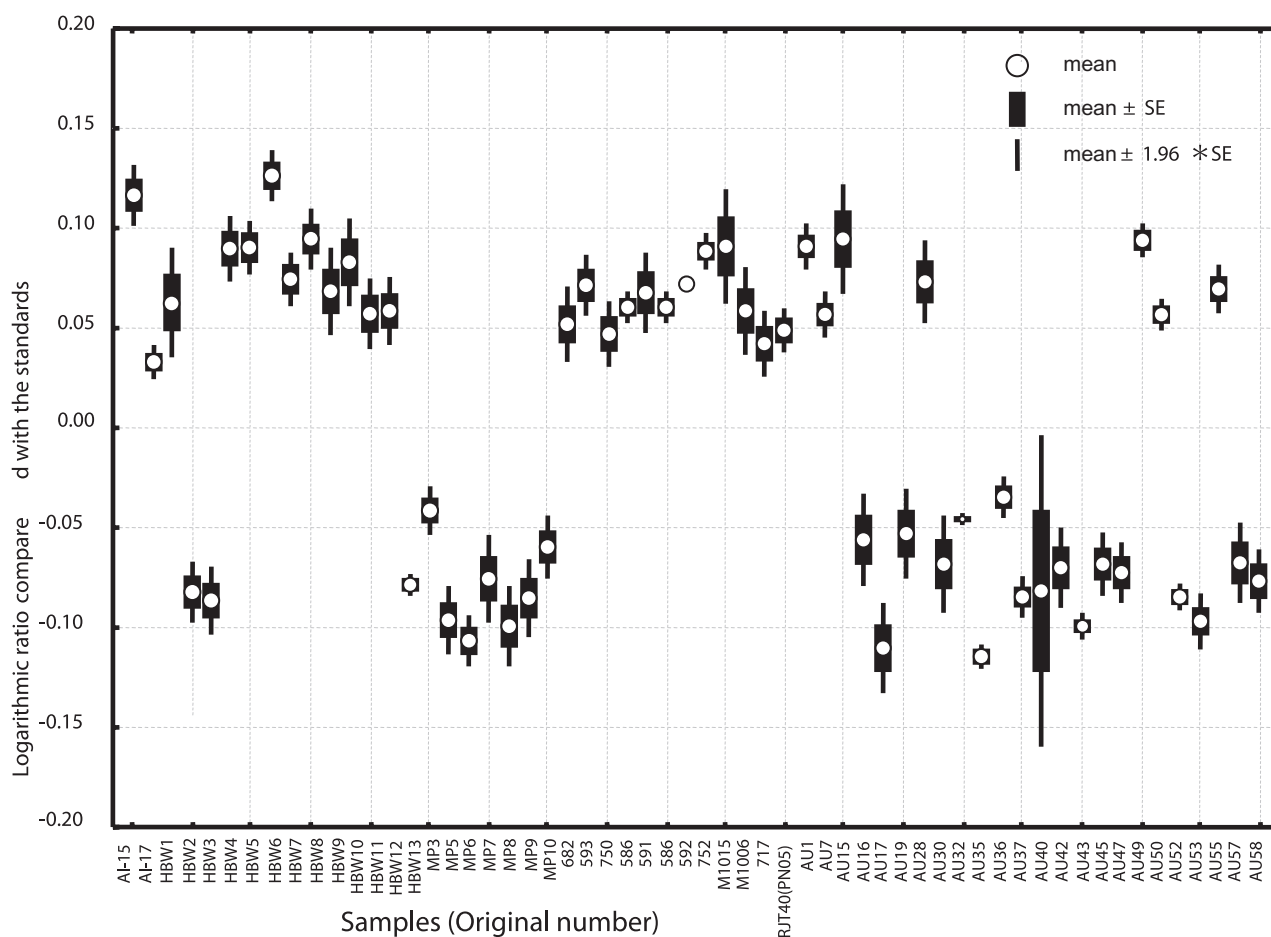


Figure 1 Comparison of bucco-lingual measurements of mandibular P3, P4, M1M, M1D, M2M and M2D, using the logarithmic ratio technique. In this study, we used the standard measurements of P3 (6.61), P4 (9.39), M1M (10.22), M1D (11.18), M2M (13.53) and M2D (13.81) from 22 Japanese wild boars in Kanagawa prefecture (Anezaki 2007).

type was the most predominant; it was detected in 12 of the 65 bone samples, including 7 of the 13 tooth samples from the village in Hoa Binh (Table 1).

