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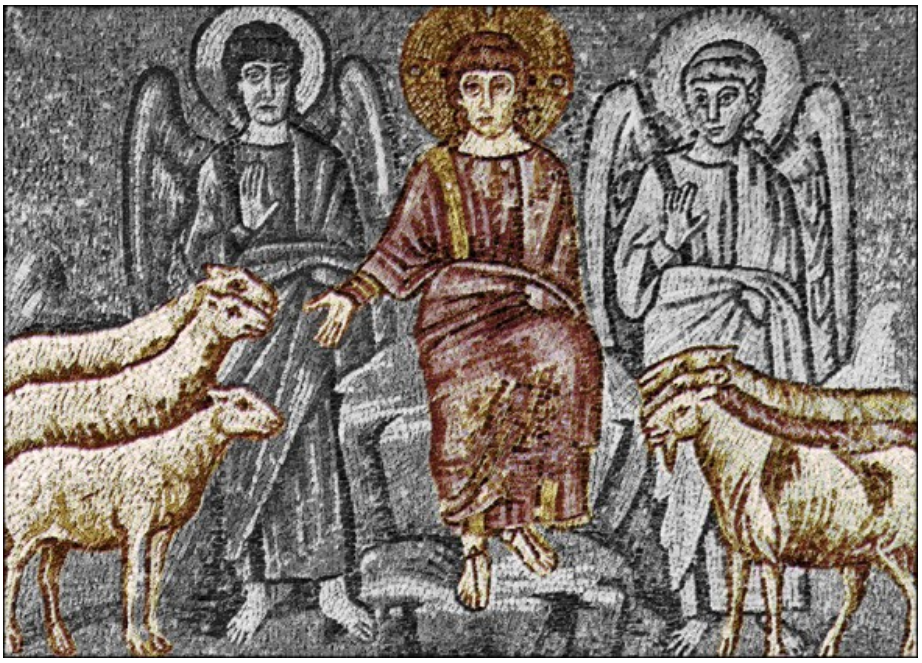
BLEATS FROM THE NORTH

Evaluation of osteological methods with support of archaeogenetics to distinguish between sheep and goats



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Bleats from the North – Evaluation of osteological methods with support of archaeogenetics to distinguish between sheep and goats

Bräkanden från Norden – Utvärdering av osteologisk metod med stöd av arkeogenetik för att särskilja mellan får och getter

A 15hp master's thesis in Archaeology, Uppsala University

Abstract

This study is an extension of the author's previous thesis (Theorell 2013) and focus on the analysed *tibia*, the *humerus* and the proximal and distal part of the *radius* of sheep bones from Gotland and goat bones from City of Falun. Methods for bone elements assessed in this study are compiled and evaluated by Zeder and Lapham (2010). The bones were then genetically species identified and the results were compared.

Seven of thirteen criteria for both sheep and goat reached 100% of correct identification. Best performing criteria were on *humerus* and distal *radius* for both sheep and goats. The results for the complete bone assessments divided for the species showed best performance on goats. Worst performing bone is the *tibia.* High performing criteria were found on all the types of bones used in this study which can be used to distinguish between sheep and goats.

Abstrakt

Föreliggande studie är en utökning av författarens tidigare magisteruppsats (Theorell 2013) och fokuserar på att analysera skenben, överarmsben samt proximal och distal del av strålbenet från fårben från Gotland och getben från Falun. Metoder för benslagen som används i denna studie har samlats och utvärderats av Zeder och Lapham (2010). Efter osteologisk analys har benen genetiskt artidentifierats och resultaten från bägge analyser har jämförts.

Sju av tretton kriterier för både får och getter visade 100% korrekt bedömning. Kriterier med bäst resultat fanns på överarmsben och distalt på strålben. Sett till bedömning av komplett benslag skilt mellan får och getter visade att metoderna presterade bäst på getter. Sämst resultat uppvisades på skenbenet. Generellt uppvisade samtliga benslag kriterier som är möjliga att använda för att särskilja mellan får och getter.

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Keywords

Sheep, Ovis aries*, Goats,* Capra hircus*, Osteology, Archaeogenetics, Ancient DNA, Gotland, Falun*

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Front page illustration

Early 6th century mosaic, "The Separation of Sheep and Goats" from the Basilica of Sant' Apollinare Nuovo, Ravenna, Italy. Modified by Theorell.

"Then home the goats to the hall were driven,
They wrenched at the halters, swift were they to run;
The mountains burst, earth burned with fire,
And Odin's son sought Jotunheim."
- From Henry Adams Bellows translation of *Þrymskviða* in the *Poetic Edda*

1. INTRODUCTION

When I last addressed the difficulties in distinguishing bones from goats and sheep (Theorell 2013) I relied on an archaeological bone material originating from the City of Visby on Gotland (1000–1500 AD). The genetically analysed samples were all identified as bones from sheep which made it impossible to study the reliability of the methods (Zeder & Lapham 2010) when identifying bones from goats. To be able to extend the work in the former thesis with additional goat bones, I have now analysed archaeological materials from the City of Falun in Dalarna (1600–1700 AD), as well as the site in Fröjel (600–1200 AD) on Gotland.

The purpose of this study is to evaluate the success rate of the osteological methods collected and evaluated in Zeder and Lapham's (2010) article. To assess the reliability of these methods on archaeological bone assemblages of sheep and goat, I use genetics to validate or reject osteological species determinations, both previous results (Theorell 2012; 2013), as well as, new osteologic and genetic analyses made for this study. If the same reliability can be achieved on archaeological bone remains, as was shown on modern materials (Zeder & Lapham 2010), it opens up for osteological re-evaluation of assemblages previously determined only as sheep/goat.

2. BACKGROUND

Today, genetic studies are used in several different fields of research. Archaeogenetics and the use of ancient DNA (aDNA) have become more popular in the last decade. Scientists have studied ancient bacterial and viral diseases, extinct plants and animals, the geographical scattering of domesticated animals and relationship between different prehistoric human populations (Pääbo et al. 2004:661, Anderung et al. 2005, Malmström et al. 2009). With new and developed technology and methods, aDNA is a field of research on the rise. Although not without serious problematic factors which, if not considered during the analysis, could render a study's results unreliable and useless (Pääbo et al. 2004; Gilbert et al. 2005).

Studies where archaeogenetics in combination with osteological analysis are used are rare but not unheard of. Archaeogenetics was, for example, used in an attempt to evaluate the

correlation between osteometrical data of metacarpal bones from cattle and gender (Svensson et al. 2008). The results showed not only the reliability in the use of osteometric sex-determination methods on metacarpals of cattle, but also the reliability of using archaeogenetic methods for identifying gender of the individual cattle, and the possibility to use bones which are deemed unsuited for osteological analysis.

