An Archaeogenetic Study of Five Ancient Siberian Individuals

Revisiting of the culture-chronology of Sakha Republic with results of mitochondrial genetic data and new radiocarbon dates.

Natalija Kashuba
Abstract


This thesis is dedicated to an archaeogenetic study of five prehistoric individuals. The sample material comes from central Yakutia, also called Sakha Republic, in the north-eastern part of Russia. The main focus of this study has been the analysis of five mitochondrial genomes, retrieved from osteological material (human bones and teeth), having an estimated age of 6845 BP to 2490 BP. The dates fall within Neolithic, Bronze Age and Early Iron Age. A brief presentation for each individual’s archaeological profile and interpretation of the burial will be provided. While a series of interpretive tests with the mitochondrial DNA material were performed and the results are presented. The neolitization of the north-eastern Eurasia will also be discussed. The correlation between the Neolithic Age, Bronze Age and Early Iron Age populations will be proposed, as well as their connections to modern populations.

Keywords: ancient mtDNA genome, Sakha Republic, Yakutia, Neolithic, Bronze Age, Iron Age

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This study was only possible with the support and active participation of archaeologists from Yakutsk, Sakha Republic. Special acknowledgement to Svetlana A. Fedoseeva, Alexander Stepanov and Sergei Fedorov. These researchers openness allowed us to work with Yakutian material and I hope that we will produce valuable data for our collaborative research.

Maja Krzewinska provided me with all the necessary guidelines for sample-processing and all relevant skill of work in a “clean” laboratory.

Great support was provided to me by the wonderful employees of the Stockholm University at the Wallenberg laboratory. Special regards have to be given to Lena Holmquist, without whom I would never have planned my journey to Sakha Republic in the first place.
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1. Introduction

1.1. Motivation and aim

The prime reasons of interest for the subject are the geological and climatological properties of the area of Sakha Republic. Not only does it yield an exciting landscape of taiga-covered tracts, ox-bow lakes and steep river banks, but it also bears a patchwork of permafrost layer that reaches up to several meters in thickness. The archaeological material is surprisingly rich and well-defined, especially artefacts made of stone and ivory. In addition, debates concerning the peopling of Yakutia and its population history are still not settled amongst the archaeologists and the geneticists: Were the first settlers related to the early Amerindians or ancient Asian populations? Another outstanding feature of this area is the amount of mammoth remains, from fragmented bones to whole carcasses frozen into permafrost, unearthed ubiquitously across Sakha Republic. Archaeology in such a region is affected by a wide range of factors, some of which I've lined up. These factors are the archaeologists’ major challenge, but also a source of inspiration.

With a rich material culture a problem might arise when there is a lack of tools to interpret the data. Current analytical methods, which can be used to interpret the archaeological data, are essential for the archaeologists of Sakha Republic in their study of the prehistoric populations of the region. Archaeogenetic studies in particular can contribute with new knowledge of the biological features of the ancient populations. Such characteristics may help to trace connections between ancient populations, as well between ancient and modern populations. More precisely, with the help of archaeogenetics, we can deduce the relation of the archaeological culture-groups between each other, determine the time span of their presence at a given area, and discuss the nature of their co-existence (for example heredity between them). At the moment, the identity of the first people who inhabited the area during the prehistoric times is trapped within the lithic and ceramic artefacts from archaeological sites and osteological material. Properties of the human osteological material are yet to be discovered using molecular-level research. Archaeo-genetic research, enables us to look upon the composition of ancient DNA. This gives an osteological sample at least two different dimensions. It allows us to study the ancient human on an individual scale and on a wider scale- as a representative for the entire population. The genetic variability of a population, in its turn, might reflect a distinct culture-group and also characterize social identity of the given ancient individual. DNA, depending on the scale of the concept, might help to deduce grand events that concerned entire populations, or to bring out a single individuals personal habits. This is why an archaeogenetic method can contribute with satisfactory data for a study of prehistoric population history within my and any other archaeological study.

The choice of material was a relatively simple task, as I've searched for the oldest human remains that were available from archaeological sites in Yakutia. Luckily, I made acquaintance with cooperative archaeologists who provided me with the osteological material from diverse prehistoric culture-group representatives from central Yakutia. This collection of samples is interesting, as the material represents several epochs and different archaeological culture-groups. The preliminary dating of the samples shows that they all fall within the time period between the Neolithic Age and Early Iron Age. Geographically the ancient individuals were found relatively close to each other, in central Sakha Republic. This means that my
research area is limited but a wide time-range is secured. With this material a study of the correlation between the various archaeological culture-groups and their genetic identity through time on the territory of Sakha Republic can be performed. I had eighteen samples to work with and an intention to extract DNA from all of them. This wasn’t, however, a probable scenario, due to unknown level of the DNA proportion and its state of preservation in the osteological material. Only after laboratory work I was able to tell how many of the samples contained enough human DNA for me to work with.

The aim of this study is to deepen the insight into the pre-historic population-groups of central Yakutia. The methods that will be used in this study (archaeogenetics and radiocarbon dating) will provide me with adequate data to increase the understanding of the subject of research. Besides providing new dates (some of the material has been dated previously), I want to focus on the determination of genetic characteristics of the studied individuals; deciding the individuals’ sex and determining the haplogroups of the individuals mitochondrion. In result, this work should provide some data on the genetic background of the ancient individuals. This information will be used to discuss the archaeological culture groups of Sakha Republic, in broad strokes. I will also study the relationship between the ancient individuals and the modern populations through a phylogeny (a genetic genealogy).

This work will allow me to confront the current archaeological chronology and population history, by adding a new interpretation based on the genetic data. Furthermore the application of these results onto the discussion of North-East neolitization is of special interest to me. The expectant outcomes of this research are the results of a test of our current molecular tool-box, and an increased understanding of the prehistory of this near-arctic region of Eurasia.

