Title

Mitochondrial DNA Analysis of Ancient Domestic Goats in the Southern Caucasus: A Preliminary Result from Neolithic Settlements at Göytepe and Hacı Elamxanlı Tepe

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Abstract
This study presents preliminary results of mitochondrial DNA (mtDNA) analyses of modern and ancient domestic goats in the southern Caucasus in order to examine their phylogenetic relationship with modern and ancient goats. For this purpose, seven ancient samples were obtained from two early agricultural villages in west Azerbaijan (Göytepe and Hacı Elamxanlı tepe, dated to ca. 6,000–5,500 cal BC, the Pottery Neolithic period), in addition to five modern goat samples in the same region. In the study, mtDNA segments of the control region (216 bp for the Neolithic samples and 481 bp for the modern samples) were amplified and phylogenetic analyses were performed using previously published reference DNA sequences. As a result, all the haplotypes found in this study were grouped in the haplogroup A of goats.

The finding of the haplogroup A among domestic goats in the southern Caucasus in the early 6th millennium BC can be interpreted as part of the geographic expansion of this lineage from the areas of initial domestication to surrounding areas that include also South and Southeast Europe. In the southern Caucasus, the haplogroup A probably continued to be a major lineage among domestic goats since their emergence in this area to the present. In contrast, this lineage has not been detected among local wild goats including *Capra aegagrus*, indicating the external origin of domestic goats. This possibility is consistent with archaeological records that indicate sudden appearance of agricultural lifeways in the southern Caucasus and cultural connections with northern Mesopotamia.

Introduction

The emergence of domestic animals has long been a major research issue in archaeological studies on the development and spread of food production economy. Among various domestic animals, this study is concerned with goats (*Capra hircus*) and aims to examine the introduction of domestic goats in the southern Caucasus by analyzing mitochondrial DNA (mtDNA) of the earliest domestic goats in the region (ca. 6,000–5,500 cal BC).

Goat husbandry is currently widespread all over the world as domestic goats are adapted to various climatic and environmental conditions (e.g., from tropical deserts and rainforests to

mountainous areas), and numerous goat breeds (more than 300) have been developed for a wide range of uses such as milk, meat, fiber, and skin (Luikart et al., 2001, 2006). On the other hand, there are several kinds of wild animals classed in the same genus *Capra* as domestic goats, including west Caucasian tur (*Capra caucasia*), east Caucasian tur (*Capra cylindricornis*), markhor (*Capra falconeri*), bezoar (*Capra aegagrus*), alpine ibex (*Capra ibex*), nubian ibex (*Capra nubiana*), spanish ibex (*Capra pyrenaica*), siberian ibex (*Capra sibirica*), and walia ibex (*Capra walie*) (Pereira and Amorim, 2010). Although these groups are given different species names, their hybrids are fertile (Pereira and Amorim, 2010). The distributions of these wild *Capra* are narrower than domestic goats and restricted to parts of west Asia, central Asia, northeastern Africa, and southwestern Europe. Although these regions are possible locations for the domestication of goats, numerous archaeological and genetic studies have suggested that all the domestic goats in the world derived from the bezoar (*Capra aegagrus*) that are distributed in west Asia, as described below.

Research background

Archaeological identifications of early domestic goats have been practiced as part of the investigations on the development of agriculture. Archaeological records from various parts of the world indicate several regions, where agricultural activities developed independently, i.e., primary agricultural centers. West Asia is one of such primary agricultural centers and provides the earliest archaeological evidence for the domestication of goats and other major livestock species (i.e., sheep, cattle, and pigs) as well as crops (e.g., wheat, barley, and legumes), dated to ca. 8,000 cal BC or earlier (Weiss and Zohary, 2011; Zeder, 2011).

In the surrounding regions, such as Europe, Africa, and central/south Asia, agricultural economy appeared later than west Asia, in association with the similar kinds of domesticates to west Asia (Zeder, 2008). This has been widely recognized to indicate the diffusion of domesticates (including goats) from west Asia to other regions. In many areas, such west Asian-type domesticates are not indigenous and thus have external origins with no doubts. However, for the regions distributed with possible wild progenitors of domesticates, it is more difficult to identify the origins of domesticates because they may have been either introduced from primary domestication centers or locally created from indigenous wild animals/plants with or without cultural influence from other regions.

Regarding the origin of domestic goats, such an equivocal situation applies to the regions distributed with wild *Capra*, as described above, because the earliest appearance of domestic

goats in west Asia does not necessarily exclude the possibility of multiple independent domestications of wild *Capra* in other regions. This problem has been effectively examined through phylogenetic studies of mtDNA.

Most of the genetic studies on the origin of domestic goats have dealt with modern samples of domestic goats and other *Capra* species. As a pioneering study, Takada et al. (1997) compared the cytochrome b gene of mDNA from six domestic goats (*Capra hircus*), one bezoar (*Capra aegagrus*), and one markhor (*Capra falconeri*), and showed a close genetic relationship between the first two, proposing *Capra aegagrus* as likely ancestors of domestic goats. This suggestion was supported by a subsequent study (Mannen et al. 2001), which increased the sample size of *Capra hircus* and *Capra falconeri* and newly added a cytochrome b sequence of *Capra ibex* in the phylogenetic analysis. As a result, *Capra falconeri* and *Capra ibex* appeared as outgroups to a cluster including both *Capra hircus* and *Capra aegagrus*.

In contrast, multiple maternal origins of domestic goats in east and south Asia in addition to west Asia were proposed by a subsequent study based on much broader samples of *Capra hircus* (n = 406) representing 88 breeds from various areas in the Old World (Luikart et al., 2001). Using such numerous samples, Luikart et al. (2001) conducted phylogenetic analyses of the HVI segment (481 bp) of the mtDNA control region and identified three divergent lineages (A, B, and C) within *Capra hircus*. These three lineages are estimated to have diverged long before the time of domestication (ca. 280–200 ka for the divergence in contrast to ca. 10 ka for the domestication), and this study found the lineage B only in east and south Asia. Mainly based on these observations, Luikart et al. (2001) suggested that there was an independent origin of domestic goats in east and south Asia in addition to west Asia (or the Fertile Crescent). This proposal has prompted several studies that seek the origin of domestic goats in east Asia (Han et al., 2010).

Subsequently, a further increase in the sample size (n = 2430) revealed greater genetic diversity of *Capra hircus* (Naderi et al., 2007). Based on the phylogenetic analysis of the HVI segment (558 bp) of the mtDNA control region, Naderi et al. (2007) identified six divergent groups (or haplogroups) that are called A, B, C, D, F, and G within *Capra hircus*. Among these haplogroups, the A group is the most abundant, accounting for more than 90% of domestic goats and distributed widely in the Old World. The other groups are minor, but their geographic distributions are not clearly structured, except for the B2 group confined to China and Mongolia and the F group to Sicily.

To supplement the data of modern domestic goats, Naderi et al. (2008) analyzed the genetic variability of modern wild goats focusing on *Capra aegagrus* (bezoar), which has been considered the primary progenitor of domestic goats by previous archaeological and genetic studies. The study analyzed an unprecedentedly large number of *Capra aegagrus* (*n* = 473) by sequencing the HVI segments (481–558 bp) of the mtDNA control region. As a result, all the mtDNA haplogroups of domestic goats (A, B, C, D, F, and G) have also been found in *Capra aegagrus*. This suggests that major genetic variations of modern domestic goats correspond to those of *Capra aegagrus*, and thus strongly indicates that domestic goats originated in west Asia where *Capra aegagrus* are distributed.

More recently, the early history of goat domestication has been investigated by ancient DNA studies (Table 1). Such studies are still rare, and the sample size is much smaller than those of modern goats. However, ancient DNA studies can provide more direct records regarding the origin and spread of domesticates on the basis of genetic records closer in time to such prehistoric processes. Table 1 shows main results of recent genetic studies of ancient domestic goats from various time periods and regions. All of these studies have identified mtDNA haplogroups common to modern domestic goats, showing their temporal and geographical distributions in the past. The early domestication of goats in west Asia is particularly indicated by the detection of the haplogroup A for five goat samples from East Chia Sabz (western Iran) dated to the 9th millennium BC (Mazdarani et al., 2014).

Aims of the study

In this research, the ancient goat samples (ca. 6,000–5,000 cal BC) are later than those of East Chia Sabz or the earliest archaeological evidence for goat domestication in the eastern Taurus and the Zagros (ca. 8,000 cal BC or earlier: Zeder, 2008, 2011). However, they come from two sites, Göytepe and Hacı Elamxanlı Tepe (Fig. 1), which represent the earliest agricultural settlements in the southern Caucasus (Nishiaki et al. 2015a and 2015b) and are suitable for our aim to examine the phylogenetic background of early domestic goats in the southern Caucasus.