As shown in the author's previous thesis (Theorell 2012), bones identified as sheep/goat which are unsuited for osteological methods to distinguish between sheep and goats are common. Out of 567 individual bones, 240 bones where of the type covered in the osteological methods available for sheep and goats species determination, whereof only 135 of these were suited for the actual osteological analysis as the others were missing the parts used in the determination. Even if archaeogenetic species determination methods may be applicable on most of these bones, the purpose here is not to determine all the bones in this assemblage.

Zeder & Lapham's (2010) study strongly indicated a high reliability in the criteria used with 91,9% on average correct identification on bones from sheep and 92,9% on average correct identification on bones from goat (Zeder & Lapham 2010:2896).

The genetic evaluation in the previous thesis (Theorell 2013) showed that these methods demand a certain degree of experience as the bones can be quite difficult to determine. The combined results in percentage of correct identification for inexperienced specialists (n=4) which varied between 52 – 100 % with the mean value of 76,6 %, and experienced experts (n=2) between 25 – 100%, with the mean value of 89,4%. However, because of the numbers of individuals in each group varied, the results are not completely compatible.

The mean values for the combined results of all criteria for bones in the previous thesis were calculated only from the results of the experienced osteologists (n=2). The *humerus* and the distal part of the *radius* both achieved 100%. The proximal part of the *radius* showed correct assessment of 87,5%, and the *tibia* performed worse with only 70% correct assessment.

The problem with the previous study was that it was only bones from sheep that were evaluated. The methods are meant to be evaluated on both sheep and goats and thus it was needed to extend the previous study with bones from goats, which is the purpose of this study.

3. MATERIAL

The material which is used in this thesis contains bones considered difficult to distinguish and bones determined as goat from four different bone elements of the limbs; *tibia, metapodia, humerus,* and *radius* (See *Table 1*).

The bones which are considered difficult have either missing bone characters or bone characters which are difficult to assess. The samples are named for easy identification of bone type and origin.

The bones analysed in this study comes from two different locations in Sweden; Fröjel on the Island of Gotland, and the City of Falun in the County of Dalarna on the mainland. Fröjel was one of the most significant trading ports on Gotland during the7th–12th century and became an important central place during the Viking Age (Carlsson, 1999).

The other samples come from eight different excavations in the City of Falun; Västra Falun 15, Falan 22, Kopparslagaren (Dnr: 4486/88), Bryggaren (Dnr: 6028/88), Bergshauptmannen (Dnr: 3718/88), Bergsfogden (Dnr: 3718/88), Slaggen (Dnr: 3578/89) and Dalpilen (Dnr: 3399/87).

Table 1 - Sample used for aDNA sequencing

Tibia	
T43	T46
T410	T411
T415	
Metapodial/Metacarpal/Metatarsal	
Mp30*	Mp35*
MC41	MC42
MC45	T283**
Mt40	Mt41
Humerus	
H41	H42
H43	H48
Radius	
R32*	R42
R47	R48
R410	R411
R418	

* Samples from Fröjel, Gotland
** Sample given by Maria Vretemark

According to historical sources, goats have been popular among households in northern Sweden and particularly in Dalarna (Myrdal 1999:88).

What connects these excavation sites is that they are part of the town that emerged around Kopparberget, a large copper mining site, which around the mid-17th century was the second largest town in Sweden after Stockholm. The excavations unearthed both urban houses with plot boundaries as in 'Bergsfogden' and farm complexes with dwelling houses and houses interpreted as storage buildings as in 'Dalpilen' (Sten 2004:65; Svedberg 1986:7).

The high concentration of copper in the surrounding earth in Falun can be seen in some of the samples. The bones have absorbed the metal and therefore have become verdigrised into green-colored bones while the preservation statuses of the bones still remain very good.
All the bones previously examined (Theorell 2012; 2013) were from Early Medieval block Apoteket in Visby on Gotland (Dnr. 7603/91) and are summarized in the final evaluation.

4. THEORY AND METHODS

When using both genetics and osteologic species determination, there is a collision between two rather different forms of natural science. The results of the genetical analysis are treated as absolute results while the results from the osteological analysis are treated as relative results (Theorell 2013:45). In other words: the results from the genetical analysis are *facts* while the results from the osteological analysis are subject to the expert and seen as estimations. This theoretical difference can be used to elevate the estimations into correct and incorrect identifications if both absolute and relative results are combined. The same theoretical approach is used in the earlier studies regarding species determinable criteria on bones from sheep and goats, however with known modern animals as absolute facts (cf. Boessneck 1969; Payne 1985; Prummel & Frisch 1986; Zeder & Lapham 2010; Zeder & Pilaar 2010).

Because of the absolute results from the genetical analysis, it is necessary to be very precise when describing how results are achieved and which steps are taken to be able to rely on the results. Therefore, the archaeogenetic method are described in a far greater detail with mixing recipes, contamination control, primer design etc. because it is important to be as transparent as possible to achieve reliable results.

From a layman's perspective it may be difficult to see the benefit of using expensive genetic methods to develop ocular methods for distinguishing between bones from sheep and goat. However, from an archaeological perspective it is far more important that osteologists learn to distinguish between sheep and goats.

Differences between sheep and goats are addressed in the previous theses (Theorell 2012:8-9; 2013:8-15) and these differences are often neglected in archaeological and osteological studies. I argued, with support of Bourdieu's concept of *habitus*, that it exist a norm among both osteologists and archaeologists resulting in an acceptance of the term sheep/goat (Theorell 2013:44p). Archaeologists and osteologists tend to choose between the animals and interpret the occurrence of sheep/goat according to what they feel like is the most probable species or what they think is the most valuable species in the material. But not only that these species have different biological needs in form of different kinds of preferred food, need for space, activity, and the ability to be trained, but also differences in how people of different cultures have perceived the animals in terms of mythology and symbology. As Jennbert (2004) points out; sheep depicted in art are absent in Scandinavian prehistory while goats occur in art connected with royalty or in terms of earthly or divine power (Jennbert 2004:161). Sheep are also absent in Norse mythology while the goat takes a more prominent

role, as both Odin and Thor each possess goats with specific powers connected to their respective heroic sagas (Jennbert 2004:161p).