Phylogenetic analysis

To determine the phylogenetic positions of the 20 novel mtDNA haplotypes, we constructed a neighbor-joining tree using the 20 novel mtDNA haplotypes and 78 previously reported mtDNA haplotypes (Ishiguro & Nishimura 2005: J1-J20, Japanese wild boar; M16-M20, Ryukyu wild boar; M21-M39, East Asian domestic boar; M40-M55 and E33, European domestic pig and wild boar; M56-M60, Korean and Taiwanese wild boars; Hongo *et al.* 2002: Viet1 to Viet17, Vietnamese domestic pig and wild boar). Figure 2 shows the two major lineages of mtDNA haplotypes: Asian (64% bootstrap value) and European. The Asian lineage was subdivided into two clusters: a Ryukyu wild boar cluster; and an East Asian cluster including Japanese wild boars, Taiwanese wild boars and Korean wild boars. The 20 novel Vietnamese haplotypes were distributed across four of the five groups in the Asian cluster: Viet20, Viet22 and Viet31 in the Korean wild boar group; Viet18–19, Viet25–28, Viet33–37 in the East Asian domestic boar group; Viet29 in the Taiwan wild boar group; Viet21, Viet23, Viet24, Viet30 and Viet32 in the Ryukyu wild boar group. No Vietnamese mtDNA haplotype was included in the Japanese wild boar group (Fig. 2). The mtDNA sequences from the 65 Vietnamese bones were distributed among several groups, suggesting that they have sequence diversity.

Relationship between results of morphological and genetic analyzes

Domestication of wild boars is characterized by reduction of body size and shortening of the cranium, especially involving the teeth (Flannery 1983). Table 1 summarizes the present comparison between morphological measurements and mtDNA haplotypes. Of the 58 present samples used for morphological measurement, the mtDNA haplotypes of the 31 large-sized bones belonged to the Korean, Taiwan and Ryukyu wild boar groups, while the 27 small-sized bones belonged to the East Asian cluster (designated as S or L in Table 1). The seven bone samples (AI-2, AI-19, MP4, 899, RJT37, AU60 and AU61) not used for morphological measurement were genetically classified into the East Asian domestic group (AI-2, MP4 and AU61), Korean wild boar group (AI19) and Ryukyu wild boar group (899, RJT37 and AU60).

Genetic relationship between Ryukyu wild boars and Vietnamese wild boars

Table 2 shows the nucleotide polymorphic sites of mtDNA haplotypes belonging to the Ryukyu wild boar group, in the present study and in previous studies (Hongo *et al.* 2002; Watanabe *et al.* 2002). The mtDNA haplotypes Nagara 5 and Nagara 13 were detected in samples of ancient *Sus* bones found in the Nagrabaru Nishi shellmidden of Ie Island (Watanabe *et al.* 2002). Five mtDNA haplotypes (Kume104, Kume105, Kume109, Kume152 and Kume156) were detected in samples of ancient *Sus* bones found in the Shimizu shellmidden of Kume Island (Watanabe *et al.* 2002). The islands of Ie and Kume are located near the island of Okinawa. The parsimonious network was constructed using the mtDNA haplotypes described above (Fig. 3). The mtDNA haplotypes detected in samples from the Ryukyu archipelago (the islands of Kume, Iriomote, Okinawa, Ie and Amami) were more closely related to each other than they were to the mtDNA haplotypes detected in samples from Vietnamese wild boars. The mtDNA haplotype M20 from Okinawa was located in the middle of the parsimonious network of mtDNA haplotypes from the Ryukyu archipelago. The mtDNA haplotype M57 from Korean wild boars was classified into the Ryukyu wild boar lineage in the tree, whereas it is classified into the Korean wild boar group with the mtDNA haplotype M56 in the previous study (Hongo *et al.* 2002; Fig. 3). The 10 mtDNA sequences from Vietnamese wild boars (Viet12–16, Viet21, Viet23, Viet24, Viet30 and Viet32) are extremely diverse. The Vietnamese mtDNA haplotype Viet14 was closely related to the mtDNA haplotypes of Ryukyu wild boars in the parsimonious network (Fig. 3).