In addition, our samples are contemporary to ancient goat DNA samples from Neolithic settlements in Bulgaria, including Kovačevo, Cavdar, and Ovčarovo-gorata (early 6th millennium: Scheu, 2012), and immediately precede those of later Neolithic sites, such as Aşağı Pınar (The Balkan Peninsula) in Turkey, Uivar in Romania (Scheu, 2012), and Baume d'Oullen in France (Fernández et al., 2012), dated to the late 6th millennium BC. Because all

of these sites are located outside the range of *Capra aegagrus* (Fig. 1), the origin of domestic goats from these sites are likely external. In contrast, the southern Caucasus is currently distributed with *Capra aegagrus*, which raises a possibility of local domestication of goats. However, according to Naderi et al, 2007, 2008, *Capra aegagrus* in the southern Caucasus are genetically distant from modern domestic goats in the same region. Specifically, modern domestic goats in the southern Caucasus belong to the mtDNA haplogroup A or B, while these two haplogroups have not been found in modern *Capra aegagrus* in the same area, which instead belong to the F group or other minor lineages. This genetic difference between domestic and wild goats suggests an alternative scenario that the origin of domestic goats in the southern Caucasus is external like those of Neolithic goats in South and Southeast Europe in the 6th millennium BC. We examine this issue by analyzing ancient goat DNA and discussing the results in light of previous studies on ancient goat DNA and archaeological records from the earliest agricultural settlements in the southern Caucasus.

Material and methods

To obtain ancient goat DNA, goat bone remains were sampled from two Neolithic sites, Göytepe and Hacı Elamxanlı Tepe, in the Republic of Azerbaijan (Fig. 1). The two sites are among the earliest agricultural settlements in the southern Caucasus, dated to the first half of the 6th millennium cal. BC (See Appendix S1 for more archaeological information).

As shown in Table 2, six bones were selected for DNA analyses from the faunal remains classed as *Capra hircus* or *Capra*/*Ovis* on the basis of morphological attributes (Nishiaki et al., 2013, 2015c). In addition, we include one sample that was morphologically identified as *Ovis aries* (domestic sheep) but turned out to be *Capra hircus* as a result of the mtDNA analysis, as described later. In total, here we report the analyses of seven ancient bones, consisting of four specimens from Göytepe and three from Hacı Elamxanlı Tepe.

Radiocarbon dates and collagen preservation

To verify the Neolithic age of the bone samples for DNA analyses, three pieces were selected for ^{14}C dating (Goy-1, Goy-2, and Hac-1 in Table 2), which was conducted at the Center for Chronological Research, Nagoya University. Gelatin collagen was extracted by following the method by Minami et al. (2013: a NaOH treatment without ultrafiltration), and the three specimens provided relatively high collagen yields (the obtained amount of gelatin collagen divided by the amount used for collagen extraction: 3.31 wt% from Goy-1, 6.45

wt% from Goy-2, and 8.85 wt% from Hac-1). This indicates good preservation of the ancient bone samples, which were probably facilitated by moderately alkaline conditions of sediments at the sites. Nine sediment samples from Levels 4 and 10 at Göytepe showed pH values between 7.6 and 8.4 (Kadowaki et al., 2015).

As a result, the two specimens from Göytepe were dated around the mid 6th millennium cal BC, while the one from Hacı Elamxanlı Tepe was older, i.e., early 6th millennium cal BC (Table 2). These new dates are consistent with previous ^{14}C dates of plant charcoal specimens (n = 46) sampled from every occupation level at Göytepe and Hacı Elamxanlı Tepe, as described above (Nishiaki et al., 2015a).

Methods for ancient DNA extraction

DNA extraction from the seven Neolithic bones primarily followed the protocol employed by Ishiguro et al. (2009) and Okumura et al. (1999). First, the exterior surface of the bones was cleaned by scraping with sterile scalpels. The cleaned surface was drilled to obtain bone powder (0.1–0.5 g), which was then suspended in 10 ml of 0.5 M ethylenediamine tetraacetic acid (EDTA) and rotated for decalcification. During this process, supernatant was removed after centrifuging at 2500 r.p.m. for 15 minutes, and the bone powder was repeatedly decalcified with 10 ml of 0.5 M EDTA until the supernatant became transparent. After the decalcification, the bone powder was treated with proteinase K (20 mg/mL), N-lauroylsarcosine (10%), and 4.5 ml of 0.5 M EDTA under rotation for two days at 37 °C. Then, the samples were centrifuged at 3000 r.p.m. for 15 minutes at 20 °C. The supernatant with ancient DNA was extracted once with phenol, followed by a treatment with chloroform to remove protein. The supernatant was then concentrated with Amicon® Ultra 30K filter device (Merck Millipore), centrifuged at 4000 r.p.m. for 20 minutes at 20 °C. Finally, the supernatant was washed with ultrapure water created by Millipore Milli-Q Gradient A10 (Merck Millipore) to obtain DNA extracts (ca. 0.1–0.15 ml) that can be used directly for PCR.

Methods for ancient DNA amplification and sequencing

A HVI segment (216 bp excluding primers) of the mtDNA control region was amplified by PCR using primers: Cap-B (5'-CATTAAACGATTTACCACATGC-3') and Cap-RII (5'-CGGGTTGCTGGTTTCAC-3'). Cap-RII has been used in Fernández et al. (2006), while Cap-B was newly designed in this study. This segment was chosen to contain the region of

the ancient samples analyzed by Fernández et al. (2006) and overlap part of the longer segments of the modern samples reported by Luikart et al. (2001) and Naderi et al. (2007, 2008). PCR amplifications were performed in a reaction volume of 50 µl containing 2 units of AmpliTaq Gold DNA polymerase (Life Technologies), 5 µl of 10xPCR buffer, 3 µl of 25 mM MgCl2, 4 µl of 2 mM dNTP, 0.2 µl of 10 mg/ml bovine serum albumin (BSA), 1 µl of 10 µM each of the primers, and 1.1µl of DNA template in sterilized water. The thermal conditions for PCR are as follows: the first denaturation at 95°C for 10 minutes, followed by 50 cycles of denaturation at 94°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 1 minute, and then final extension at 72°C for 10 minutes. The PCR products were checked by electrophoresis with 1 % agarose gel and then purified with illustra ExoProStar (GE Healthcare Life Sciences). The purified DNA products were directly sequenced with the same primers and a BigDye Terminator Cycle Sequencing Kit (Lite Technologies) at the Center for Gene Research, Nagoya University.

See Appendix S2 for the authentication of ancient DNA experiments.

Modern DNA experiments

Modern domestic goats in Azerbaijan were sampled for comparison with the ancient samples described above. The modern goat samples were obtained at a small, local farm (40°58'16.16"N 45°42'21.31"E) besides Göytepe. Five individuals were selected among the indigenous breeds in the farm, and their hair was collected as samples for DNA extractions (Table 2).

After cleaning the goat hair samples (n = 5), hair roots were used to extract DNA with a QIAamp DNA Micro Kit (Qiagen), following the protocols of the kit.

A HVI segment (481 bp excluding primers) of the mtDNA control region was amplified to correspond to the region analyzed by Luikart et al. (2001) and Naderi et al. (2007, 2008). We used two primers, Cap-F and Cap-R, which have been employed in Naderi et al. (2007, 2008). PCR amplifications were performed in a reaction volume of 20 µl containing 1.25 units of KARATaq EXtra DNA polymerase (Clontech), 4.4 µl of 5xPCR buffer, 1.4 µl of 25 mM MgCl2, 0.6 µl of 10 mM dNTP, 1 µl of 10 µM each of the primers, and 2 µl of DNA template in sterilized water. The thermal conditions for PCR are as follows: the first denaturation at 92°C for 10 minutes, followed by 35 cycles of denaturation at 92°C for 1 minute, annealing at 50°C for 1 minute, and extension at 72°C for 45 seconds, and then final extension at 72°C for 10 minutes. The PCR products were checked by electrophoresis with 1 % agarose gel and

then purified with illustra ExoProStar (GE Healthcare Life Sciences).

The purified DNA products were directly sequenced with the same primers and a BigDye Terminator Cycle Sequencing Kit (Life Technologies) at the Center for Gene Research, Nagoya University.