In contrast, in both archaeological and historical sources it seems like the sheep is, in terms of economy, a more valued animal than the goat. After the introduction of Christianity in Scandinavia, sheep are highly valued, both as livestock and as a symbol for the new religion. The goat is reduced to an animal fit only for people of low social status and still into modern time seen as 'a poor man's cow' (Theorell 2013:15).

With osteological methods, which are proven to be reliable when examined, we can gain access new perspectives of the past relationship between sheep and goats and humans.

4.1. Osteological analysis

The method used in this thesis is basically the same as used in the previous thesis (Theorell 2013) but with some alterations. The osteological analysis was performed by the author in the osteological laboratory of Uppsala University - Campus Gotland using methods described in Zeder & Lapham (2010). Genetic species determination is then performed on the same bones in the DNA laboratory DBW, Uppsala University - Campus Gotland. The results from the osteological analysis are then compared to the results from the archaeogenetical analysis.

The osteological analysis is focused on distinguishing between sheep and goat by analysing the *tibia*, the *metapodials*, the *radius* and the *humerus* (Zeder & Lapham 2010:2889-2892). The tibia possesses four different species-specific characters on the distal part of the bone. The metapodials were divided into metacarpals and metatarsals, however samples that were impossible to identify either as carpal or tarsal were considered only as metapodials. The metapodials and the metacarpals possess three characters while the metatarsals possess a fourth.

The first character of the metapodials is identified by the measurement of the distal trochlea and is the only character that is measured (Zeder & Lapham 2010:2892) while all other characters are determined by ocular analysis of morphological differences in the bones. The radius consists of characters both on the proximal and the distal part of the bones. The proximal and the distal part were assessed as two different bones since it is uncommon for the bones to be found complete (Theorell 2012:67p; 2013:73-76). In this study, two of the samples R41 and R48 were found as complete bones, which is an example of the pristine preservation of the bones from Falun. Lastly, the *humerus* is assessed through the use of four different species-specific characters from the distal part of the bones.

The osteological identification of the complete sample can result in *Capra* (Goat), *Capra?*, *Ovis* (Sheep), *Ovis?* and *Ovis*/*Capra*. A majority of the criteria for respective sample which indicate Capra (or Ovis) will result in Capra (or Ovis) for the overall assessment of the respective sample. However, when there is no majority amongst the criteria but more Capra (or Ovis) the result will be shown as Capra? (or Ovis?). If the same number of criteria for both Ovis and Capra is reached the sample will be seen as Ovis/Capra and indeterminable (see *Figure 1*). There are mainly two reasons for an unsuccessful identification; bones damaged from taphonomic factors and morphological characters which does not stand out as either goat or sheep.

Bone	Criteria 1	Criteria 2	Criteria 3	Result	Species
Bone1	C	C	C	3 C	Capra
Bone 2	O	O	O	3 O	Ovis
Bone 3	C	C	O	2 C, 1 O	Capra?
Bone 4	O	O	C	2 O, 1 C	Ovis?
Bone 5	C	-	O	1 C, 1 O, 1 -	Ovis/Capra

Figure 1. Example of determining species from criteria. Five different designations depending on the results from the criteria.

4.2. Genetic analysis

The archaeogenetical analysis consists of several steps before a result can be achieved and are presented below under separate headings.

4.2.1. Contamination control

There are several key problematic factors which strongly affect the archaeogenetic analysis whether the results can be seen as reliable or unreliable. The main adversary in genetical studies is the high risk of contaminated samples. We have to take great measures to be sure to analyse DNA only from the sample and not DNA from the surroundings. Brown and Brown (2011) have identified five sources for contamination; contamination resulting from handling during burial, movement of DNA between buried specimens, handling by archaeologists, osteologists and other specialists, cross-contamination with amplicons from previous PCRs (see section *4.2.4. PCR (Polymerase chain reaction)*) and the use of contaminated plastic ware or reagents (Brown & Brown 2011:138). These sources of contaminations are explained in detail in the previous thesis (Theorell 2013:27p).

Because of the risk of contamination, it is necessary to take precaution against them. All laboratorial work are carried out in two different ancient DNA laboratories, one for extraction

of DNA and one laboratory for post PCR, and all work is performed in protective clothing, mouth guard and double set of plastic gloves. All laboratory consumables are sterile and radiated with UV light and the work area is cleaned with bleach and ionized water before and after each step in the analysis.

To be able to discover contaminations among the samples a set of contamination controls are used. All samples are radiated with UV light and a thin layer is removed from the sample before drilling into the bone. All master-mixes are prepared in bulk. A negative control, in this study a bone from a chicken, which should not work with the primers, is set up for the extraction. In both the extraction and PCR set up, a blank control without bonepowder/ DNA is used with the same mastermix as the other samples. If there is any indication of DNA in the negative or blank sample, the samples are deemed contaminated and will not be sent for sequencing, only results with at least two identical sequences from different PCRs from each bones were used in the final data analysis.

4.2.2. Primer design

The first thing to do in archaeogenetical analysis is to decide what sort of system should be used. In this analysis we want to extract mitochondrial DNA (mtDNA) and find species-specific sequences of mtDNA in both sheep and goats. To be able to do this we use a conservative primer system designed from a region of the mitochondrial genome named Cytochrome B. With a conservative primer system, the primers are applicable on different species, the primers seek out and connect themselves to the corresponding sequence of the Cytochrome B during PCR, the area between the primers is species-specific (see *Figure* 2) and it is this specific area we want to study (Theorell 2013:29).

For this study I use the same forward and reverse primers as in the previous thesis; ***"5' CTA GAA ACA TGA AAC ATT GG 3' "***(Forward) and ***"5' GAT ATT TGT CCT CAT GGT A 3' "*** (Reverse) based on the sequences GU068049, NC005044 and NC001941 in GenBank (NCBI 2010). The target sequence is 50 base pairs long between position 14500 – 14549 in the Cytochrome B gene (Hassanin et al. 2010; Fraser 2011 unpublished; Theorell 2013:29).