DISCUSSION

In 2002, Hongo *et al.* reported that mtDNA sequences isolated from large-sized skeletons from two research institutes in Hanoi were related to mtDNA sequences of Ryukyu and Korean wild boars. In the present study, to elucidate the relationship between morphological measurements of *Sus* bones and the mtDNA haplotypes detected in them, we examined 65 modern *Sus* bones stored in three research institutes and a village in Vietnam. Based on comparison of buccolingual measurements of mandibular P3, P4, M1 and M2, using the logarithmic ratio technique, we divided the 58 bones into two groups: large- and small-sized bone groups. The large-sized bone samples show hap

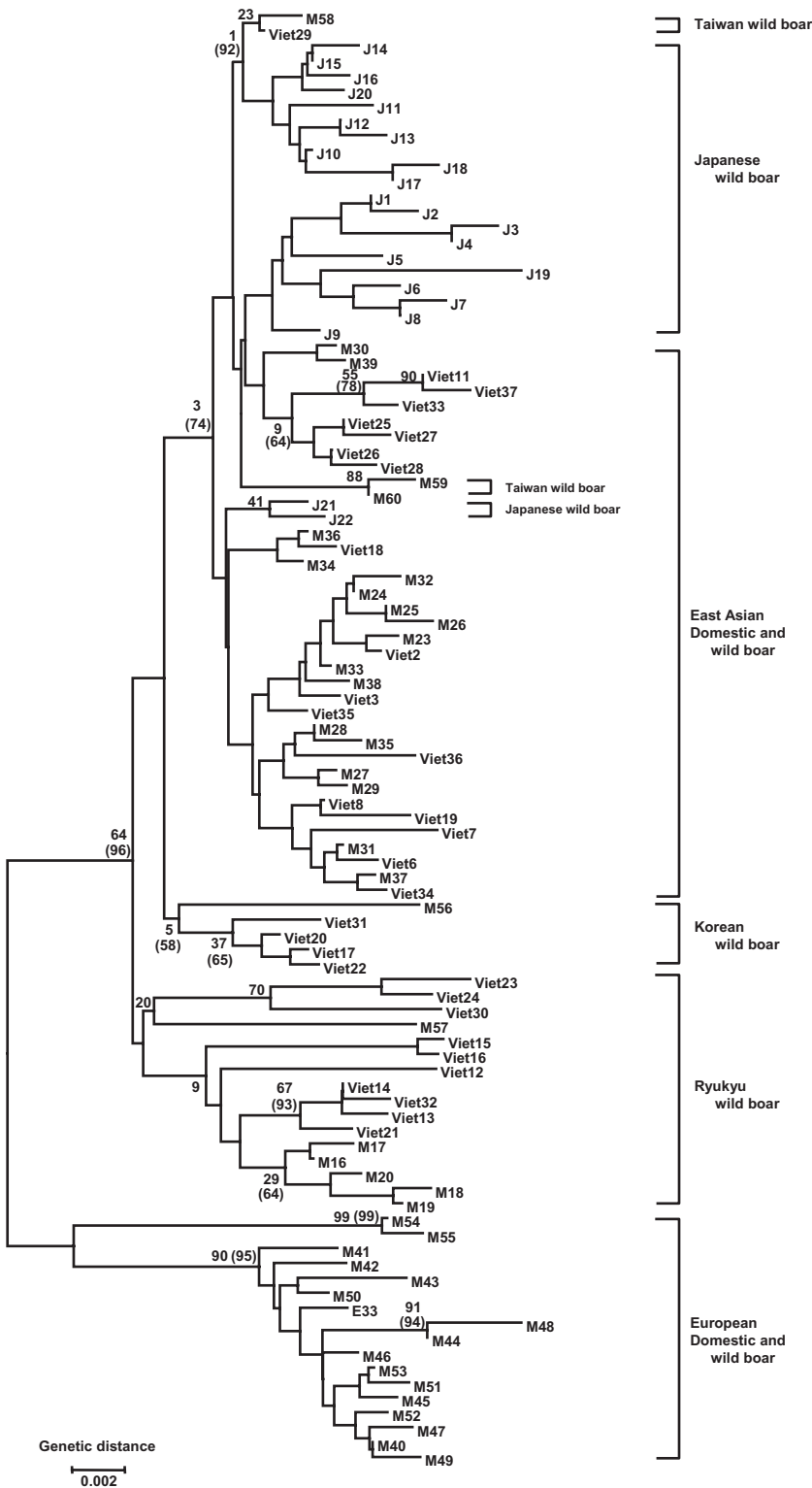


Figure 2 Neighbor-joining (NJ) phylogenetic tree constructed by the NJ method using the 572- to 574-bp mtDNA control region of 20 novel mtDNA haplotypes detected in the present study and 78 previously reported haplotypes (Hongo *et al.* 2002; Ishiguro & Nishimura 2005). Bootstrap resampling was performed 500 times, and bootstrap probabilities are shown on the corresponding branches. Numbers in the parenthesis indicate the interior branch test of phylogeny.