Alignment of DNA sequences and phylogenetic analysis

The results of the DNA sequencing were aligned with reference sequences of modern domestic and wild goats reported in Naderi et al. 2007 and 2008. We selected sequences including 22 reference sequences of modern domestic goats covering their major mtDNA haplogroups (A, B1, B2, C, D, F, and G) in addition to 26 sequences of six different species of wild *Capra* and three sequences of *Ovis aries*, as listed in Table 3. We constructed a phylogenetic tree based on a 216 bp segment of mtDNA control region.

The ancient haplotypes (AA-1 and AA-2) identified in this study were also aligned with ancient mtDNA sequences reported in previous studies (Table 1). We selected Neolithic samples that are roughly contemporaneous with ours (the 6th millennium cal BC), including those from Kovačevo, Cavdar, Ovčarovo-gorata, Aşağı Pınar, Uivar, and Baume d'Oullen (Table 1). In addition, we included earlier Neolithic samples (the 9th millennium BC) from East Chia Sabz (Mazdarani et al., 2014). We constructed a phylogenetic tree based on a 130 bp segment of mtDNA control region, which overlaps among the compared samples.

We used MEGA 6 (Tamura et al., 2013) to align the sequences and to construct a phylogenetic tree, which was created by the neighbor-joining method (Saitou and Nei, 1987) under the Kimura-2-parameter model (Kimura, 1980).

Results: Mitochondrial haplogroup of modern and Neolithic goats in the southern Caucasus

Identification of ancient DNA haplotypes

As shown in Table 2, we successfully amplified 216 bp of the HVI control region of five ancient samples. The obtained sequences were differentiated into two haplotypes (AA-1 and AA-2: AA representing Azerbaijan Ancient). The two haplotypes differ from each other by only three substitutions, which are all transitions between A and G or between C and T, at positions 16006, 16019, and 16045 (Table 4). The reliability of our identification of the ancient DNA haplotypes is discussed in Appendix S3.

A single sample (Hac-7) was morphologically identified as *Ovis aries*, but it produced the

same haplotype (AA-1) as three samples of *Capra hircus* (Goy-1, Goy-2, and Goy-4). This difference between genetic and morphological identification is discussed in Appendix S4.

Identification of modern DNA haplotypes

From the five modern domestic goat samples, the 481 bp of the HVI control region was analyzed, and five haplotypes were differentiated (AM-1, AM-2, AM-3, AM-4, AM-5: AM representing Azerbaijan Modern) (Table 2). The haplotype AM-4 shows the sequence identical to the corresponding region of a domestic goat (GenBank ID: EF618493) reported in Naderi et al., 2007. On the other hand, the haplotypes AM-1, 2, 3, and 5 are unique sequences (Table 4).

Phylogenetic analysis with modern reference samples

To examine phylogenetic relationships, a neighbor-joining phylogenetic tree was constructed (Fig. 2) by including the haplotypes identified in this study (Table 4) and the comparative DNA sequences, including modern domestic goats, wild *Capra* groups, and modern domestic sheep (Table 3).

As a result, the sequences of domestic sheep were clearly separated as an outgroup. In addition, all the ancient and modern haplotypes found in this study (AA-1, 2, and AM-1, 2, 3, 4, 5) were grouped in a cluster of the haplogroup A of *Capra hircus* and *Capra aegagrus*. The other major haplogroups (B, C, D, F, and G) also formed distinct clusters, each of which consists of both *Capra hircus* and *Capra aegagrus*. On the other hand, other wild *Capra* groups, such as *Capra cylindricornis* and *Capra falconeri*, were separated from the above major haplogroups. These topological patterns are consistent with previous phylogenetic analyses of modern goats based on longer segments of the control region (481 bp) and larger sample size (2430 sequences of *Capra hircus*, 473 sequences of *Capra aegagrus*) (Naderi et al., 2007, 2008).

To examine genetic relationship between domestic and wild goats in the southern Caucasus, six samples of *Capra aegagrus* in Azerbaijan were also included in the phylogenetic analysis (EF989466, EF989553, EF989583, EF989584, EF989585, EF989633 in Table 3). As shown in Fig. 2, all the six samples do not belong to any of the major haplogroups and are clearly separated from both the modern and Neolithic domestic goats in Azerbaijan.

Phylogenetic analysis with ancient goat DNA from other Neolithic sites

As shown in Fig. 3, the two Neolithic haplotypes (AA-1 and AA-2) found in this study clustered with other Neolithic haplotypes that had been identified as the haplogroup A in previous studies. Within this group, the two haplotypes (AA-1 and AA-2) of our samples do not cluster tightly, but AA-1 is moderately separated from most of other A-lineage samples.

These haplotypes in the A group are clearly separated from another cluster that includes samples identified as the haplogroup C in previous studies (Fernández et al., 2006; Scheu, 2012).

Discussions

Phylogenetic background of Neolithic domestic goats in the southern Caucasus

The results of the above analyses indicate that our samples from Neolithic domestic goats in the southern Caucasus belong to the haplogroup A. This is suggested by phylogenetic relationship with modern references (Fig. 2) as well as other Neolithic goat samples (Fig. 3).

The presence of A-lineage domestic goats in the southern Caucasus during the early 6th millennium BC is expectable in light of the space-time distribution of this lineage indicated by previous studies. The A-lineage goats have been found in three Neolithic sites in Bulgaria (i.e., Kovačevo, Cavdar, and Ovčarovo-gorata), dated to the early 6th millennium BC (Scheu, 2012) and also in East Chia Sabz, the central Zagros, dated to the 9th millennium BC (Mazdarani et al., 2014). These results, coupled with archaeological records on the domestication of goats (Zeder, 2008, 2011), suggest the initial domestication of the A-lineage goats in west Asia in the 9th millennium BC and their subsequent appearance in the southern Caucasus and Southeast Europe by the early 6th millennium BC.

The A-lineage goats have also been detected at Tachti Perda (Georgia), another archaeological site in the southern Caucasus, dated to 1,400–700 BC (Table 1: Scheu, 2012). At present, the haplogroup A is a dominant lineage in the southern Caucasus, according to the results of this study (haplotypes AM 1–5) and Naderi et al. 2007.

On the basis of these observations, we suggest that the haplogroup A has been a major lineage among domestic goats in the southern Caucasus since their emergence in this area to the present. A single specimen belonging to the haplogroup B was found in the modern samples by Naderi et al. 2007. Admittedly, the sample size of modern and ancient domestic goat DNA from the southern Caucasus is still small (n = 10 for modern samples and n = 11 for ancient samples). Thus, the future increase in modern and ancient samples could find

other lineages in the southern Caucasus although they are likely to remain minor in comparison with the haplogroup A.

Despite the limitation in current datasets, early domestic goats in the southern Caucasus may be genetically distinguishable from the groups that spread to Europe. This is suggested by 1) the absence of the haplogroup C in the southern Caucasus in contrast to its presence at Aşağı Pınar and Baume d'Oullen and 2) the slight difference of the AA-1 (found in four of our samples: Table 2) from other ancient haplotypes in Europe in the 6th millennium BC (Fig. 3).

Origin of domestic goats in the southern Caucasus

Although the southern Caucasus is currently distributed with *Capra aegagrus*, they are phylogenetically distinct from modern domestic goats in the same area (Naderi et al., 2007, 2008), indicating the external origin of domestic goats in the southern Caucasus. This scenario is not inconsistent with the results of this study, which indicates that domestic goats in the southern Caucasus are likely to have been dominated by the haplogroup A since their emergence in the area and genetically distant from *Capra aegagrus* in the region.

The haplogroup-A *Capra aegagrus* is currently distributed in the areas from east Anatolia to northwest Iran (Naderi et al., 2008), where the earliest domestication of goats has been indicated by archaeological records (Zeder, 2008, 2011). This suggests that the initial domestication of the haplogroup-A goats took place somewhere in east Anatolia and northwest Iran, and domestic goats of the haplogroup A subsequently spread to surrounding areas, including the southern Caucasus.

This scenario can be confirmed by increasing the sample size of *Capra aegagrus* from the southern Caucasus (currently $n = 6$). It is also important to analyze ancient wild goats at the time or before the emergence of domestic goats in the region. Wild goats have been reported from several Middle and Upper Palaeolithic sites in the southern Caucasus. Among them, wild goats from Ortvale Klde and Dzudzuana Cave have been morphologically identified as Caucasian tur (*Capra caucasia*) (Adler et al., 2006; Bar-Oz et al., 2007), while those from Hovk-I, closer to Göytepe and Hacı Elamxanlı Tepe, are reported as *Capra aegagrus* (Bar-Oz et al., 2012).