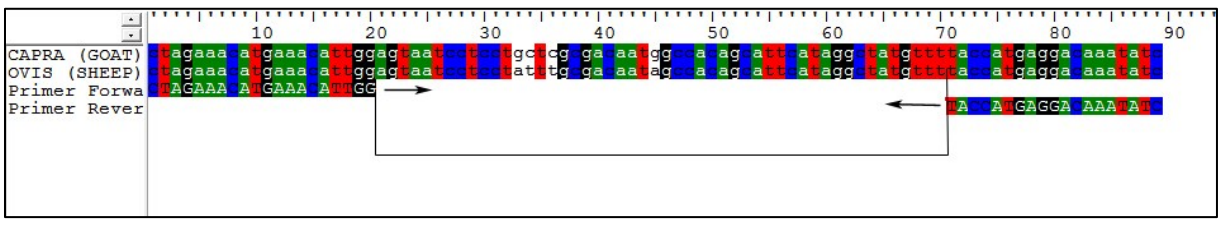

Figure 2. Picture of the target sequence with the bording primers. Differences between the species is highlighted white

4.2.3. Extraction

To extract DNA from the samples I use the "Silica Spin-Column Extraction Method" (Yang et al 1998; Bouwman & Brown 2002 and modified by Svensson et al. 2007). With the use of a fine drill, 75-100 mg of bone powder is collected. The sample is then mixed with a lysation buffer, consisting of 0.5M EDTA pH8, 1M UREA and 100 µg Proteinase K, and incubated overnight in 55°C. The EDTA will break down the hydroxyapatite in the bone powder while the UREA will break down and lysate proteins and the Proteinase K will split the peptide bonds into protein chains.

The extracted DNA sample still consists of organic material which could affect the PCR reaction. To purify the sample we use '*QIAquick PCR Purification Kit*', a commercial package for purifying samples before the PCR. With the use of a silica filter, buffers and a centrifuge, it is possible to discard unwanted organic remains from the samples. First we bind the DNA to the silica filter with the use of a PB buffer with high concentration of salt. Next, unwanted organic material is removed from the silica filter by the PE buffer consisting of 80 % ethanol and high concentration of salt and only DNA remains in the filter. Lastly we use an EB buffer (10mM Tris-HCl) to extract the pure DNA sample from the silica filter (Theorell 2013:30).

4.2.4. PCR (Polymerase Chain Reaction

DNA extracts from the samples are amplified in a process called PCR or Polymerase Chain Reaction. The idea behind this method is to copy the specific sequence of DNA several times until a large quantity is achieved. These PCR products can later be visualized in a gel and subsequently be sequenced if determined reliable. To make this possible specific components are needed. The primers mentioned above, free deoxyribonucleic triphosphates (dNTP) or artificial nucleotides as building blocks, and an enzyme called Taq DNA polymerase which together with a PCR buffer and $MgCl_2$ will replicate the nucleotides from the specific sequence template. The PCR recipe totals 25 µl and consists of 2,5 µl of 10x buffer (Naxo), 1,5 µl of each primer (0,3 µM), 0,25 µl of dNTPs (200 µM, Invitrogen™), 2 µl of $MgCl_2$ (5 mM, Naxo), 0,2µl of Smart Taq (2 U, Naxo), 2 µl of DNA extract and is diluted with ddH_2O.

Heat is necessary for the reaction. When the samples are exposed to cycles of different temperatures the DNA in the sample reacts in a pre-determined pattern. First, the samples are heated to 95°C for 10 minutes. During this period, the double helix of the DNA molecule splits apart leaving two strings of DNA. Then follows 45 cycles of the following temperatures: 94°C for 30 seconds, 50°C for 30 seconds and 72°C for 30 seconds. Finally, the

reaction ends with 72°C for 7 minutes. During these cycles, the original molecules of DNA separates, binds with the primers and the polymerase creates the equivalent strings of DNA from the free deoxyribonucleic triphosphates. And for 45 cycles, this reaction repeats itself resulting in a large amount of PCR products with the correct sequence of nucleotides (Theorell 2013:31p).

4.2.5. Gel electrophoresis

To visualize the PCR results and assess which samples to be sequenced is possible through a method called electrophoresis. When DNA molecules are placed in an electric field they start to move towards the positive pole. This phenomenon occurs because the DNA molecule in itself contains a negative charge. There is also a correlation between the speed of this movement and the length, or mass, of the molecule. To use this correlation for visualization, the PCR product is dyed and placed in an agarose gel. The gel used for electrophoresis in this study is a 2 % agarose gel made out of agarose sugar from a Japanese algae and 1X TBE buffer. Small cavities in the gel cause a certain resistance for the DNA sequences when the gel is placed in a field of electricity. Shorter sequences meet less resistance than long sequences thus shorter sequences travel farther in the gel than long sequences. Since we know the amount of nucleotide base pairs in the sequence we want to study, we also know the length the sequence will travel in the agarose gel. With the help of a DNA ladder with known length of DNA sequences we can compare the ladder with the dyed PCR product in the gel. The dye consists of an acid stain called GelRed™ which binds to DNA and will fluoresce when exposed to ultraviolet light with a bright orange colour. If everything has worked according to plan we have a PCR product with the right length of DNA sequence. Only sequences of correct length and without traces of contamination in the blank samples are permitted to be sequenced (Theorell 2013:32p).

4.2.6. Sequencing

Sequencing is needed to visualize the sequences in terms of readable data. Sequencing requires the samples to be purified from remaining dNTPs and primers. This is achieved with the use of ExoSAP, a substance which consists of the two enzymes exonuclease I and Shrimp Alcaline Phosphate (SAP). When ExoSAP is mixed with the samples and then heated, first to 35°C and then to 95°C, the exonuclease I will remove the dNTPs and the SAP will remove the primers.

While the extraction, PCR and electrophoresis are done in the DNA laboratory of Uppsala University - Campus Gotland, the laboratory work needed to be able to sequence the DNA

samples are accomplished by Macrogen Inc. Macrogen Inc. is a company specialized in the biotech industry and a service provider for genome research. Macrogen Inc. will sequence the samples through a genetic method called 'Sanger sequencing'.

Sanger sequencing mixes the amplified sample with primers, DNA polymerase, dNTPs and ddNTPs (Dideoxynucleotides). The ddNTPs are fluorescence in a different colour for each nucleotide and also terminates the sequence elongation at the specific nucleotide. When using the same technology as with the PCR, these ddNTPs will bind randomly to positions in the sequence during the replication which will result in a large amount of sequences in different length. To read the sequence, a new form of electrophoresis is used where the sequences travels through a gel with the help of electrical current through a capillary tube. Short sequences travels farther than long sequence and with the help of a laser it is possible to translate the fluorescent ddNTP's every unique position in form of a chromatogram resulting in a read-able sequence (Shendure & Ji 2008:1135).