Figure 1 Yakutia is shaded bright-green. It is the largest republic in Russian Federation. Figure modified by author, original is taken from website althistory.wikia.com.

1.2. The structure of the work

To accomplish the aim of this study I want to provide dating for the selected human remains and to extract and study the mitochondrial DNA from these ancient individuals, belonging to
several culture-groups and epochs. I am going to compare the dates that I receive with the current archaeological time line and interpret the genetic data, reviewing the culture-group chronology of the suited populations. Two supervisors will be guiding me through this study, in order to successfully merge archaeology and genetics within one project. First I am going to present the archaeological background and the thesis statement. Since the solidity of the results depends on the quality of the preserved DNA in the samples, the thesis statement will be presented in several independent points. Secondly, material and methods will be presented, including an introduction to the properties of the mitochondrial DNA and work within population genetics. A thorough description of each ancient sample provider will be given. The presentation of the results will be done through gathering them within the part of the fourth part of the thesis- *discussion*. The final part of the work consists of a summarizing chapter and further research propositions. Last comes the appendix and the literature list.

1.3. Archaeological Background

1.3.1. History of research

It is unfortunate that, despite all the archaeological material, the archaeology of northern parts of the world isn’t particularly well known to the scientific community, especially considering that the culture-groups and sites of Siberia and Russian Far East are rarely brought up in archaeological discussion. This neglect has partially influenced me in the motivation for choosing this research area.

The sample material hails from Central Yakutia, or Sakha Republic, with city of Yakutsk as administrative centre. The climate of Sakha Republic is continental, with contrasting temperatures. It yields extremely cold subarctic winters and very hot summers. A special feature of the regional cryosphere is a permanent layer of permafrost, reaching several meters in thickness. The permafrost together with the rivers are the key landscape features of Siberia. The largest river in Sakha Republic is River Lena. It has numerous tributaries, also large rivers, such as Vilyuy and Aldan. They may serve as transport routes through taiga forest both in summer and during winter. The river system also reflects the rout of archaeological surveys, which scientists have undertaken during latest hundred years. The areas adjacent to rivers were considered a preferable environment for settlement of prehistoric culture-groups. Thus, water-bodies have defined how the archaeological research was performed in Yakutia. A map of discovered sites, the result of a series of archaeological expeditions since 1953 is illustrative of this notion (figure 2).

Each dot on the map represent an archaeological site. It’s easily noticeable that the most of the areas between the rivers is blank, which is now a notion that puts the representativeness of the discovered sites under question. It is evident that the position of the prehistoric sites might have been aggravated by past decisions to arrange archaeological expeditions along the rivers, excluding the territory between them (Pestereva & Argunov 2014).

The topography of Yakutia is nevertheless a result of a long-term river activity. The old age of the rivers can be deduced by the size and composition of the river banks, which change from several tens of meters high hanging cliffs to oxbow lakes with broad areas of sand beaches. The panorama of an archaeological location plays a role in the archaeological discussion. Some of the sites locations hold dramatic scenery (figure 3).
Figure 3 Fragment of the map of archaeological sites from Prilensk Archaeological Expedition in 60-s and 80-s. (Mochanov 2010: 169).

Figure 2 A meander of the Lena River. Photo by author.
Difficulties might arise in understanding foreign archaeological schools because of the different socio-political influences in academic disciplines. It is worth to mention that the history of the studies of archaeology of Yakutia is divided into three phases: first phase- the pre-revolutionary, second phase- the period from the positioning of soviet power until the Great Patriotic War, and third period -the post-war period (Abramova 1981: 109). Almost every work that I've read mentions archaeologists, historians and enthusiasts from the first two periods, who have made an effort that contributed to the archaeological knowledge that Sakha scientists have today (such as Alexey Pavlovich Okladnikov and Anatoly Derevyanko). The intention of this introduction is not just to acknowledge these people, but also because of how essential these people are of the current phase of the archaeological school in Yakutia. The accepted schedule for the archaeological periods in Yakutia is based on the dating of sites and typologically defined cultures. The dating of the sites and artefacts is mainly done through following the stratigraphy of the excavated surfaces on a site. Layers (or horizons) are later explained and dated with help of quaternary geological data. A preoccupation with the classification typology is evident and it, despite providing boundaries between the cultures and ages, results in a plain chronology, where cultures and population-groups are unified and replacing each other. These methods cause problems for the management of content of all the culture-groups. For example, some specific type of artefacts from the Yumyuakhtakh culture, which is presumably Neolithic, are also found in layers from Bronze Age and consequently marked as “post-Yumyuakhtakh”. This Bronze Age culture is also known as Sugunnakh culture (Alekseev & Djakonov 2009: 38, 26).

Another example is that some researchers have taken an irresolute position towards the Mesolithic Age, by splitting the Stone Age into only two periods: Palaeolithic Age and Neolithic Age. I use the term Mesolithic as a time period between Late Palaeolithic and the Neolithic Age, a stage of economic and technological evolution between these two periods. The current attempts to squeeze in the term “Mesolithic” are perplexing. This can be seen for example in two different descriptions of the Mesolithic Sumnagin culture, which simultaneously is being set to be an Upper Palaeolithic culture, sliding into the Earliest Neolithic (Mochanov 1973: 243). However, in more current works, this culture is also presented as a Mesolithic culture (Alekseev & Djakonov 2009: 26). This reflects that, even if it is important to provide a solid time frame that cultures easily fit into, it also is important to research the difference between the social and ecological changes that different epochs stand for. The current differentiation of culture-groups may be set under question. The absence of Mesolithic Age in the discourse is correlating with the lack of discussion of neolitization, yet it is one of the most intriguing socio-economic changes during the prehistoric times. The reason for this might be that the concept of “culture” is set to fall within the classical archaeological tradition. A distinct culture is expressed through artefacts and is carried by a certain group of people (Webster 2008: 12). The change from one culture-period to another is explained with migration of a new population-group into the area and/or later diffusion (Trigger 2006: 246). This has been the accepted framework of the archaeology in Sakha Republic and it is “culture-historical” in theory. I will follow this framework to a big extent, because a legitimate re-interpretation of the archaeology of Sakha Republic wouldn't fit into the time frame of a master thesis.