Despite the limitation of the current genetic records, the external origin of domestic goats in the southern Caucasus is also suggested by archaeological records. First, the earliest evidence for agricultural economy in the southern Caucasus dates to ca. 6,000 cal BC

(Nishiaki et al., 2015a), which is 2,000–3,000 years after than the initial domestication of animals and cereals at several areas in the Fertile Crescent (Zeder, 2008, 2011; Wilcox, 2013). During this time gap of two to three millennia, there is currently no archaeological record indicating indigenous development of plant/animal domestication in the southern Caucasus. Several sites dated (or estimated) to the early Holocene in the southern Caucasus are either cave/rockshelter sites or camp sites without any architectural remains or evidence for domesticated plants or animals (Meshveliani et al, 2007; Meshveliani 2013; Arimura et al, 2010). In contrast, the earliest sites with agro-pastoral economy in the southern Caucasus, such as Göytepe and Hacı Elamxanlı Tepe, are associated with numerous mud-brick buildings and other architectural facilities, e.g., hearth and storages, and left as anthropogenic mounds resulting from repeated constructions of settled villages at the same spot (See Appendix S1). In this way, current archaeological records suggest that agricultural lifeways appeared suddenly in the southern Caucasus (Nishiaki et al., 2015a, 2015c; Lyonnet et al., 2015).

In association with this sudden appearance of agricultural villages, several lines of evidence suggest long distance contacts between the southern Caucasus and the Fertile Crescent (particularly northern Mesopotamia), where agriculture started earlier. The first is the possible introduction of emmer wheat (*Triticum dicoccum*). This domesticated type of wheat probably corresponds to hulled wheat that is common at early agricultural villages in the southern Caucasus, including Göytepe and Hacı Elamxanlı Tepe (Nishiaki et al., 2013, 2015c; Kadowaki et al., 2015). However, its wild progenitor (*Triticum dicoccoides*) is not distributed in this region (Weiss and Zohary, 2011). Thus, emmer wheat is likely to have been introduced to the southern Caucasus from agricultural centers in the Fertile Crescent.

The second is the possible import of painted fine wares to the southern Caucasus. Painted fine wares are very few and stand out among numerous coarse wares with limited decorations at early agricultural villages in the southern Caucasus (Fig. 4: Palumbi, 2007; Nishiaki et al., 2013; Badalyan and Harutyunyan, 2014). Because fine wares with similar patterns of painted decorations are common cultural elements at Neolithic sites in northern Mesopotamia or east Anatolia (e.g., Halaf and Samarra cultures), they may have been transported to the southern Caucasus through trades or population movements.

The third is a type of arrowheads shaped in the form of trapeze (Fig. 5), called also transversal arrowheads or geometric microliths (Badalyan and Harutyunyan, 2014). Trapezes have been found more frequently at Hacı Elamxanlı Tepe (early 6th millennium cal BC) than at Göytepe (mid 6th millennium cal BC) and thus represent part of material culture at the

very beginning of agricultural lifeways in the southern Caucasus (Nishiaki et al., 2013, 2015b, 2015c). Because trapezes of similar form and production technology exist in the region from northern Mesopotamia to southern Iran in the late 7th millennium to the early 6th millennium BC, they may be interpreted as evidence for cultural contacts between the southern Caucasus and the region to further south.

Such a cultural link is also suggested by a unique architectural structure, which we call "snowman-shaped building" (Fig. 6: Nishiaki et al., 2015a, 2015b, 2015c). This name derives from a building plan consisting of two round rooms attached to each other. Because the two round rooms are different in size (ca. 5 m and 2 m in diameter), they show a snowman-shaped plan. This type of building structure characterizes Hacı Elamxanlı Tepe (older than Göytepe) and has been reported from several other early agricultural settlements in the southern Caucasus (Nishiaki et al., 2015c). Interestingly, a snowman-shaped plan is also recognizable in the late Pre-Halaf or Proto-Halaf settlements at Halula, northern Syria (towards the end of the 7th millennium BC: Akkermans and Schwartz, 2003: 104). One of the circular buildings in this layer has two circular rooms connected with each other with a passage (Akkermans and Schwartz, 2003: 104-110). Despite some differences in architectural elements, the occurrence of a snowman-shaped house at Halula at the same time with Hacı Elamxanlı Tepe is notable along with the aforementioned evidence that indicates cultural contacts between the southern Caucasus and northern Mesopotamia.

Conclusion

The distribution of *Capra aegagrus* in the southern Caucasus raises a possibility that goats were indigenously domesticated in this region. However, currently available genetic and archaeological data actually indicate an alternative scenario that domestic goats were introduced to the southern Caucasus as part of the spread of agricultural economy during the early 6th millennium BC, and this economic influence was likely associated with inter-regional cultural linkage, which was manifested in the occurrence of foreign pottery traditions as well as the widespread similarity in lithic technology and architectural structures. Although a sudden appearance of agricultural lifeways in the southern Caucasus may have involved population movement with cereals and livestock, further investigations are necessary to clarify how local hunter-gatherers were involved in the process of agricultural spread to the southern Caucasus.

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Supporting Information

Appendix S1: Research background of Göytepe and Hacı Elamxanlı Tepe

Appendix S2: Authentication of ancient DNA experiments

Appendix S3: Reliability of ancient DNA haplotypes

Appendix S4: Discrepancy between genetic and morphological identification of goat

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Table 1. Previous studies of ancient goat mtDNA, ordered by the age of the samples (oldest at the top)

Site	Country	Approximate date	mtDNA haplogroup[1]	Reference	GenBank ID
East Chia Sabz	Iran	8500–7500 BC	A (5)	Mazdarani et al., 2014	KC404854–8
Kovačevo	Bulgaria	6200–5600 BC	A (4)	Scheu, 2012	
Cavdar	Bulgaria	6000–5500 BC	A (1)	Scheu, 2012	
Ovčarovo-gorata	Bulgaria	5700–5500 BC	A (1)	Scheu, 2012	
Aşağı Pınar	Turkey	5500–5000 BC	A (10), C (3)	Scheu, 2012	
Uivar	Romania	5250–5050 BC	A (1)	Scheu, 2012	
Baume d'Oullen	France	5300–4900 BC	A (9), C (10)	Fernández et al., 2006	DQ847506–11
Malkayası Cave	Turkey	5000–4200 BC	A (1)	Scheu, 2012	
Drama-Merdžumekja	Bulgaria	4500 BC	G (1)	Scheu, 2012	
Pietrele	Romania	4450–4250 BC	A (1)	Scheu, 2012	
Lenk	Switzerland	2900–2600 BC	B1 (1)	Schlumbaum et al., 2010	GQ342248–50
Kanlıgeçit	Turkey	2700–2200 BC	A (6), G (1)	Scheu, 2012	
Tachti Perda	Georgia	1400–700 BC	A (6)	Scheu, 2012	
Van-Yoncatepe	Turkey	1000 BC	A (7)	Akis et al., 2014	KF771887–93
Bancheng and Xiaoshuangguchcheng	China	500 BC	A (7), B1 (2), D (1)	Han et al., 2010	GU356623–32
Rostino	France	1200–1400 AD	A (21)	Hughes et al., 2012	JN007874–94

1: The numbers of DNA samples are shown in parentheses.

Table 2. Ancient and modern samples analyzed for mtDNA in this study

Lab. Code	Material	Morphological identification	Sampling location	Context	Level	Bone elements	^{14}C date (BP)	Cal BC ±1σ	Amplification	mtDNA haplotype	GenBank ID
Ancient											
Goy-1	Bone	*Capra hircus*	Göytepe	4BI-17	5	Mandible	6418±29	5407±40	Success 216 bp	AA-1	LC050643
Goy-2	Bone	*Capra hircus*	Göytepe	4BIIX-10	9	Mandible	6630±30	5571±32	Success 216bp	AA-1	
Goy-4	Bone	*Capra hircus*	Göytepe	4BI-113Q	10	Phalanx			Success 216bp	AA-1	
Goy-5	Bone	*Capra / Ovis*[1]	Göytepe	4BIIX-128	14	Pelvis			Success 216bp	AA-2	LC050644
Hac-1	Bone	*Capra hircus*	Hacı Elamxanlı tepe	M10-44	1	Humerus	6975±30	5860±50	Failure		
Hac-2	Bone	*Capra hircus*	Hacı Elamxanlı tepe	M10-60	2	Humerus			Failure		
Hac-7	Bone	*Ovis aries*[1]	Hacı Elamxanlı tepe	M10-20	3	Scapula			Success 216bp	AA-1	
Modern											
Az-1	Hair root	*Capra hircus*	Local farm near Göytepe						Success 481bp	AM-1	LC050638

Az-2	Hair root	*Capra hircus*	Local farm near Göytepe	Success 481bp	AM-2	LC050639
Az-3	Hair root	*Capra hircus*	Local farm near Göytepe	Success 481bp	AM-3	LC050640
Az-4	Hair root	*Capra hircus*	Local farm near Göytepe	Success 481bp	AM-4	LC050641
Az-5	Hair root	*Capra hircus*	Local farm near Göytepe	Success 481bp	AM-5	LC050642

1: The results of mtDNA analyses suggest that Goy-5 and Hac-7 are *Capra hircus*. See Appendix S4 for discussions.