4.2.7. Sequence alignment and species determination

The results from the sequencing are retrieved as computer files which are prepared to be edited with specific genetic software for genetical analysis. For this study I use Sequence Scanner Software 2 (version 2.0) developed by Applied Biosystems and a sequence alignment editor called 'BioEdit' (version 7.2.5) developed by Ibis Biosciences. The results can range from good readable sequences to sequences which are difficult to read or even sequences which have failed and are impossible to use. The program translates the chromatogram to a readable sequence; however for ancient DNA you cannot trust that it is correctly translated. You have to study both the chromatogram and the sequence to retrieve a correct and readable result (Theorell 2013:34p). For each sample you get two sequences, a forward and a reverse sequence that you have to align with the sequence alignment editor, you also need to align the results from several different PCRs of the same bone sample in order to confirm that the results are reliable. The final result is then compared to the known sequence of each animal to confirm the results (see *Figure 2*).

4.2.8. Osteological and genetic comparative studies

The results of the osteological and the genetic analyses for all bones analysed in this study were correlated, and converted into correct, incorrect or indeterminable answers for each criterion.

Data from the *humerus*, *tibia* and *radius* from this analysis were used together with the data from the same bone elements from the 2013 analysis (Theorell 2013). The percentage of

correct assessments was calculated for each criterion, and bone element. The results were divided into two different categories: one separated by species and the other with the combined totals of both species.

5. RESULTS

5.1. Genetic analysis

It was noted in the osteological analysis that several of the Falun bones had absorbed copper because of high concentration of copper in the earth. This phenomenon was observed as the bones had become verdigrised with a clear green colour. This also affected the incubated sample with a clear green colour. This is a known taphonomic factor, however it was unknown if DNA was preserved in the bone or if the concentration of metal could affect the PCR amplification. Therefore I took extra precaution in rinsing these particular samples several times during the DNA purification step during the extraction. All the affected bones gave successful results, thus it seems as the metal had not interfered with the preservation of DNA, or with the PCR amplification.

The results are based on successful sequencing of the samples, only sample with at least two identical results from different PCR amplifications were used (See *Table 2*). Four samples (R418, Mp35, T43 and T283) are not used when summarizing the results. For sample R418 we obtained an unclear and distorted sequence and thus forcing us to disregard the sample. For sample Mp35 it was not possible to extract and amplify DNA. In both the first and second PCR no results could be observed in the electrophoresis and thus the PCR products were never sent for sequencing. For T45 and T283 a result was achieved however only from a single PCR so these bones were not used in the comparative analyses. A total of nineteen samples remain, consisting of 16 samples of goat and 3 samples of sheep.

5.2. Osteologic analysis

The results from the *humerus, tibia* and *radius* in this study (See *Table 2*) are combined with the results from the previous thesis (Theorell 2013) and thus we acquire a total of 26 samples. The other bones (*Metapodia, metatarsal* and *metacarpal*) analysed both osteologically and genetically here will not be addressed in the following comparisons as I do not have a comparable material from the previous study. Detailed results of the osteological and genetic analyses for all criteria and bone elements divided by goat and sheep can be seen in the appendix (See *Table 7* in *Appendix*).

The *tibia* samples (n=10) are species determined to four goats and six sheep. The *humerus*

samples (n=6) are species determined to four goats and two sheep. The *radius* samples consist of six proximal parts, two distal parts and two complete bones. For this study's benefit, the complete radius bones are not seen as individual bones but as proximal (n=8) and distal (n=4) parts and were species determined to four goats and four sheep for the proximal parts, and two goats and two sheep for the distal parts.

Table 2 - Sample results of osteological and archaeogenetical analysis of bones from Fröjel and Falun devided by bone element.

Bone Sample	**Results** **Osteological analysis**	**Archaeogenetical Analysis**
Tibia		
T43	Capra (3 C)	-
T46	Capra (3 C)	Capra
T410	Capra? (2 C, 1 O)	Capra
T411	Ovis? (2 O, 1 C)	Ovis
T415	Capra (3 C)	Capra
Metapodial/metacarpal/Metatarsal		
Mp30	Capra? (2 C, 1 O)	Ovis
Mp35	Capra? (2 C, 1 O)	-
MC41	Capra? (2 C, 1 O/C)	Capra
MC42	Capra? (2 C, 1 O)	Capra
MC45	Capra (4 C)	Capra
T283 (MC)	Capra (4 C)	-
MT40	Capra (4 C)	Capra
MT41	Capra (4 C)	Capra
Humerus		
H41	Capra (4 C)	Capra
H42	Capra (3 C, 1 O/C)	Capra
H43	Capra (3 C, 1 O)	-
H48	Capra (4 C)	Capra
Radius		
R32 (Distal)	Ovis/Capra (1 C, 1 O)	Ovis
R42 (Distal)	Capra (2 C)	Capra
R47 (Proximal)	Capra (4 C)	Capra
R48 (Complete)	Capra (4 C, 2 O	Capra
R410 (Proximal)	Capra (3 C, 1 O)	Capra
R411 (Proximal	Capra? (2 C, 1 O, 1 O/C)	Capra
R418 (Proximal)	Ovis/Capra (1 C, 3 O/C)	-

Assessed criteria in brackets. C = Capra (Goat), O = Ovis (Sheep), O/C =Indetermined.

Although the sample size for each bone element and species are small, as well as of different numbers in both categories, it still is possible to get some indications of how well the different criteria perform, both on the individual species and on the combined results.

5.3. Comparative analysis

To be able to determine if the osteological methods are reliable we need to take a closer look at the performance of the individual species determinable criteria which I have used to identify the bone sample. First we observe the performance of the criteria divided between goats and sheep (See *Table 3*). Here we can see an overall high performance of more than half of the criteria.

Table 3 – Assessment of criteria with results from 2013 and 2014 divided for species

		Goat				Sheep		
Bone	**Criteria**	**n**	**% Corr**	**% Wrong**	**% O/C**	**n**	**% Corr**	**% Wrong**	**% O/C**
Humerus	1	4	100			2	100		
	2		100				100		
	3		100				100		
	4		50	25	25		100		
Tibia	1	4	100			6	83		16
	2		75	25			33	66	
	3		100				33		66
Radius (p)	1	4	75		25	4	100		
	2		75	25			75		25
	3		75	25			75		25
	4		50	50			100		
Radius (d)	1	2	100			2	100		
	2		100				50	50	

n = quantity, Corr = Correct, O/C=Indeterminable

A total of seven criteria for goat reached 100% of correct identification (*Humerus* 1-3, *tibia* 1 and 3, and distal *radius* 1 and 2). There are also seven criteria which reached 100 % on sheep although different criteria from goats (*Humerus* 1-4, proximal *radius* 1 and 4, and distal *radius* 1).