Table 2 Nucleotide variation of Vietnamese wild boar lineage

| Haplotype | Nucleotide positions ^a | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | Source | | | | | | | | | | | | | | | | | |
|-----------|-----------------------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|--------|-----|-----|-----|-----|-----|-----|-----|-----|-----|--------------------|---------|------------------|-----------|---|---|---|--|
| | 138 | 182 | 215 | 249 | 261 | 268 | 279 | 283 | 295 | 302 | 303 | 307 | 323 | 324 | 325 | 332 | 343 | 349 | 378 | 388 | 389 | 391 | 392 | 414 | 444 | 453 | 460 | 463 | 490 | 492 | 499 | 502 | | 531 | 543 | 561 | 585 | 606 | 638 | 641 | 658 | 690 | 693 | 703 | | | | | | |
| J1 | - | C | T | G | C | C | C | C | G | C | A | T | A | T | C | C | C | A | T | C | C | A | T | C | C | A | T | C | C | A | T | C | C | A | T | C | C | G | C | T | A | A | Japanese wild boar | | | | | | | |
| M57 | - | T | . | . | T | . | . | T | . | . | . | C | . | . | . | . | . | G | . | C | T | . | . | A | . | C | . | . | . | C | . | . | . | C | . | . | C | . | . | C | . | . | G | G | Korean wild boar | | | | | |
| M16 | - | T | . | . | . | . | T | . | . | . | C | . | . | . | . | . | . | G | . | G | C | . | . | A | . | C | . | . | . | C | . | . | . | C | . | . | C | . | . | C | . | . | G | G | Anamni Island | | | | | |
| M17 | - | T | . | . | . | . | T | . | . | . | T | . | . | . | . | . | . | G | . | G | C | . | . | A | . | C | . | . | . | C | . | . | . | C | . | . | C | . | . | C | . | . | G | G | | | | | | |
| M18 | - | T | C | . | . | . | T | . | . | . | T | . | . | . | . | . | . | G | . | G | C | . | . | A | . | C | . | . | . | C | . | . | . | C | . | . | C | . | . | C | . | . | G | G | Iriomote Island | | | | | |
| M19 | - | T | C | . | . | . | T | . | . | . | T | . | . | . | . | . | . | G | . | G | C | . | . | A | . | C | . | . | . | C | . | . | . | C | . | . | C | . | . | C | . | . | G | G | | | | | | |
| M20 | - | T | . | . | . | . | T | . | . | . | C | . | . | . | . | . | . | G | . | G | C | . | . | A | . | C | . | . | . | C | . | . | . | C | . | . | C | . | . | C | . | . | G | G | | | | | | |
| Viet13 | - | T | C | . | . | . | T | . | . | . | C | . | . | . | . | . | . | G | . | G | C | . | . | A | . | G | C | . | . | T | . | . | C | . | . | C | . | . | C | . | . | G | G | Vietnam | | | | | | |
| Viet14 | - | T | C | . | . | . | T | . | . | . | C | . | . | . | . | . | . | G | . | G | C | . | . | A | . | G | C | . | . | . | C | . | . | . | C | . | . | C | . | . | C | . | . | G | G | | | | | |
| Viet15 | - | T | . | . | . | . | T | . | . | . | G | . | C | . | . | . | . | G | . | G | C | . | . | A | . | C | . | . | . | C | . | . | . | C | . | . | C | . | . | C | . | . | G | G | | | | | | |
| Viet16 | - | T | . | . | . | . | T | . | . | . | G | . | C | . | . | . | . | G | . | G | C | . | . | A | . | C | . | . | . | C | . | . | . | C | . | . | C | . | . | C | . | . | G | G | | | | | | |