Table 3. Reference mtDNA sequences of goats and sheep used in the phylogenetic analysis

Species	Haplogroup[1]	Country	GenBank ID	Reference
Domestic goats				
Capra hircus	A	India	AY155721	Joshi et al., 2004
Capra hircus	A	Italy	EF618134	Naderi et al., 2007
Capra hircus	A	France	EF617779	Naderi et al., 2007
Capra hircus	A	Jordan	EF618200	Naderi et al., 2007
Capra hircus	A	Iran	EF617945	Naderi et al., 2007
Capra hircus	A	Iran	EF617965	Naderi et al., 2007
Capra hircus	B1	Laos	AB044303	Mannen et al., 2001
Capra hircus	B1	Azerbaijan	EF617707	Naderi et al., 2007
Capra hircus	B2	Mongoria	AJ317833	Luikart et al., 2001
Capra hircus	B2	China	DQ121578	Liu et al., 2006
Capra hircus	C	India	AY155708	Joshi et al., 2004
Capra hircus	C	Switzerland	AJ317838	Luikart et al., 2001
Capra hircus	C	Spain	EF618413	Naderi et al., 2007
Capra hircus	C	China	DQ188892	Liu et al., 2006
Capra hircus	D	India	AY155952	Joshi et al., 2004
Capra hircus	D	Austria	EF617701	Naderi et al., 2007
Capra hircus	D	China	DQ188893	Liu et al., 2006

Capra hircus	F	Sicily	DQ241349	Sardina et al., 2006
Capra hircus	F	Sicily	DQ241351	Sardina et al., 2006
Capra hircus	G	Iran	EF618084	Naderi et al., 2007
Capra hircus	G	Turkey	EF617727	Naderi et al., 2007
Capra hircus	G	Egypt	EF618535	Naderi et al., 2007
Wild *Capra*				
Capra aegagrus	A	Iran	EF989163	Naderi et al., 2008
Capra aegagrus	A	Iran	EF989167	Naderi et al., 2008
Capra aegagrus	A	Turkey	EF989185	Naderi et al., 2008
Capra aegagrus	B	Iran	EF989192	Naderi et al., 2008
Capra aegagrus	B	Turkey	EF989200	Naderi et al., 2008
Capra aegagrus	C	Iran	EF989231	Naderi et al., 2008
Capra aegagrus	C	Turkey	EF989348	Naderi et al., 2008

Table 3 (continued)

Species	Haplogroup[1]	Country	GenBank ID	Reference
Capra aegagrus	D	Iran	EF989368	Naderi et al., 2008
Capra aegagrus	D	Iran	EF989369	Naderi et al., 2008
Capra aegagrus	D	Turkey	EF989617	Naderi et al., 2008
Capra aegagrus	F	Turkey	EF989645	Naderi et al., 2008
Capra aegagrus	G	Iran	EF989391	Naderi et al., 2008
Capra aegagrus	F	Azerbaijan	EF989466	Naderi et al., 2008
Capra aegagrus	Others[2]	Azerbaijan	EF989553	Naderi et al., 2008
Capra aegagrus	Others[2]	Azerbaijan	EF989583	Naderi et al., 2008
Capra aegagrus	Others[2]	Azerbaijan	EF989584	Naderi et al., 2008
Capra aegagrus	Others[2]	Azerbaijan	EF989585	Naderi et al., 2008
Capra aegagrus	Others[2]	Azerbaijan	EF989633	Naderi et al., 2008
Capra cylindricornis		Daghestan	AJ317868	Luikart et al., 2001
Capra cylindricornis		Daghestan	AJ317869	Luikart et al., 2001
Capra cylindricornis		Daghestan	AJ317870	Luikart et al., 2001
Capra falconeri		Tadjikistan	AJ317872	Luikart et al., 2001
Capra falconeri		Turkmenistan	AJ317873	Luikart et al., 2001
Capra caucasica		Georgia	AJ317875	Luikart et al., 2001
Capra sibirica		Pakistan	AJ317874	Luikart et al., 2001
Capra ibex nubiana		Israel	AJ317871	Luikart et al., 2001

Domestic sheep			
Ovis aries	Spain	AM279285	Fajardo et al., 2007
Ovis aries		AF039577	Hiendleder et al. 1998
Ovis aries		AF039578	Hiendleder et al. 1998

1: According to Naderi et al., 2007, 2008

2: Haplotypes that do not belong to the haplogoups A, B, C, D, F, or G.Table 4. Polymorphic sites of the ancient (AA) and modern (AM) goat haplotypes in comparison with a reference sequence (NC_005044)

NC_005044	15728	15747	15807	15811	15818	15821	15835	15843	15846	15873	15883	15885	15887	15893	15894	15898	15913	15920	15930	15947	15950	15960	15965	15969	14973	15974	15975	15976	15977	15981	15982	15983	15992	16006	16008	16010	16011	16019	16027	16028	16037	16040	16045	16083	16149
	A	G	C	T	A	A	A	A	T	A	T	A	C	T	A	G	A	A	C	C	C	A	C	C	G	T	C	C	T	T	A	G	G	T	T	C	C	A	A	A	T	C	T	C	A
AA-1	—	—	—	—	—	—	—	—	—	—	—	—	—	C	·	·	G	G	T	T	T	·	T	T	·	C	T	T	C	C	G	·	A	·	·	T	T	·	G	·	C	T	·	T	—
AA-2	—	—	—	—	—	—	—	—	—	—	—	—	—	C	·	·	G	G	T	T	T	·	T	T	·	C	T	T	C	Y	G	·	A	C	·	T	T	G	G	·	C	T	C	T	—
AM-1	G	A	T	·	·	·	G	·	·	·	·	·	T	C	·	·	G	G	T	T	T	·	T	T	·	C	T	T	C	C	G	A	A	C	C	T	T	·	G	·	C	T	·	T	G
AM-2	G	A	T	·	·	G	·	G	·	G	·	·	·	C	·	·	G	G	T	T	T	·	T	T	A	·	T	T	C	C	G	·	A	C	·	T	T	G	G	·	C	T	C	T	·
AM-3	G	A	T	C	·	·	·	G	·	·	·	·	·	C	·	·	G	G	T	T	·	N	T	T	·	C	T	T	C	C	G	·	A	C	C	·	T	G	G	G	C	T	·	T	·
AM-4	G	A	T	·	·	·	·	G	·	·	·	·	·	C	·	·	G	G	T	T	T	·	T	T	·	C	T	T	C	·	G	·	A	C	·	T	T	G	G	·	C	T	C	T	·
AM-5	G	A	T	·	N	·	G	·	N	·	N	N	T	C	N	N	G	G	T	T	T	G	T	T	·	C	T	T	C	C	G	A	A	C	C	T	T	·	G	·	C	T	·	T	G

A dot represents the identity with a base of the same position of NC_005044.

Y: C or T

N: Unidentified

—: Not amplified in the ancient haplotypes (AA-1 and AA-2)

Figure 1. Locations of Göytepe and Hacı Elamxanlı Tepe and other sites discussed in the text. A grey-shaded area shows the modern distribution of *Capra aegagrus* (Naderi et al., 2008). The earliest domestication of goats is considered to have taken place in a black-filled area, according to zooarchaeological records from Neolithic sites, such as Nevalı Çori and Ganj Dareh (Zeder, 2008, 2011) and the modern distribution of *Capra aegagrus* belonging to the haplogroup A (Naderi et al., 2008).