We also can compare the columns of incorrect identifications where a total of five criteria on bones from goats were incorrectly assessed as sheep. For sheep, only two criteria were incorrectly assessed as goat during the osteological analysis.

The second criterion of *tibia* was incorrectly assessed in both goats and sheep.

The last column is the percentage of undetermined criteria. Most undetermined criteria were found among bones from sheep with a total of four criteria which were difficult to assess and while only two criteria from goats were difficult to assess (See *Table 3*).

In table 4 I do the same analysis but with the combined of sheep and goats.
Once again there are criteria with a high performance. The best performing criteria were *humerus* 1-3 and distal *radius* 1 with 100% correct assessment for both sheep and goats. However we should also take notice of *tibia* 1 (90%) and proximal *radius* 1 (87,5%) which should be seen as very high performing criteria. This also means
that we have high performing criteria on all the bone categories in this study (see *Table 4*).

Table 4 – Assessment of criteria with results from 2013 and 2014

Bone		**Criteria**	**n**	**% Corr**	**% Wrong**	**% O/C**
Humerus		1	6	100		
		2		100		
		3		100		
		4		66,7	16,7	16,7
Tibia		1	10	90		10
		2		50	50	
		3		60	40	
Radius (p)		1	8	87,5		12,5
		2		75	12,5	12,5
		3		75	12,5	12,5
		4		75	25	
Radius (d)		1	4	100		
		2		75	25	

n = quantity, Corr = Correct, O/C = Indeterminable

It is not enough for bones to have high performing criteria if the overall assessment for each bone does not lead to a correct identification. To evaluate this I calculated the mean value for the total assessment of correct, incorrect, and indeterminable scores for each bone from the previous thesis (Theorell 2013) and from the bones in this study to see if the use of the criteria results in correct assessments of the bones. Table 5 shows the results divided between sheep and goats. The methods perform well for both sheep and goats. Best performance is seen on goats with 100% correct assessment of the distal *radius*, 91,7 % of the *tibia* and 87,5% of the *humerus*. Performance of sheep is also good with 100% correct assessment of the *humerus* and 87,5% of the proximal *radius*. Lowest performance is seen in the tibia of sheep with 49,7%, and 68.75 % of the proximal radius in goats (see *Table 5*).

Table 5 - Mean value of percentage for assessment of bone divided for species										
				Goat					Sheep	
Bone		**n**	**% Corr**	**% Wrong**	**%O/C**		**n**	**%Corr**	**%Wrong**	**% O/C**
Humerus		4	87,5	12,5			2	100		
Tibia		4	91,7	8,3			6	49,7	44	5,5
Radius (p)		4	68,75	25	6,25		4	87,5		12,5
Radius (d)		2	100				2	75		25

n = quantity, Corr = Correct, O/C = Indeterminable

As with the criteria, it is also necessary to see how the method performs overall on the combined bone samples of both species (see *Table 6*).

Table 6 - Mean value of percentage for assessment of bone					
Bone		**n**	**% Corr**	**% Wrong**	**% O/C**
Humerus		6	91,7	8,3	
Tibia		10	66,4	29,7	3,3
Radius (p)		8	78,1	12,5	9,4
Radius (d)		4	87,5		12,5

n = quantity, Corr = Correct, O/C = Indeterminable

The method seems to perform best on the *humerus* with a correct assessment of 91,7% and the distal *radius* with 87,5%. The worst performing bone is the *tibia* with a result of 66,4%. The *tibia* is clearly affected by a high percentage of incorrect assessment (29,7%) while the best performing bones had 8,3% incorrect assessment (*Humerus*) and no incorrect assessments at all for the distal *radius,* however 12,5 % were indeterminable (see *Table 6*).

6. DISCUSSION

Travelling to the County Museum of Dalarna to acquire samples of goats from Falun was a gamble motivated not by osteological research but from historical research of Swedish agriculture performed during my previous thesis (Theorell 2013) This should be seen as a gamble since the preservation status of the bones were unknown, and I was not sure how well the osteological methods would work as bones from sheep can be mistaken for bones from goats. When the bones had been osteologically analysed several times with use of Zeder and Lapham's (2010) compiled methods and still indicated a large quantity of bones from goat, it

seemed as if the change of location was a success and several bones were sampled to be analysed with archaeogenetical methods. The sequenced samples returned and proved that 17 out of 19 sequenced samples had been correctly identified with the use of the osteological methods it also proved that it was possible to use Zeder and Lapham's (2010) compilation of methods to sample bones from goats from an archaeological bone material, and that it was possible to extract DNA from the archaeological bones of Falun. The incorrectly identified samples, whereof one sample was indeterminable and the other was incorrectly identified as goat, were taken from the Fröjel archaeological bone material. As of yet, the sampling from Falun has achieved a very high success rate.

The results showed a relatively high performance rate of several of the criteria used to distinguish between sheep and goat. The best performing criteria for both sheep and goat were first three criteria for *humerus* (100%) and the first criterion for the distal *radius* (100%). When comparing the results from sheep and goats it seems that most criteria perform well on both species. Still, there are some criteria with remarkably low performance if we study criteria for sheep. The worst performing criteria on sheep is the second and third criteria of the *tibia*. Only 33% were correctly assessed respectively with a staggering 66% incorrect assessment of the second criterion. Compare that result with the result from the *tibia* from goats which had a correct assessment rate at 100% for the first and third criteria and 75% for the second criterion.

However, we should note that there is an important difference between the species. It seems that incorrect assessments are more common on bones from goats. A total of five of the thirteen criteria are affected from incorrect assessments. When studying the results from sheep it tends to result in more undeterminable criteria which are affecting the assessments. According to Zeder and Lapham's (2010) study, criteria on goat are easier to detect and assess than criteria for sheep since the criteria for goats are more distinct in their shape. This is clearly seen in the number of undetermined criteria for sheep in the result. Perhaps the distinct appearance of the criteria for goats appears more sheep-like when the character is expressed less goat-like. That would explain the numbers of incorrect assessed criteria on goats.

The highest performance was achieved on the *humerus* with 91,7% correct assessment for both sheep and goats (87,5% for goats and 100% for sheep). The worst performance was observed on the *tibia* with 66,4% correct assessments for both sheep and goats despite a very high performance on bones from goat (91,7% correct assessment). The overall performance of the *tibia* is greatly affected by the very low performance on sheep with only 49,7% correct assessment. Both proximal and distal part of *radius* performed quite well with 78,1% correct

assessment of the proximal part and 87,5% for the distal part.