1.3.2. Suggested culture chronology

The discussion of similar problematics for the Palaeolithic in Yakutia brings the most anxiety to the archaeological discourse, which is one of the reasons why I will not mention the Palaeolithic Age in this essay. Also, Palaeolithic Age, being much discussed, isn't represented by osteological objects. Furthermore, I don't have the competence and knowledge on the subject, that are necessary in order to decently criticise or reference the many publications on
the subject of Palaeolithic in Yakutia. There is by definition a limit for the Palaeolithic Age - it is seen through material culture. An example for this “typological” kind of boundary is ceramics (Mochanov 1973: 252). The Palaeolithic-Neolithic border is set by the presence of ceramics on the sites. Any archaeological culture that is identified “younger” than the Palaeolithic Age has correlative names to the common concepts of culture-chronology starting from the Neolithic Age. The Bronze Age and the Iron Age correlate with the common concept, supported by the chemical composition of the artefacts correlating with the names of these epochs. These last three periods are of the most interest to me, since the samples in my work are associated to these epochs. The chronology of the cultures may easily bedazzle with the entitlements, names of the cultures, which are mostly Yakut toponyms. Some of them are also Russian, since the first Russian colonists came to this area in the end of 16th century. They settled by the middle of 17th century, contributing with slavic (from slavic languages) toponyms (Alekseev 1992: 174-175). In the further presentation of the accepted chronology I will use as many published culture names as possible, in some cases I will make a transcription. The oldest and youngest dates for each culture show the time of presence of each culture-group on the territory of Sakha Republic. The most ancient culture that I encountered with my samples is the Syalakh culture (Сыалахская культура) and it dates to between 4870 ± 170 and 3490 ± 150 years BC. It existed for 1380 years and is considered the biggest culture complex in the early Neolithic Age.

Belkachi culture (Белькачинская культура), sometimes called Belkachinsk culture, falls between the following dates, 4100 ± 300 to 2160 ± 150 years BC. Its period is about 1940 years and is defined as a middle Neolithic culture-group. Belkachi site (excavated in 1964) is a fine example of how complex the stratigraphy of excavations can be. It has four levels, the youngest being dated to the Iron Age. The oldest couldn’t be dated conclusively but in the lower layers lithic artefacts, are described as Middle Neolithic (Mochanov 2010: 79-80). The dates for Yumyuakhtakh culture (Ымыяхтахская культура) are 2900 ± 450 and 1025 ± 235 years BC, it stretched for as much as 1880 years, and is defined as a Late Neolithic culture. Although, these are the three Neolithic culture-complexes of Yakutia. Bronze Age starts with the Ulakhan-Segelennyakh culture (Ула-хан-сегеленняхская культура), the culture with the pearled ceramics is dated to be 2175 ± 425 to 1350 ± 350 years BC, stretching for about 830 years. Next on the time line is Ust’-Mil’ Bronze Age culture (Усть-Миль), dated to be between 1380 ± 120 and 10 ± 100 BC and covering a period of about 1370 years. The final Bronze Age culture of Sugunnakh (post-Yumyuakhtakh culture) is dated to 325 ± 375 years AD (Alekseev & Djakonov 2009: 38, 26). A more recent culture-chronology is based on radio carbon dates received from 30 archaeological sites of Sakha Republic and its nearby territories (Alekseev & Djakonov 2009: 27) (figure 4).

![Figure 4](image)

**Figure 4** Chronology of culture-groups in prehistoric Sakha Republic. By author.
1.3.3. An overview of the archaeological material in Sakha Republic

The preservation conditions naturally rob the archaeologists of organic artefacts. Both the climate, with large temperature shifts between summer and winter, and the geological activity of the permafrost layers are keen to destroy them (Argunov & Pestereva 2015). Luckily, stone, bone and ceramics are less affected by these factors and are rather well-preserved. There is a vast lithic material gathered on the Stone Age of Yakutia. It reflects a well-developed local technological tradition. The tables with stone and bone-tools from the various culture-complexes are frequently published for example by Mochanov Yu. A. These objects are used in the typological work for culture-determination, together with the ceramics from the Neolithic and later sties. A gender perspective is present in the analyses of the artefacts assemblage from the Neolithic sties. The style and amount of the artefacts are similar for both female and male burials. Examples of these are artefacts in bone and stone that are interpreted as arrow heads, scrapers, nucleuses and various blades. (Lidochen 2001: 206) This quantitative data may add an important aspect to the discussion of the economic and social aspects of the neolithic populations.

There is a large variety of ceramic pottery remains (Kuzmin & Orlova 2000: 356-364) starting from the Syalakh culture-complex. Its presence is ineluctably connected with the Neolithic Age, however it is unclear if pottery reflects a different kind of economy than that of pre-pottery culture-groups (Kuzmin 2002: 37). Typologically pottery is well-defined, all of the cultures, mentioned above, have distinct style of pottery (Kistenjov 1990). Pottery of the Syalakh culture is round-based with a characteristic net-pattern on the body of the vessels (Mochanov 1973: 252). It is also called net-impressed pottery (McKenzie 2016: 170). Traits of fire are present. The rim is flat and straight. There are interesting suggestions that the net-imprints on the pots are traces of textile (figure 5).