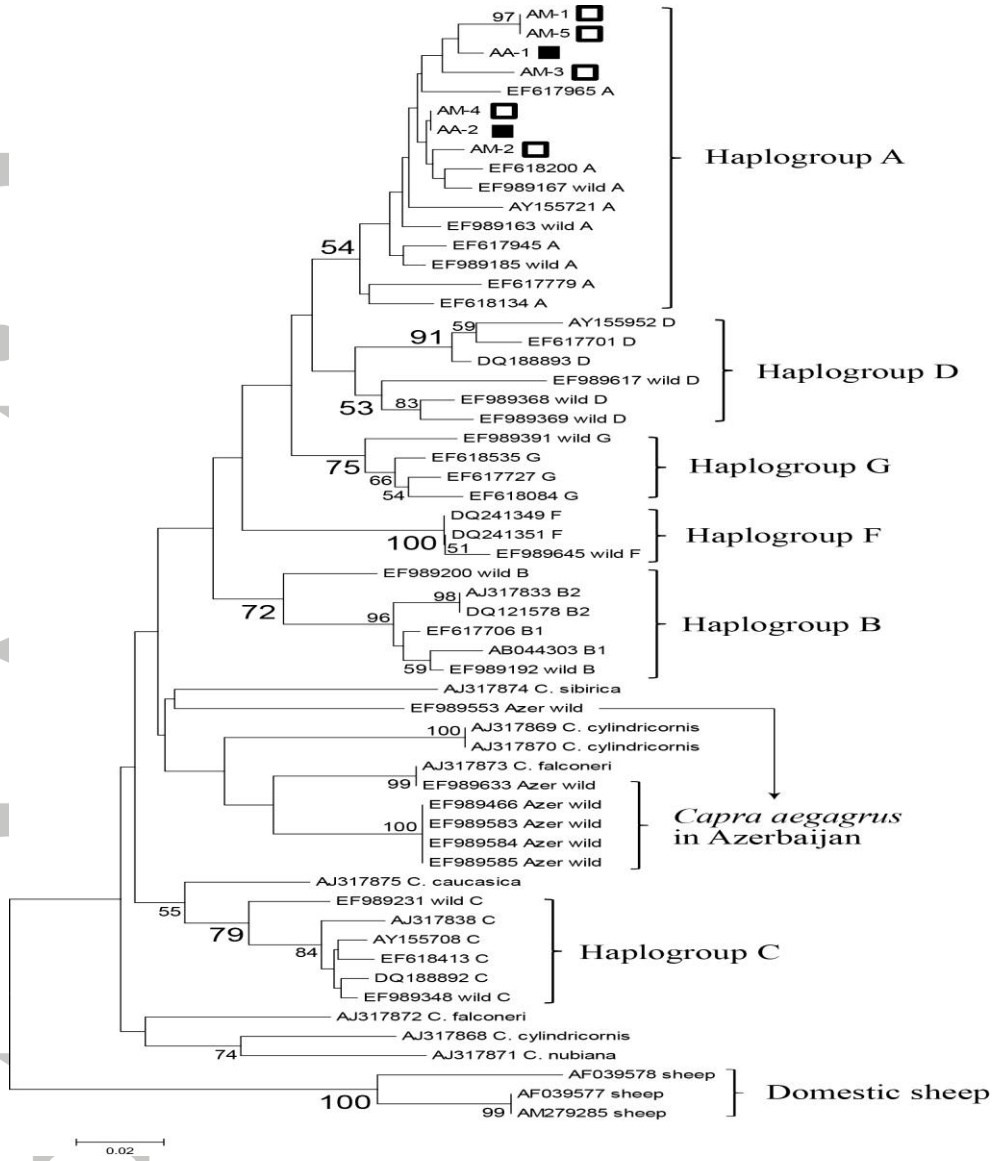

Figure 2. Neighbor-joining phylogenetic tree of the haplotypes identified in this study (Table 3: ■Neolithic, □modern) and modern goat and sheep DNA sequences (Table 2) based on a 216 bp segment of mtDNA control region. Bootstrap values higher than 50 % from 1,000 pseudo-replicates are shown.

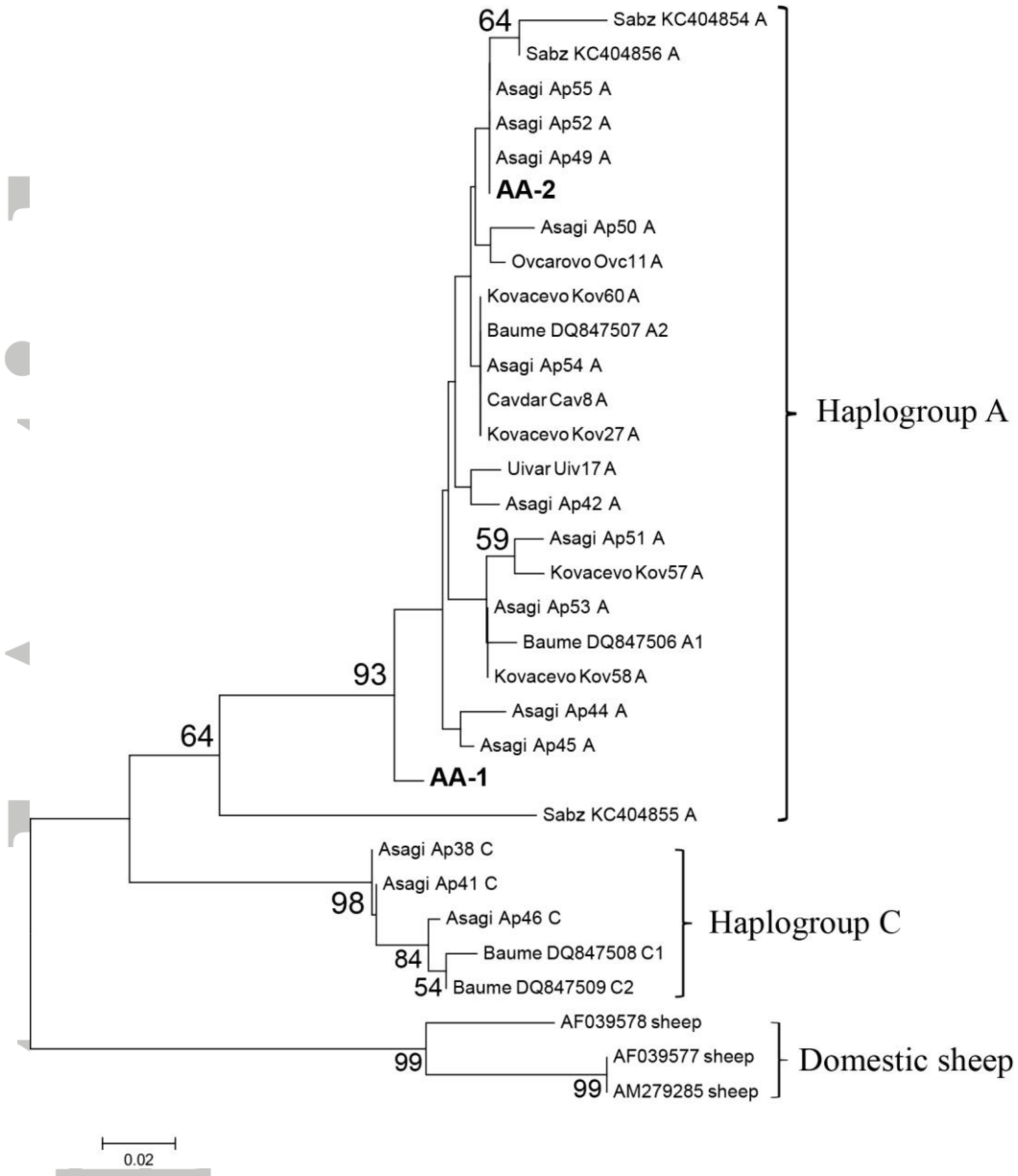

Figure 3. Neighbor-joining phylogenetic tree of the ancient haplotypes identified in this study (AA-1 and AA- 2) and other ancient sequences of goats dated to the 6th millennium BC or before (Table 1) based on a 130 bp segment of mtDNA control region. Bootstrap values higher than 50 % from 1,000 pseudo-replicates are shown.