| Viet12 | - | T | C | . | . | . | . | . | . | . | G | C | . | . | . | . | . | G | . | G | C | . | . | A | . | C | . | . | T | . | . | C | . | . | . | C | . | . | C | . | . | G | G | | | | | | | |
| Viet21 | - | T | C | . | . | . | T | . | . | . | C | . | . | . | . | . | . | G | . | G | C | . | . | A | . | C | . | . | . | T | . | . | C | . | . | . | C | . | . | C | . | . | G | G | | | | | | |
| Viet32 | - | T | C | . | . | . | T | . | . | . | C | . | . | . | . | . | . | G | . | G | C | . | . | A | . | C | . | . | . | T | . | . | C | . | . | . | C | . | . | C | . | . | G | G | | | | | | |
| Viet23 | - | T | . | . | . | . | T | . | . | . | T | . | . | . | . | . | . | G | . | G | C | . | . | A | . | C | . | . | . | . | C | . | . | . | C | . | . | . | C | . | . | C | . | . | G | G | | | | |
| Viet24 | - | T | . | A | . | . | T | . | . | . | T | . | . | . | . | . | . | G | . | G | C | . | . | A | . | C | . | . | . | . | C | . | . | . | C | . | . | . | C | . | . | C | . | . | G | G | | | | |
| Viet30 | - | T | . | . | . | . | T | . | . | . | C | . | . | . | . | . | . | G | . | G | C | . | . | A | . | C | . | . | . | A | . | . | . | C | . | . | . | C | . | . | C | . | . | G | G | Ie Island | | | | |
| Nagara5 | - | T | . | . | . | . | T | . | . | . | C | . | . | . | . | . | . | G | . | G | C | . | . | A | . | C | . | . | . | . | C | . | . | . | C | . | . | . | C | . | . | C | . | . | G | G | | | | |
| Nagara13 | - | T | . | . | . | . | T | . | . | . | C | . | . | . | . | . | . | G | . | G | C | . | . | A | . | C | . | . | . | . | C | . | . | . | C | . | . | . | C | . | . | C | . | . | G | G | | | | |
| Kume104 | A | . | . | . | . | . | T | . | . | . | C | . | . | . | . | . | . | G | . | G | C | . | . | A | . | C | . | . | . | . | C | . | . | . | C | . | . | . | C | . | . | C | . | . | G | G | | | | |
| Kume105 | A | . | . | . | . | . | T | . | . | . | C | . | . | . | . | . | . | G | . | G | C | . | . | A | . | C | . | . | . | . | C | . | . | . | C | . | . | . | C | . | . | C | . | . | G | G | | | | |
| Kume109 | A | . | . | . | . | . | T | . | . | . | C | . | . | . | . | . | . | G | . | G | C | . | . | A | . | C | . | . | . | T | . | . | A | T | . | . | C | . | . | . | C | . | . | C | . | . | G | G | | |
| Kume152 | A | . | . | . | . | . | T | . | . | . | C | . | . | . | . | . | . | G | . | G | C | . | . | A | . | C | . | . | . | . | C | . | . | . | C | . | . | . | C | . | . | C | . | . | C | . | . | G | G | |
| Kume156 | A | . | . | . | . | . | T | . | . | . | C | . | . | . | . | . | . | G | . | G | C | . | . | A | . | C | . | . | . | T | . | . | . | C | . | . | . | C | . | . | C | . | . | C | . | . | G | G | | |

^aNucleotide position 1 corresponds to the first position of the complete DNA sequences of mtDNA control region described by Okumura *et al.* 2001. Dots indicate the nucleotide identity with Japanese wild boar haplotype J1.