The *tibia* is quite interesting in the perspective of reliability. On the one hand, it's highly reliable when it comes to identifying bones from goats. On the other hand it's highly unreliable when it comes to the identification of sheep. These two differences balance each other in the combined percentage for both animals and results in a reliability which is not good but not quite as bad as it could have been. It is worth taking a closer look at these particular bones to see why they performed so badly in the osteological evaluations.

The performance of the *humerus,* both divided between the species and the overall assessment, make it apparent that the criteria used are reliable for this bone element from sheep and goat. The fourth criterion however does not seem to perform as well as the other three criteria regarding goats. Not only did the use of the fourth criterion result in incorrect assessment but also were found indeterminable in some samples. One reason for this result is that the morphological character on the bone in goats is affected somehow by other factors such as behavioural patterns. The goat is, according to Sjödin (1970:92), a highly active animal which prefer to skip and jump and this behaviour may affect the articulated parts of the bones in the joints and thus also the characters of *humerus*.

The performance of the *radius* differs between the proximal and distal parts of the bone. The results show the distal part to be reliable for species determination with 87,5% correct assessment for sheep and goats combined and performed very well with goats (100%). It can be argued that the results for both distal and proximal parts of *radius* should be merged since it is the same bone. However it is more common to find the parts separately in archaeological bone assemblages since the preservation of the bones is seldom at the level of complete *radius* in the material. Often we find the unfused distal parts of the *radius* in assemblages from young sheep or goats (Theorell 2012:53-64). According to Zeder and Lapham's (2010) study the unfused *radius* does not affect the reliability of distinguish the *radius* as sheep or goat since the characters develop after six months (Zeder and Lapham 2010:2904).The difference in reliability between the distal and proximal part may also be due to the different numbers of criteria. The distal part of the *radius* has only two criteria to assess while the proximal part has four criteria. This difference can have a significant impact on the statistics since we calculate on mean values of the assessments. It is also possible to argue, since the four proximal criteria is part of a major joint in the anterior extremities and connects with the distal part of the *humerus* which also had a criterion with low performance, that the movement of the joint may affect the development of the bone characters and may affect a post mortem osteological assessment.

The results depend on specific key components which we have to address because these components affect the outcome of the study. The osteologist is for example always a factor when bones are identified. This was discussed in both Zeder & Lapham's (2010:2898p) study and in the previous thesis (Theorell 2013) and it is recommended to not only use the pictures shown in the compiled methods in Zeder & Laphams (2010) article but also try to understand the pictures with the use of actual bone references in the laboratory. But even so, as both the students and the trained osteologists encountered difficulty in assessing the known sheep and goat bones housed in the reference collection of the osteological laboratory at Uppsala University - Campus Gotland.

But not only experience affects an osteological analysis since the osteologist also needs to perform well during the circumstances. There is reason to believe that an overstrained osteologist will not perform as well as a well-rested osteologist. The methods need not only to be useful, but also instil a sense of security for the osteologists. If the osteologist feels unassertive for the result, it is reason to believe he or she will not be able to distinguish between the species and thus still use the sheep/goat term as a result. This phenomenon was seen in the osteological analysis in the previous thesis (Theorell 2013:68-82). Knowing the bone criteria and what traits to look for may be more important than an overall experience of osteological analysis.

The difficulty to assess the tibia from sheep may be the cause of different behavioural patterns between the species. Sheep tend to wander greater distances while goats tend to be more stationary (Sjödin 1994:228p; Sjödin et al. 1970:84). This difference may affect the tibia or the *humerus* and radius causing a greater wear on the different part of the bone and thus affect the species-specific characters making them more difficult to identify. Zeder and Lapham's study concludes that sheep have features which are less strongly expressed than goats resulting in a higher proportion of undetermined identification of sheep (Zeder & Lapham 2010:2896, 2902). Sheep also tend to starve more often than goats which could affect the bones (O'Connor 2000:102, Theorell 2013:7). When analysing an archaeological bone material we have to adapt to circumstances created by past handling of the livestock. According to written sources, it was custom for the animals to feed from the last remaining fodder, produced during the warmer period of the year, when winter, cold and snow kept the animals from grazing (Myrdal 1999:275p). Whereas goats have no problem eating whatever nutrition they can find, the sheep tend to sort out and eat the most nutrient fodder and refusing the rest of the fodder leading to starvation despite the appearance of plenty of food (Theorell 2013:11p).

The breeding of sheep and goats in Scandinavia may also affect the performance of Zeder and Lapham's collected and evaluated methods. These methods are evaluated with the use of a modern skeletal material from flocks of both wild and domestic goats and sheep originating primary from Iran and Iraq but also samples from USA and China (Zeder & Lapham 2010:2888). The breeding of sheep and goats at these different geographic locations may differ from the breeding in Scandinavia since the Scandinavian sheep and goats needs a natural resistance against a colder climate. The native breed of Scandinavian sheep's resistance to cold climate were the main reason the first organised introduction of fine-woolly sheep failed during the 18th century in war-torn Sweden (Theorell 2013:10). If the breeds of Scandinavia differ morphologically from the breeds used in the evaluation of the methods, it may also affect how well the criteria perform when the methods are used on a Scandinavian archaeological bone material. This factor may explain why some criteria are incorrectly identified or tend to be indeterminable.

In the earlier thesis (Theorell 2013), it was also argued that the computed results were affected by the limited amount of bone samples. The same argue can be said of the results in this study. When a single diverging identification results in a steep shift in the percentage, it is difficult to come to a clear conclusion of the reliability of the results. It can be argued that these results could be scalable in the sense of multiplying the amount of samples would render a similar percentage result of the analysis. This is supported of the high percentage of reliability seen in Zeder and Lapham's (2010:2896-2903) assessment of the bone characters as well as the results in the previous thesis (Theorell 2013:41-44). However we should not forget that the analysis basically can be reduced to a choice of two species. One incorrect assessment does naturally inflict significant impact on the statistics for the individual character and, because of the few criteria, also a significant impact on the result of the complete assessment of the bone. A most terrifying feat in a method, and it is imperative that we strive to instil some safeness in the user; the safety of numbers.

7. CONCLUSION

It is concluded that it was possible to use the methods compiled in Zeder and Lapham's (2010) study to find bones from goats in an archaeological bone material.