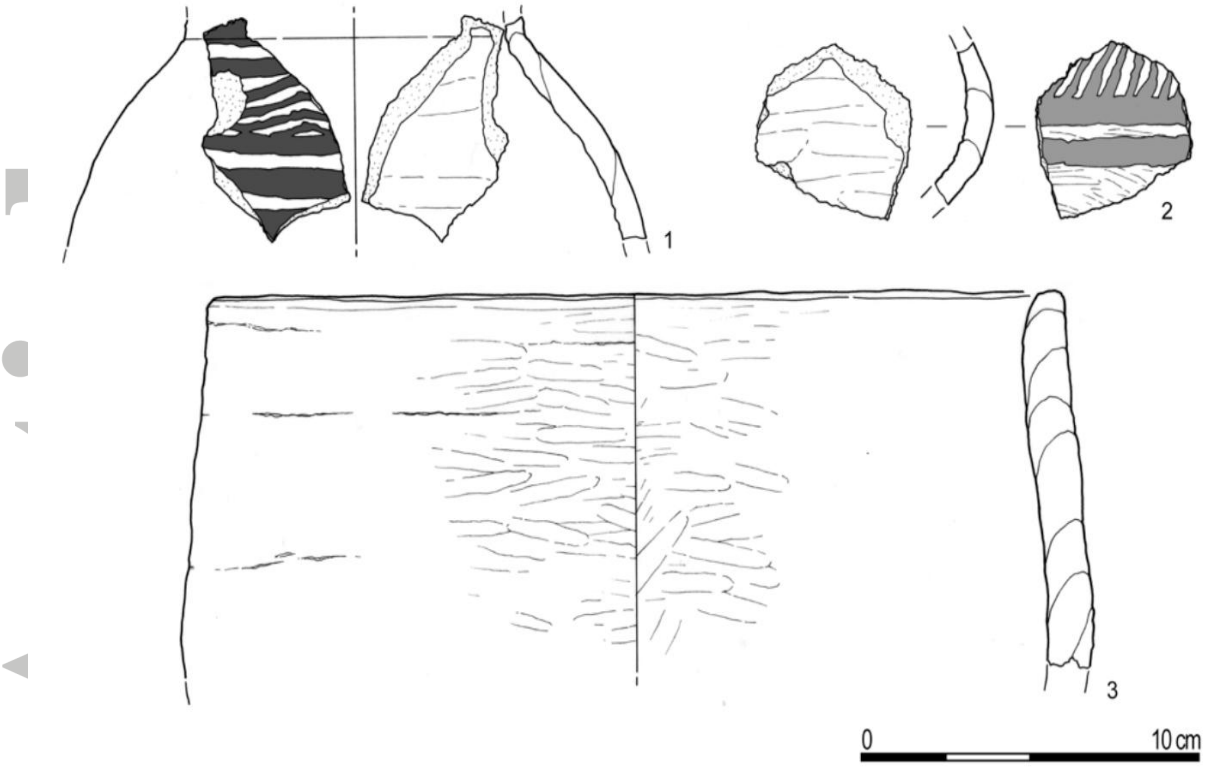

Figure 4. Painted fine wares (1 and 2) and a coarse ware (3) excavated from Hacı Elamxanlı Tepe (Nishiaki et al., 2013).

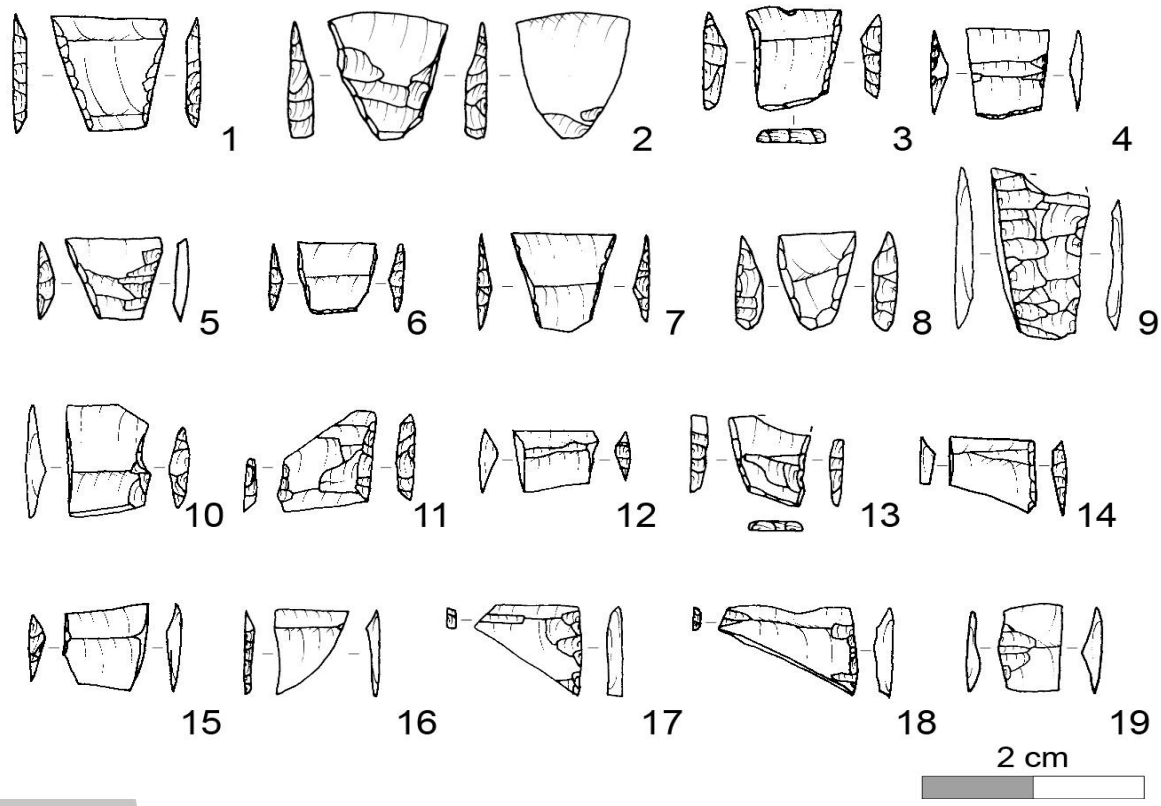

Figure 5. Trapezes (chipped stone tools interpreted as transversal arrowheads) excavated from Hacı Elamxanlı Tepe.