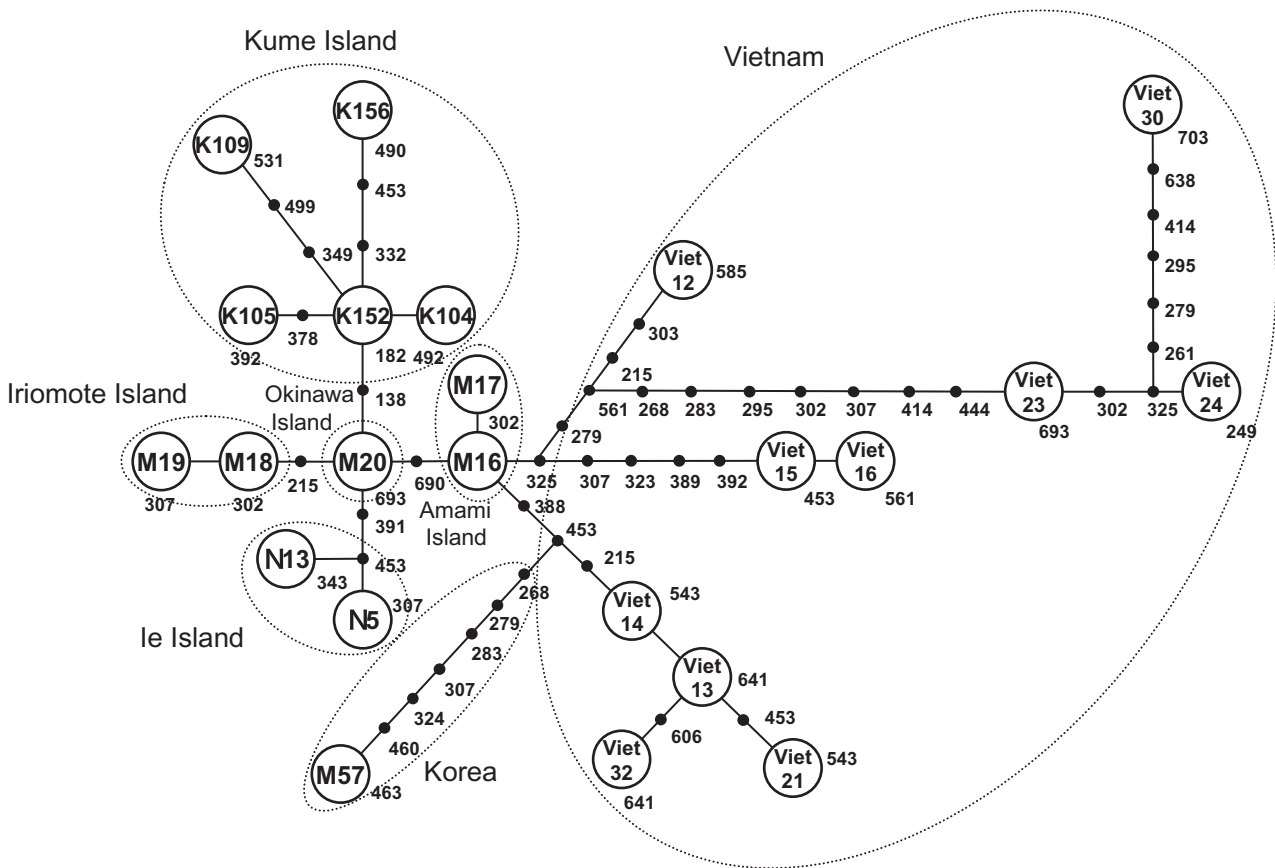


Figure 3 Parsimonious network constructed using 23 mtDNA haplotypes with Ryukyu wild boar lineage. The 23 mtDNA haplotypes comprise five novel mtDNA haplotypes found in the present study (Viet21, Viet23, Viet24, Viet30, Viet32), five mtDNA haplotypes found in Vietnamese *Sus* bones (Viet12, Viet13, Viet14, Viet15, Viet16; Hongo *et al.* 2002), seven mtDNA haplotypes from ancient *Sus* bones (N5, Nagara5; N13, Nagara13; K104, Kume104; K105, Kume105; K109, Kume109; K152, Kume152; K156, Kume156; Watanabe *et al.* 2002) and five mtDNA haplotypes from modern Ryukyu wild boars (M16, M17, M18, M19, M20; Watanabe *et al.* 1999). The nucleotide position numbers indicate substitutions in Table 2.

lotypes that were found mostly from Ryukyu, Korean and Taiwan wild boars, and none of them had haplotypes of Japanese wild boars, whereas the small-sized bones belonged to East Asian domestic and wild boar group. These results suggest that Vietnamese wild boars share a common ancestor with East Asian wild boars.