The Belkachi pottery typically has a rounded or a pointed-base and an evident rim. There is a great variety of ornaments, that look like comb imprints and stamps (Mochanov 1973: 255). The Yumyuakhtakh culture has a round-based pottery with a net-pattern (figure 6). The Ulakhan-Segelennyakh pottery is richly embellished (figure 7). At the later stages of this culture-complex bronze artefacts appear. This stage is a transition stage to the Bronze Age and the Ust’-Mil’ culture-complex, where bronze objects are common. The pottery is as well present, but it is plain in style, compared to the Neolithic pottery, with reduced embellishment.

The image is copyright protected and cannot be displayed here in the digital version.

Figure 6 Ceramic fragments from Syalakh complexes. Mochanov 2010: 255.
Much attention has been given to the prehistoric osteological material not only from Sakha Republic, but from entire Siberia. One research worth mentioning is a taphonomic analysis of bone damage pattern, where special attention has been given to the perimortem damage. The treatment of the bones at the time near to death may reflect the dietary habits, and explain ecology of the prehistoric populations or human activity on the sites (Turner et al. 2013: 351, 28). This type of analysis is also used to affirm human presence at the very oldest of Palaeolithic sites. Unfortunately, I have not found a representative summary of this research for the material from my sites. As for central Yakutia, a quantity of different animal species are represented in the material. However, most of the discovered animal bones belong to elk and reindeer, about 90% (Pestereva & Argunov 2014). Human material is uncommon. The individuals, that I have got my samples from, are rare examples of human remains for some of the above described culture-groups. Most of these burials contain animal bones as well, from dog, hare or a smaller carnivore. I will give an extensive presentation on the burial sites in a later chapter.
1.3.4. What is a Neolithic Age within Yakutian complexes

The economy and the social structure of prehistoric groups of Sakha Republic are described in conformity with the theoretical approach. Therefore, I will clarify the general differences in the theoretical angles, concentrating on the particularly relevant definition of the Neolithic Age. I have already mentioned the absence of the Mesolithic Age in the chronology and the connected liquidation of the “neolitization” from the discourse, which is probably connected to it.

The concept of neolithic revolution has common roots in all archaeological schools, however the current interpretations of this phenomenon are different. In the Western archaeological school neolitization is associated with a series of revolutionary steps that changed the social and economic organization of prehistoric societies. A neolithic society is described by the actualization of such factors as a settled (or semi-settled) way of life, accumulated food production (through practising agriculture and pastoralism), increased population and the need for change in social hierarchy and political structures (which might be outcomes of all the economic changes) (Pluciennik & Zvelebil 2007: 467). The duration of these events falls within the period from the end of the Mesolithic Age throughout the Neolithic Age. Each part of the world has a different time scale for these events, however on a broader time-scale these events seem to have occurred neck-and-neck at several locations around the world. A simultaneous population growth can be traced at these early neolithic sites (Bocquet-Appel 2011: 560-561). In Northern Europe it occurs between 7000 BC and 4000 BC. During this time, the Mesolithic hunter-gatherer economy shifts to a pastoral-agricultural one and these alterations in prehistoric society can be traced in the archaeological, genetic and environmental source material.

The main difference in the Russian archaeological school is that the presence of ceramics labels the neolitization. Ceramics is a sign of technological progress, according to Marxist principle, which inevitably means a more advanced kind of society (Jordan & Zvelebil 2010: 48). The society is considered to have constantly developed from its primitive stage into a more convoluted one (superior to primitive) and it is reflected by adaptation of more complex technologies, such as ceramics. The economy is not discussed on the same plane as it is in the western archaeology, where the signs of farming or animal husbandry (increased production) make a prehistoric complex a “neolithic” one.

There are different exemplifications of the neolitization. In Europe establishment of the neolithic economy occurred relatively late in comparison to the Near East, where an agrarian society was already formed by 7000 BC (Whittle 2001: 136,137). The domestication of grasses- the predecessors of wheat and barley along with the domestication of goat, sheep and cattle are considered the main achievements in the region of the South-western Asia. This kind of agriculture development in this region can be traced by some of the discovered tools, for example blades that have indications of having been used as sickles (Pluciennik & Zvelebil 2007: 471). Domestication of the different species depend on their regional belonging, as well as human effort. Traces from some of the species, especially plants, are sometimes hard to find. We cannot either be sure about the extent of agricultural production. Therefore it is important to remember that we only have a fragmented picture of the prehistoric economy. Which were the driving factors for the change to agro-pastoralism from hunting and gathering? These numerous inquiries within the neolitization discourse make it seem as too ambitious to tackle. However, there is material from both a pre-neolithic and a post-neolithic period that we can gain knowledge from. By comparing the two periods, we can learn how they differ and correlate.

This notion brings me back to what kind of economy the prehistoric societies in Sakha Republic have practiced, while mastering the ceramic technology. The appearance of pottery in Siberia might have occurred before the adaptation of agriculture or pastoralism. However some researchers suggest that such early pottery is a sign of neolitization, at least in South-Eastern Siberia (Yanshina & Shewkomud 2012: 249). There are some culture-complexes in the world that are exemplary for this kind of economy. This oldest ceramic, pre-dating the
Neolithic Age even by the Russian standards, is found in the eastern part of Eurasia. Notable examples are ceramics from China, dated to 18300 BP and in Eastern Russia, with oldest dates up to 16000 BP (Boaretto et al. 2009). It is even argued that pottery technology has spread to Europe from the utmost eastern part of Eurasia, before the neolitization (in the economic sense) occurred (Jordan and Zvelebil 2010: 50). The pottery is found in Europe much later, especially Northern part of Europe. And when it comes to Scandinavia the first pottery is found in hunter-gatherer complexes. The earliest pottery is of the comb Ware type and it is found in Scandinavia (Sweden, Norway and South-East Finland) and Western Russia. A site in the North-Western part of Norway on the coast of Barents Sea dates to 6570 BP (Stilborg & Holm 2016: 326). Typical Comb Ware pottery is large (the capacity is up to 70 litres) and has a big variation of the amount and style of decorations: imprints and holes (Stilborg & Holm 2016: 326). Matching elements in the ceramics allow to draw a conclusion that populations of Scandinavia and Western Russia were in close contact during the Late Mesolithic and Early Neolithic Ages. In the Southern Scandinavia earliest ceramics is found on the sites of the Ertebølle culture-complexes, which are dated to be between 5000 BC and 4000 BC (Stilborg & Holm 2016: 332). The economy was based on marine resources, but even hunting and gathering inland took up a big part (Stilborg & Holm 2016: 333). These examples might become useful in the discussion of the economy of the Neolithic Age in Sakha Republic.