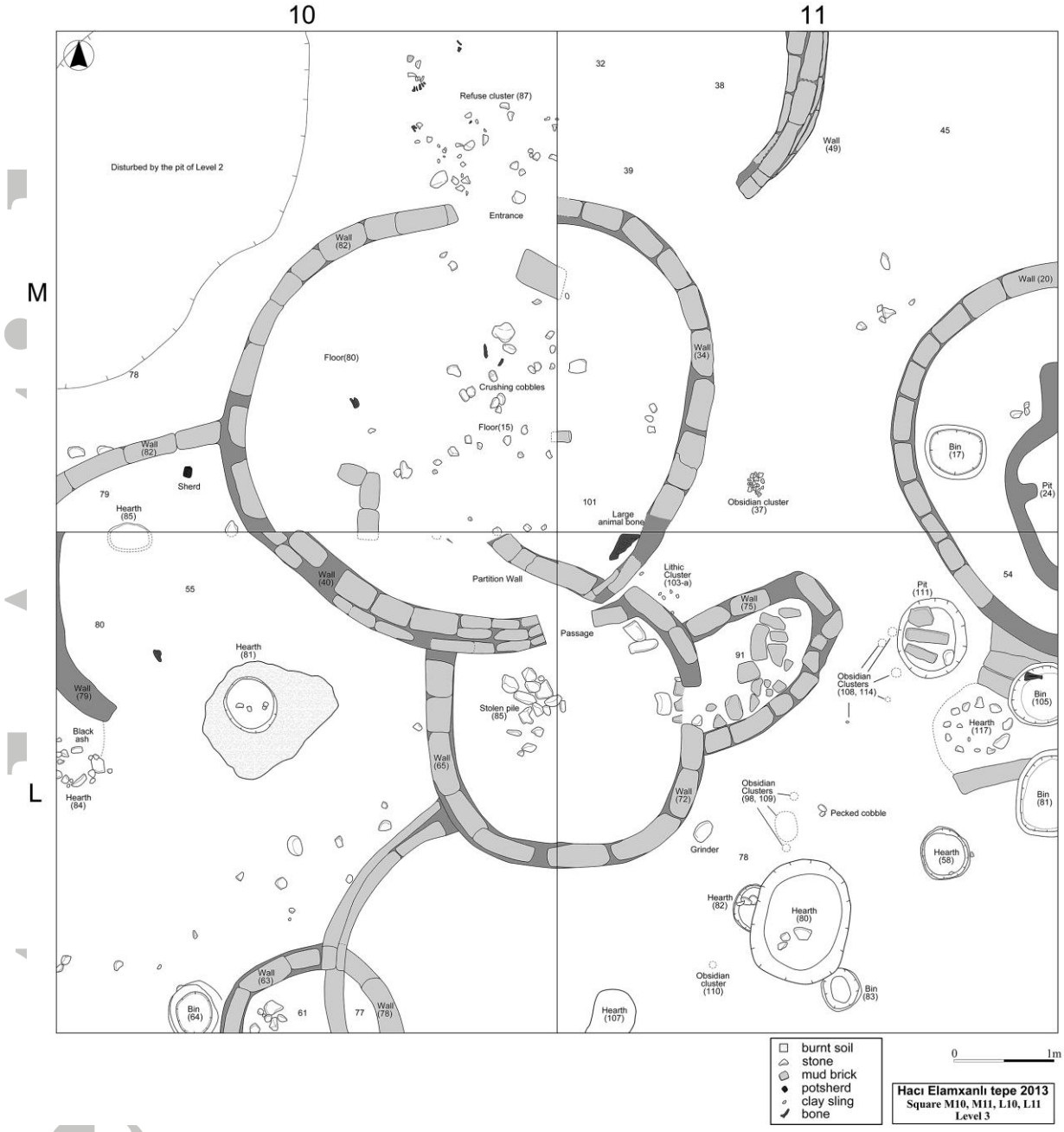

Figure 6. Plan of mud-brick buildings and other architectural features, including a snowman-shaped building, in Level 3 of Hacı Elamxanlı Tepe.

Appendix S1: Research background of Göytepe and Hacı Elamxanlı Tepe

The archaeological investigations of these sites have been conducted by a joint Azerbaijani-Japanese research group since 2008, directed by two of the co-authors (F. G. and Y. N.). These two neighboring sites, 1.5 km apart from each other, are located on the alluvial plains in the middle course of the Kura River at the altitude of 400–430 meters a.s.l.. The mean annual precipitation in this area is about 300 mm (World Meteorological Organization 2015), and the range of monthly temperature is -2.3–6.5 °C in January and 19.5–31.7 °C in July (See Kadowaki et al, 2015 for more descriptions of the local geology, modern climate, and vegetation).

The sites are anthropogenic mounds (called tepe in the local language), which were created as a result of repeated constructions and occupations of mud-brick buildings that constituted the ancient settlements. Göytepe measures ca. 145 m in diameter and 8 m in height, while Hacı Elamxanlı Tepe is a smaller mound covering an area of 60 m x 80 m with a height of 1.5 m. Recent excavations at Göytepe during 2008 and 2013 took place over an area of 1000m^2 and uncovered ca. 11 m-thick anthropogenic deposits, which were divided into fourteen occupation levels (Levels 1–14 from the top) (Guliyev and Nishiaki, 2012, 2014). Radiocarbon dates for the charcoal remains from these levels indicate the range of ca. 5650–5450 cal BC for the successive settlements at Göytepe (Nishiaki et al., 2015a). At Hacı Elamxanlı Tepe, a square of 10 m x 10m near the top of the mound was excavated to reveal ca. 2 m-thick deposits, in which four occupation levels were identified (Levels 1–4 from the top) (Nishiaki et al., 2013, 2015b, 2015c). Radiocarbon dates for the four levels range between ca. 5950 and 5800 cal BC, predating the dates of Göytepe (Nishiaki et al., 2015a). In this way, these two sites are likely to represent successive settlements during the first half of the 6th millennium cal. BC, corresponding to the Neolithic period in the archaeological chronology.

The excavations of Göytepe and Hacı Elamxanlı Tepe uncovered Neolithic settlements consisting of numerous mud-brick buildings that are mostly round in the plan form. These round buildings are associated with hearths and storage facilities, around which various artifacts and biogenic remains are densely distributed. The artifacts include chipped and ground stone tools, bone tools, and pottery, while biogenic remains consist of animal bones and charred botanical remains that resulted from daily domestic activities, such as tool production, cooking, cereal processing, and animal butchering (Kadowaki et al., 2015; Nishiaki et al., 2013, 2015c). Faunal remains mostly consist of livestock species, dominated by goats and sheep followed by cattle and pigs. Charred botanical remains include domesticated species, such as naked and hulled

wheat/barley and legumes. Agro-pastoral economy is indicated not only by these biogenic records but also by the recovery of agricultural tools (e.g., sickles used for cereal harvesting), cereal processing tools, as well as numerous storage facilities for cereals and tools.

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Appendix S2: Authentication of ancient DNA experiments

The DNA extraction and amplification were conducted at Nagoya University Museum (Nagoya, Japan), where no genetic or zoological samples of goats or sheep had been treated before this study. To avoid contamination between modern and ancient samples, the extraction and amplification of modern DNA were conducted in a room separated from those dedicated for ancient DNA samples. We also temporally separated the experiments of modern and ancient samples (i.e., we did not treat modern and ancient samples in the same day).

For the experiments of ancient samples, separate locations in Nagoya University Museum were dedicated for different procedures, i.e., the creation of bone powder, the DNA extraction, the PCR amplification, and the electrophoresis. The DNA sequencing was conducted at a different building (the Center for Gene Research, Nagoya University). To avoid inter-sample contamination, amplified DNA products or the equipment used for the amplification have never been transported to the areas for pre-PCR procedures. The working areas and equipment dedicated to ancient DNA analyses were cleaned with bleach.

The results were confirmed by repeating the DNA extraction and amplification. We treated only one sample with its negative control at a time of PCR amplification. In addition, the results of the four samples (Goy-1, 2, 4 and Hac-7 in Table 2) were replicated under the same protocols at laboratories of Naotaka Ishiguro (Faculty of Applied Biological Sciences, Gifu University, Japan), where the rooms and equipment for ancient DNA experiments had been set up (Ishiguro et al., 2009, 2014; Okumura et al., 1999). The DNA extraction and the PCR amplification were conducted in separate rooms dedicated to the treatment of ancient DNA samples.

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Appendix S3: Reliability of ancient DNA haplotypes

The successful amplification of ancient DNA in this study (five out of seven samples) may have been fostered by good preservation of DNA in the analyzed bone samples. This is indicated by the relatively high wt% (greater than 3 %) of gelatin collagen extracted from the three samples (Goy-1, Goy-2, and Hac-1), as described in Material and methods (See also Götherström et al. 2002; Campos et al. 2012). Other factors for DNA preservation (Burger et al., 1999), relevant in this case, may include moderate alkaline condition of the sediments, in which the bones were buried (Kadowaki et al., 2015), and the cool, dry climate in the Caucasus region, where ancient DNA of domestic animals have been successfully analyzed in previous studies (Larson et al. 2007; Scheu, 2012).

The amplified sequence of AA-2 is the same as the corresponding part of a modern haplotype (AM-4) except for Y at 15981 in AA-2. Although this might raise a possibility of contamination from the modern sample, the experiments of the modern sample Az-4, which produced AM-4, and the ancient sample Goy-5, which produced AA-2, were spatially and temporally segregated with different equipment. In addition, a carry-over contamination from the experiment of Az-4 to that of Goy-5 is unlikely because they were temporally intervened by the experiments that yielded a different haplotype AA-1 from the samples Goy-1, 2, 4 and Hac-7.

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Appendix S4: Discrepancy between genetic and morphological identification of goat

All the samples in this study showed the DNA sequences aligned with goats belonging to the haplogroup A. However, the sample Hac-7 was morphologically identified as domestic sheep (*Ovis aries*) (Table 2). This sample showed the same mtDNA haplotype (AA-1) as that found in the samples Goy-1, 2, and 4, which were classed as *Capra hircus* morphologically.

It is unlikely that this was caused by a contamination of goat DNA to a sheep sample on the basis of our precautions (See Appendix S1). It is also unlikely that sheep and goats have very similar nucleotide sequences in the amplified segment (216 bp) of the control region because the phylogenetic analysis clearly separates sheep from goats (Fig. 2).

Above all, it is often difficult to distinguish between *Capra hircus* and *Ovis aries* through the morphological observation of fragmented bones. In fact, the majority of bone remains from Göytepe and Hacı Elamxanlı Tepe were classified as an undistinguishable category, *Ovis aries*/*Capra hircus* (Nishiaki et al., 2013, 2015). It is thus suggested that the sample Hac-7 is actually *Capra hircus*. Such cases have been reported previously (Kahila Bar-Gal et al. 2003; Hughes et al., 2012), and the results of this study add another case that suggests a significance of the collaboration between genetic and morphological examinations of archaeological faunal remains.

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