Domestication from wild animals to domestic animals generally involves morphological changes such as reduction in body size and shortening of the cranium, including changes in tooth size (Flannery 1983). The present results of comparison between morphological results and phylogenetic analysis are consistent with the general theory of the domestication process. The morphological measurements clearly divided the present samples into two groups: large-

sized bones corresponding to wild boar lineage, and small-sized bones corresponding to domestic pig lineage. No intermediate form existed in the present samples. There are many native domestic pig breeds in Vietnam, including Mong Cai, and Meo examined in this study (Thuy *et al.* 2006). Unfortunately, it is difficult to identify Vietnamese pig breeds from the shapes of their bones. Several wild boar subspecies inhabit East Asian countries such as China and Vietnam, and it has been suggested that domestication of pigs from local populations of wild boars occurred between 6000 and 9000 years ago (Xu 1950). Domestic Vietnamese pig breeds have been derived from several wild boar subspecies with the purpose of obtaining a stable supply of animal protein. In the present study, none of the bone or tooth samples in the small bone group

showed evidence of Ryukyu wild boar lineage. The modern samples collected were divided into two size groups without overlap and there was no individual with an intermediate size between the two groups. This result suggests that modern Vietnamese domestic pig breeds are distinct from the local wild boar population, and no interbreeding occurred between the domestic and wild population. It is unclear why no wild boars with Ryukyu wild boar lineage were domesticated in Vietnam in ancient times. Perhaps the Ryukyu wild boar lineage was also once domesticated, but the type was later wiped out by other lineages.

The origin of Ryukyu wild boars has been debated for many years, because no wild boars genetically related to Ryukyu wild boars have been found in areas near Ryukyu, such as Kyushu Island, Taiwan and China. It is unknown whether descendants of the ancestor of Ryukyu wild boars still inhabit Taiwan and China, although wild descendants of the ancestor of Ryukyu wild boars have been found in Vietnam (data not shown). It is thought that in prehistoric times when the Ryukyu archipelago was part of the Asian continent, wild boars with Ryukyu wild boar lineage were widely distributed on the Asian continent. There have been several opportunities for the ancestor of Ryukyu wild boars to migrate to the Ryukyu archipelago from the Asian continent via a land bridge (Kizaki & Oshiro 1980; Ujiie 1986). After the Ryukyu archipelago became separated from the Asian continent, Ryukyu wild boars evolved into a unique form on the islands they inhabited. The skeletons of Ryukyu wild boars are morphologically smaller than those of Vietnamese wild boars (Hongo *et al.* 2002). The reduction of the morphological size of the skeletons is due to the island-isolation effect on the isolated Ryukyu archipelago. However it is difficult to assess the direct pressures leading to the size reduction. Imaizumi (1973) speculated that Ryukyu wild boars may be a relic of continental wild boars, as are some other endemic wild boar species on the Ryukyu Islands. The mtDNA sequence diversity found among Ryukyu wild boars supports Imaizumi's hypothesis that Ryukyu wild boars are a unique species established on the isolated Ryukyu archipelago (Fig. 3). The mtDNA sequences of Ryukyu wild boars on different Ryukyu Islands are distinguished from each other by nucleotide substitutions at 1–9 different sites (Fig. 3). The mtDNA diversity among wild boars on different islands of the Ryukyu archipelago has probably been influenced by geographic changes such as union or separation between various islands that continually

occurred in prehistoric times (Kizaki & Oshiro 1980; Ujiie 1986). The five mtDNA sequences previously identified in ancient *Sus* bones excavated from the Shimizu shellmidden on Kume Island (K104, K105, K109, K152 and K156) all possess the unique 139-A insertion (insertion of nucleotide A at position 138; Table 2; Watanabe *et al.* 2002). The 139-A insertion has also been found in the Korean wild boar haplotype M56 (Fig. 3). These results suggest that Kume wild boars and Korean wild boars are derived from a common ancestor on the Asian continent that is also the ancestor of Vietnamese wild boars. Further morphological and genetic analysis of *Sus* bones will provide important information about the domestication history and geographical distribution of domestic pigs and wild boars in prehistoric times.

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