1.2.5. Neolithic Economy of Sakha Republic

Prehistoric economy of both the Palaeolithic and Neolithic Ages is described as homogeneous and dependant on the climate and faunal diversity. Permafrost in the area wouldn't allow agriculture, therefore people had to tend to hunt and gathering. The most important source of all products and a substantial part of the diet is considered to have been the reindeer. Especially this is often mentioned by the most cited authors (Mochanov and Fedoseeva). Also, many artefacts that were interpreted as fishing attributes were found on the sites of Belkachi, Syalakh and Yumyuakhtakh cultures. Such artefacts include fishing hooks, weights (presumably used with nets) and harpoons. A particularly interesting discovery is an imprint of burned fish scales on the inside wall of a ceramic pot from the site of Yumyuakhtakh (Everstov 1988: 68). Even if fishing tools are common kinds of artefacts, fishing is considered to have been only a seasonal part of the Stone Age diet. The explanation for this is that the ice during the winter was too thick to be penetrated and the nets wouldn't hold in a cold temperature (Everstov 1988: 68, 69). However, it is also true that fish bones are of a modest size and are particularly hard to find, unless specifically looked for. One more feature that adds to the discussion is a burial found at the site of Onnyos, where a human is buried together with a dog (Kozlov 1980: 55-61). If there were domesticated animals during the Neolithic Age in the Sakha area, a pastoral economy might have been unreasonably overlooked. Despite all this, based on the overlook of the literature, the economy of the people of the Neolithic is described as being based on hunting and gathering and to a little extent depending on seasonal fishery (based on the presence of fishing tools).

I think that the neolitization process in the near-arctic regions of Eurasia isn't a less exiting one than in other areas of the world. Neolithic economy in such severe environment could have had a completely different style than a classic neolithic one. More about the neolitization in Sakha Republic may be discovered if the usage of pottery and a sedentary (or semi-sedentary) life-style is discussed. For such a study there is a substantial archaeological material to analyse. And to create a more accurate picture of the prehistory of the area, molecular level research could be implemented. I would like my study to add knowledge of population history in order to understand the prehistory of the Sakha area. This was the archaeological introductory part of my research subject. In addition to this material there is a broad range of genetic studies, concerning the population development and history of the area, which I will describe in the next chapter.
1.4. Earlier published population studies of Yakutian and its neighbouring regions

1.4.1. Who are the Sakha people? Modern DNA studies and historical background

In the North-East Asia, the Sakha population is one of the largest in numbers, compared to the many surrounding indigenous populations with distinct cultures and genetic backgrounds. From written historical sources it is well known that the current Russian population isn't indigenous to Yakutia, as mentioned earlier. This explains why studies of Yakutia's population usually involve working with non-European groups, where the Sakha people form the biggest of population. The recurring aim for the researchers is to find out the origin of the Yakutian population, whether the people have migrated into Yakutia or if they originated local groups from the area. If the Sakha are not “indigenous” to the area where do they have their roots and what other population is genetically the closest to them?

The question has been raised due to the fact that the Yakutian culture, traditional economy and language isn't similar to other population groups in the area. Yakutian language is a Turkic language, it is closely connected to, for example, Mongolian. The traditional economy is based on cattle- and horse-breading. To compare, neighbouring populations of various groups (figure 8) have reindeer herding and hunting/fishing central to their life-style. Groups with this kind of economy are considered to be the indigenous groups of the Sakha Republic area.

The material for research of genetic history of Yakutians is human remains. In the case of Sakha, there is a significant amount of remains from the different periods of the pre-Christian culture. A wooden casket appeared to be a good enough protection for the body, as the permafrost allows the body to mummify. Another way of taking care of the dead are the traditional shaman burials, the arangas. The bodies were placed in wooden boxes above the ground, placed in trees (Crubézy et al. 2010). Once there is DNA data from the individuals there are several methods to explore the population history. For example Yakutian DNA can be compared to the DNA of the neighbouring populations. MtDNA groups that are found in Yakutian populations are evidently showing that Yakutians are closer to the Mongolian and Central Asians groups, than to the Palaeoasiatic groups (Koryaks, Chukchi, and Itelmen) - their closet neighbours (Pakendorf et al. 2006: 335). This means that the Yakutians are a
distinct group, which could have experienced a migration event. The migration is supported by such factors as genetic variability of Y-chromosomal SNPs (a male marker) which is low in the Yakutian population. This means that the founder male group for this population was rather small. This is contrasted by the surrounding groups’ (Tuval and Yukaghir) high Y-chromosomal diversity (Pakendorf et al. 2006: 346). In the mtDNA similar pattern is found, showcasing ones again that the Yakutian founder group wasn’t very big, resulting in today’s population being relatively homogenous (Pakendorf et al. 2006: 348). This again points at a rapid migration event of a smaller group to the area, with no considerable admixture with the indigenous population (Pakendorf et al. 2006: 348). Since the mtDNA is pointing towards a southern origin of the Yakut people, their origin might be connected to the populations of Southern Siberia and Central Asia, where most common haplogroups are C, D5a, F, G2a (Pakendorf et al. 2006: 349).