17 out of 19 samples from Falun had been correctly identified through the use of osteological methods. However, in comparison to the results of the previous thesis (Theorell 2013), it is reason to believe that the performance is affected by factors such as species, time, space and quantity and there are indications that bones from sheep are more difficult to distinguish than

the bones from goats. Bones from Scandinavian sheep and goats may closely resemble the bones from sheep and goats in Iran and Iraq but may also have morphological differences. It is also evident that a limited number of analysed samples are sensitive to the smallest of irregularities although this hinder can be bypassed by more research and a larger amount of samples. But lastly, it is the eyes that study the bones that make the results. We can strive to quantify the reliability of the methods but at the end it is the user of the methods who makes the last call. But it also should be concluded that there are reliable criteria and overall good results from assessment of the complete bones, and that osteologists should distinguish between the species more often and drastically limit the use of sheep/goat in our scientific literature, and thus make it possible to open up new archaeological assemblages for re-evaluation and delve deeper into the unexplored history of our four-hoofed friends, sheep and goats alike.

8. SUMMARY

This master thesis is an extension on the previous thesis (Theorell 2013) in which several bones from sheep were evaluated by combining osteological assessment of the bone samples with archaeogenetic analysis. It was noted that all bones in the previous study were bones from sheep which is why this study focused on finding an archaeological bone assemblage containing bones from goats. According to historical research on Swedish agriculture it would be possible to find such assemblages in the Swedish county Dalarna. The bones in this study originate from several excavations in the City of Falun and are dated to 17^{th}-18^{th} century. The purpose with this thesis was to evaluate osteological methods compiled and evaluated in Zeder and Lapham's (2010) study with the use of archaeological bone remains. This was possible due to the use of ancient DNA and archaeogenetical species determining analysis.

A total of 19 bone samples were genetically species determined and 16 were determined to be from goats. The results from the *humerus, radius* and *tibia* from sheep in the previous thesis (Theorell 2013) were compared to the results of the new bone samples of goats analysed here to evaluate the performance of the specific characters on the bones presented in Zeder and Lapham (2010). The mean value of the osteological assessment for the complete bone sample was then calculated to study the percentage of correct and incorrect assessments as well as indeterminable bones.

The results showed that seven of thirteen criteria for both sheep and goat reached 100% of correct identification; *Humerus* 1-3, *tibia* 1 and 3, and distal *radius* 1 and 2 and *humerus* 1-4, proximal *radius* 1 and 4, and distal *radius* 1 respectively. When studying the results for the

combined species, the best performing criteria were *humerus* 1-3 and distal *radius* 1 with 100% correct assessment for both sheep and goats. Also *tibia* 1 (90%) and proximal *radius* 1 (87,5%) should be seen as very high performing criteria.

The results for the complete bone assessments divided for the species showed that the best performance is seen on goats with 100% correct assessment of the distal *radius*, 91,7 % of the *tibia,* and 87,5% of the *humerus*. Performance of sheep is also good with 100% correct assessment of the *humerus* and 87,5% of the proximal *radius*. In sheep the *tibia* performed poorly with only 49,7% correct assessment, whereas in goat the proximal *radius* performed poorly with 68,75% correct assessment.

When combining the species, the method seems to perform best on the *humerus* with a correct assessment of 91,7% and the distal *radius* with 87,5%. The worst performing bone is the *tibia* with a result of 66,4%.

It is evident that, while several of the assessments were correct, there are differences in the performance between the species. Assessments of bones from goat tend to get more incorrect assessed criteria while the assessments of bones from sheep tend to result in indeterminable criteria.

In the discussion it was argued that the same reliability as in Zeder and Lapham's (2010) study could not be seen since the material is different in terms of time, space, breeds and quantity. Even behaviouristic factors such as activity and feeding are argued to affect the appearance of the bones.

The thesis concluded that the methods compiled in Zeder and Lapham's (2010) study are useful when assessing goat bones from an archaeological bone assemblage as 16 out of 19 samples were correct assessed. The reliable criteria and overall good results from assessment of the complete bones should be seen as an invitation to osteologists to limit their use of the term sheep/goat and distinguish between the species more often, as it would be possible to open up new archaeological assemblages for re-evaluations and explore the different relationships humans have had with goats and sheep in the past.

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10. FIGURE INDEX

Figure front page. An early 6th century mosaic from the Basilica of Sant' Apollinare Nuovo in Ravenna, Italy. *"Cristo divide le pecore dai capretti."* The figure is modified by Theorell. [Online source]
http://upload.wikimedia.org/wikipedia/commons/1/14/Ravenna%2C_sant%27apollinare_nuovo_cristo_divide_le_pecore_dai_capretti_%28inizio_del_VI_secolo%29.jpg?uselang=sv
Last assessed: 2014-06-01

Figure 1. Example of determining species from criteria. Figure created by Theorell for this study.

Figure 2. Picture of the target sequence with the bording primers. After Theorell 2013:29.

11. LIST OF ABBREVIATIONS

aDNA	Ancient DNA
DNA	Deoxyribonucleic acid.
ddH_2O	Double-distilled water
dNTP	Deoxyribonucleic triphosphates
ddNTP	Dideoxyribonucleic triphosphates
EDTA	Ethylenediaminetetraacetic acid.
mtDNA	Mitochondrial DNA.
n	Quantity
NCBI	National Center for Biotechnology Information
PCR	Polymerase Chain Reaction.
UV	Ultraviolet light.

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13. APPENDIX

Table 7 - Detailed results divided for goat and sheep in Falun

Bone	Criteria	*Goat* n	O/C	Correct	Wrong	*Sheep* n	O/C	Correct	Wrong
Radius	1 (Proximal)	**4**	1	3	-		-	-	-
	2 (Proximal)		-	3	1		-	-	-
	3 (Proximal)		-	3	1		-	-	-
	4 (Proximal)		-	2	2		-	-	-
	1 (Distal)	**2**	-	2	-	**1**	-	1	-
	2 (Distal)		-	2	-		-	-	1
Metapod	1		-	-	-		-	1	-
	2		-	-	-	**1**	-	-	1
	3		-	-	-		-	-	1
Metatarsal	1	**2**	-	2	-		-	-	-
	2		-	2	-		-	-	-
	3		-	2	-		-	-	-
	4		-	2	-		-	-	-
Metacarpal	1	**3**	1	2	-		-	-	-
	2		-	2	1		-	-	-
	3		-	3	-		-	-	-
Tibia	1	**3**	-	3	-	**1**	-	1	-
	2		-	2	1		-	1	-
	3		-	3	-		-	-	1
Humerus	1	**3**	-	3	-		-	-	-
	2		-	3	-		-	-	-
	3		-	3	-		-	-	-
	4		1	2	-		-	-	-

n = quantity, O/C = indeterminable