The period, which is needed for a certain population to develop its modern diversity, can be counted out using the mutation rate. However, the fluctuations of the results using this method might be considerable. For example one of the mitochondrial haplogroup is to have had its founding event in Yakut population 1,286 years +/- 800 years ago. The Y-chromosome provided a founder event proposition of 880 years BP. These results may still be counted as more or less compatible (Pakendorf et al. 2006: 349). In 13th century Asia was to a big extent under the rein of Mongol Empire. The expanding nature of this empire might have given the founder group for yakutian population a push in the northerly direction. Interesting is the fact, that in Yakutian language there are some mongolian loan-words. However, the language might have been enriched with mongolian words at any other time in history. The relationship of Mongol Empire to the presumed founder group is unknown, therefore mongol expansion should not be used as the reason for proto-yakut migration north. However, such a theory has a right to exist and can be at some point tested by genetic research to find out just how close the relationship between mongol people and proto-yakut population might have been.29 The same source is mentioning a study, where the Yakut male expansion is thought to have happened around 3800 BP, yet it isn’t given much attention, not the least because of the lack of historical, linguistic, archaeological or osteological evidence (Pakendorf et al. 2006: 349-350). The size of the founder population has been suggested. For example the female founder population is thought to have been about 150 individuals (Zlojutro et al. 2009: 480). And these do belong to the common haplotypes of the area (C and D groups). The Y-chromosomal gene flow and origin is not as easy to trace and remains an object of future studies (Zlojutro et al. 2009: 476, 481). This makes it hard to determine the origin of the Yakutian population based on the Y-chromosomal types and mtDNA haplogroups.

1.4.2. Ancient DNA studies of Sakha population. Sakha neighbours.

If Sakha are not indigenous to the territory, are any of the neighbouring groups? The above described studies are done, using modern DNA. Work on more ancient samples has also been done. For example, recently excavated burials from 15th to late 18th century and a number of museum samples. This study gave more support to an already existing theory of a relatively small founder population. Also a region of the populations’ origin was proposed, the Cis-Baikal region of Siberia, where the Yakut group is ought to have formed before the 15th century (based on a South Siberian mitochondrial origin) (Crubézy et al. 2010: 10-11). The most frequent occurring mtDNA haplogroups in the material are the following: C (especially C4, that is found within the modern populations of Evenk, Tuval, Buryat and Tofalar) and D (D5 that is present in Mongolian, central Asian and South Siberian populations) (Crubézy et al. 2010: 5-8).

The conclusion that can be drawn from population studies of Yakut history is that the Yakutian population haven’t been living in the Sakha republic territory for more than 700 years. The Yakutian samples sometimes show that they have European mitochondrial haplogroups. This can be explained by the intermarriage with the Russians after the 15th century (specifically Russian women, who married Yakutian men) (Pakendorf et al. 2003: 220). It is a good example of how DNA marker, such as mitochondrial DNA might reflect historical events and support historical written sources.
The interest to find an essentially indigenous population of Yakutia is not easy to satisfy. This northerly territory is home to several population groups with distinct language, culture and economy. Some of these are practicing a nomadic, or semi-nomadic lifestyle, which makes the territory that such a population inhabits much bigger than expected.

1.4.3. Before the Sakha. Prehistoric population of North East Eurasia and beyond.

A number of prehistoric individuals from archaeological sites in Sakha Republic have been analysed. A neolithic site of Rodinka (dated to 3600±60 BP) contained a burial of a woman. This study (published in 2005) uncovered the quantity and the haplogroup of the studied individual. Mitochondrial DNA had 377 base pairs from the HV1 region, which allowed to determine that the haplogroup it belongs to is C (Ricaut et al. 2005: 459). This haplogroup is found both in the Asian (Siberian) population and the American. This gives a general picture that these populations are maternally connected with each other, still the amount of data isn't enough to conclude anything more specific. Attempts on determining the haplotypes of ancient individuals have also been done for two of the individuals from the Kyordyughen burial (from 2000–1000 B.C.), which I will also be working with (Fedorova et al. 2008: 392). The grave has been dated with the help of comparative typology of the archaeological description of the burial. An incomplete mitochondrial genome (HVS1 sequence) was produced from both of the sampled individuals. The individual 4 (N4b2 in my numbering) was assigned a haplogroup A4, which is one of the Asian haplogroups. The individual 5 hasn't been assigned a haplogroup, but the sequence allows authors to speculate that it belongs to one of these haplogroups: D or G2a. The A4 haplogroup is found in the Kazakh population mostly, with smaller variations it is also found in several siberian populations. However, of most archaeological interest is that the two individuals from this burial have different haplogroups and so do not share maternal ancestry (Fedorova et al. 2008: 395). Y-chromosome studies of pre-historic siberian population have also been performed, but the decay of human osteological material allows much less than scientists prefer (Keyser et al. 2007). The most ancient DNA from Eurasia (Altai region, Mal’ta site) has been extracted from remains of an individual dated to about 24000 BP. Interestingly, data showed that this individual shares genetic information with early inhabitants of Europa, despite territorially being positioned half the continent away from first hunter-gatherers. The mitochondrial haplogroup, assigned to the individual is U- a widely recognised group within for example for Mesolithic hunter-gatherers of Scandinavia (Raghavan et al. 2014).

The population studies of Siberian groups have also been used within research, concerning the peopling of the Americas. Main questions are 1) if any of siberian population groups could have been the ancestors of the modern Amerindians and 2) if so- when the migration occurred. It is commonly assumed that the migration occurred between 17,000-34,000 BP from the Eurasian continent to Beringia refuge area, and later on into Americas- where the indigenous American population developed into several population groups (Torroni et al. 1993: 605). The haplogroups that are common for both northern Siberian populations and indigenous populations in Americas are A, C and D. The haplogroup B (common in Sothern Siberian and all but Alaskan American groups) is the exception that is not at all present in Northern Siberian or Alaskan populations. This mitochondrial lineage must have had a more southern location, and it’s indeed common in Southern Siberian populations, for example the Mongolian population (that lies geographically south to Yakutia) that carries all the haplogroups related to populations of the Northern Eurasia and Americas (Kolman et al. 1996: 1332; Merriwether & Ferrell 1996: 243). This is why the North Siberian groups are not considered founder populations for Amerindians, even if they are close neighbours to each other. Even if the Siberian populations are not considered to be the founder peoples for Amerindians, it is necessary to mention that it is considered that populations of the Americas and Siberia are genetically close groups, with common haplogroups and other genetic markers
And it is possibly due to both of these groups having a common origin (Kolman et al. 1996: 86). These theories could be applied when more ancient samples from the North-East Eurasia are going to be discovered. Mitochondrial DNA from Siberian samples that I am working with might give insight into the genetic history of prehistoric population of this region, and help to strengthen some of the above described theories.

1.5. Thesis statement

With this study I hope to increase our understanding of the pre-historic populations of Yakutia. There are several aspects that I find problematic within the population history and archaeology of Yakutia, and this motivates me to conduct research on the archaeological material from this area.

First is that the culture-groups are decided with typological differentiation of the lithic and ceramic artefacts. The system that was created by the first archaeologists is still being used as a solid foundation to all current research. This means that when new sites are found and excavated they are categorized using the above described method of arranging.

Next, much of the dating is either done incorrectly or on samples that might not be representative for the culture, rather than for a specific site (materials such as charcoal and animal bones). The method of carbon dating was not always available for the archaeologists in Sakha Republic, this is why the geological dating was often used. This kind of method is not ultimate for dating sites positioned in demanding topographical locations (river banks, known for the level of geological activity through erosion and sedimentation.) Since quaternary geology has for a long time been a permanent base for archaeological chronology, culture-group identification was tied to the usage of geological stratigraphy, the layering of the sites, as direct records of the history of the area. Of course, the artefacts positions may have been displaced thorough the levels by anthropogenic or geological activities. Stratigraphy is not optimal to be used as a direct parallel to how the culture-groups replaced each other in the area, as the artefacts do not necessarily connect to a population that carried them. In result, the cultures are described as "being brought", and the past cultures simply seize to exist, become replaced- which is a direct and unilateral interpretation of the Yakutian archaeological past. Stratigraphy is a good method, but it should definitely be used together with other studies from the archaeological tool-box.

The dating methods, being unstable enough, are easy to be compromised with, for the profit of determining a definite culture group and assign it a period. In result the cultures that are described in the literature are “absolute” cultures that never developed through time and were always replaced by a group of a more technologically advanced migrants. This might explain why the neolitization is not really brought up in the archaeological literature of the area, and why the Mesolithic Age (as a transition period) isn't discussed. Yet it is exactly what might be interesting to trace- the development of culture on a certain territory through time. Not only through stratigraphy and artefacts but also through the population genetics, dietary patterns, paleo-climatology and other interdisciplinary studies.

When it comes to the population history, according to the archaeological output, every culture-group had a distinct population, which became displaced by the next one. If this conclusion is correct, we will see a lot of different population groups moving around the prehistoric Yakutian territories. The direction and origin of such migrations are also interesting to trace. The mitochondrial DNA would allow to investigate such large scale population events (such as migrations or population bottle-necks).

I find the discussion concerning the economy and societal structure of the prehistoric populations overruled (which may be due to the lack of data). Each and every one of these prehistoric population-groups are characterized as reindeer-hunters and fishers, with no permanent settlements and voluntary seasonal migration. Archaeology has now many
methods available to produce more data and analyse the one that exists already. There are traditional methods, based on typological differentiation or stratigraphy. Some methods are interdisciplinary, such as archaeogenetics. The application of the newer methods might bring new structure to the vast archaeological material of Yakutia, by challenging the framework of the older methods. Using several methods might provide for better understanding for any given archaeological dilemma. The Yakutian data needs a re-interpretation, using the results from different new methods of analysis.

The historic and pre-historic osteological human material from North-East Siberia is scarce, but it is still representative and useful for many different scientific disciplines. I will only address archaeology, specifically archaeo-genetics, leaving out many other questions that this material can hold answers to.

Even though some studies of ancient individuals have been implemented, which I have mentioned in an earlier chapter, the genetic history of the area is not yet well described. More material, especially prehistoric material, is needed in order to address the problems that I discussed above. My individuals come from the area of Central Yakutia and a period from the Neolithic Age to the Early Iron Age. This material justifies the work that I have set to do, and it is the following.

Dating of the human remains: Since the current culture-chronology was established using an uncertain set of dates, it should not be used as a framework into which archaeological artefacts can be put in. I am therefore interested in providing the material within the study with new dates. The dating of the collagen from the bones will put the current culture and period systematization to a test.

Individual’s sex: The genetic analysis will allow to check some of the osteological results from examining of the anthropological remains. Genetically deciding the sex of an ancient individual is more accurate analysis than the osteological examining (if the DNA amount in a sample is sufficient).

The genetic history of the area and the genetic background of the individuals: By looking at the individual’s haplogroups, the intercontinental connections can be traced, and the mtDNA expertise may allow us to address the question of Americas populating. Since it is considered that the peopling of Americas happened in Palaeolithic Age, it is interesting to test whether any of the representatives of Neolithic populations that I work with share DNA with the Amerindians. How ancient is the routes from Eurasia to the American continents? Not to forget, that the mtDNA will allow to only discover the maternal background for the representatives of the culture-groups and the periods, which will still certainly clarify the population history. However, just using mtDNA as a marker for kinship between groups is questionable as I will explain in the method chapter.

By comparing genomes of mitochondria from the studied individuals, we can see if they had a common maternal background, how far back in time they were related. Two of the Late Neolithic individuals, from the Kyordyghen site, are central for my thesis. The genetic properties of the individuals in these burials are statistically more significant than the ones from other sites with single burials. This site may be considered much more representative for a population-group and, belonging to the Late Neolithic Age, is a good reference to compare the more ancient burials with. It would also be interesting to find out if the two individuals were related. A large scaled population dynamics of the area, based on the DNA research may help to trace big-scale population events.

Genetics of the neolithization: Finally, I think that it is necessary to raise the discussion on the subject of neolithic transaction of the prehistoric culture-groups of Northern Asia, specifically the Sakha Republic. This is important for the grounding of the current working theory of culture-group replacement. Are these defined culture-groups also reflected in the genetic data of the representatives from these populations? The answer to this question might increase our understanding of the socio-economic patterns of the given prehistoric populations.
2. Materials and methods

The material that I will be working with is rare. The results of this study will become easier to explain if a short essay on genetic research will be provided. I will start with characteristics of the properties of the DNA and continue with the material presentation.

2.1. MtDNA characteristics

Intercellular DNA is found not only in cell’s nucleus, but also in mitochondria, organelles found in the cytoplasm of a cell. These organelles exists in several copies in eukaryotic cells. Mitochondria are repeated with a variable occurrence in different types of cells; the number varies from hundreds to thousands (Robin & Wong 1988: 512). A mitochondria's main role is of a "cellular power plant", although its involvement in other processes, like cell death (apoptosis) have been recognised (Newmeyer & Ferguson-Miller 2003: 482).

Studies around mitochondrial DNA (mtDNA) started more than half a century ago, when it was discovered (Nass & Nass 1963 and Schatz et al. 1964). The mtDNA is made up by the same bases as nuclear DNA. DNA (short for deoxyribonucleic acid) is a large molecule that carries the inheritance information and codes the protein construction. It is built up by the four following nucleobases: A-adenine, G-guanine, C-cytosine, T-thymine. These are paired up A-T, G-C and these pairs have a particular order (Sadava et al. 2009: Chapter 11). The nuclear DNA molecule is organized into chromosomes. One chromosome may contain tens or millions of these nucleotides. Units in DNA are called genes and genome is the entire set of genetic material in a cell (Matisoo-Smith & Horsburgh 2012: 22, 28). The first complete human mitochondrial genome (entire genetic material) was published by Anderson S. et al, showing its composition of 16,569 base pairs (Anderson et al. 1981: 457). There are between 1 to 15 copies of mtDNA in a mitochondrion (Satoh & Kuroiwa 1991: 140). The research of mitochondrial genome proposes its evolutionary background being a symbiotic relationship of a eubacteria and a cell. It's genetically closest relatives are the thea-proteobacteria (Burger 2003: 709).

A structure of the mitochondrial DNA is different to nuclear DNA. Its genome is arranged into a circular molecule, with its 16,569 base pairs divided into regions. The regions are different in both the structure and their function. Figure X is depicting these regions, including the D-loop (displacement-loop), the one marked in light blue, which is central for population studies. The remaining (and the largest part) of the circular molecule is made up by the coding regions. These are the L strand (LSP), the so-called light-strand and the H (H1 and H2), the heavy strand (HSP). The strands are named so because of the uneven amount of the nucleotide content, the H strand contains more guanine (G) than the LSP (Falkenberg et al. 2007: 682).
The D-Loop is relatively short, it consists of 1.100 base pairs of non-coding proteins. This region is called the control region, where sequences responsible for DNA transcription and replication (promoters of the HSP and LSP) are located (Clayton 1991: 473). It is made up by the HVR1 and HVR2 (the hyper-variable regions 1 and 2) that have positions 16024–16383 and 57–372. In the HVR1 and HVR2 the DNA mutations are stored (Stoneking 2000: 1029-1032). The time span between the different mutations occurring is called the mutation rate. For the human mitochondria it is estimated to be \( \frac{3}{705} = 0.0043 \) per generation (95% confidence interval [CI] .00088 – .013), or .32/site/1 million years (95% CI .065 – .97) (Sigurðardóttir 2000: 1608). The mutation rate is high, which provides a big number of variability in this region of mitochondria. Just by looking at the D-Loop makes it possible to distinguish genetic variations. This is the base for the population genetic studies. It is although done by distinguishing the genetic divergence between the mtDNA and the mutation rate allows to place mutations on a time scale. This is one of the mitochondrial characteristics that are used in such studies. Another important mitochondrial characteristic is that the mitochondrial genome is inherited through maternal line. The male mitochondria are deleted at one of the first stages of fertilization. The paternal mitochondria are tagged with a special protein during spermatogenesis and get automatically destroyed in the oocyte (Sutovsky 1999: 372). This means that all mitochondria in our bodies are uniparental. It allows us to trace the individuals’ maternal background many generations back. In fact, mtDNA lineages can be traced all the way back, to one single haplogroup that can serve as common root to all of them. This is the genetics behind the “mitochondrial Eve” theory (Birky 1995: 113331-11338). The fact that there are multiple copies of the mitochondrial genome in a cell makes this DNA more accessible, as there is bigger chance of at least some of the mitochondria being preserved (Brown & Brown 2011: 177). These are the main reasons that make mitochondrial DNA particularly interesting for archaeogenetics.

The variety in the mitochondria is found through comparing of the mtDNA single nucleotide polymorphisms (SNPs). The arrangement of the SNPs differs the most in the hyper-variable regions. However it is possible to compare the entire mitochondrial genome, which might provide more accuracy in placing of the mtDNA within a haplogroup. Haplogroups are created, based on the different arrangement of the mitochondrial DNA. Population studies allows us to trace populations’ most common haplogroups. And, since the mitochondrial DNA has a higher mutation rate than the nuclear DNA, there are more of these polymorphisms in the DNA and also more variations to follow and also set a time scale with the help of the mutation rate. For example, the mitochondrial Eve (with the help of Molecular
The mitochondrial population studies are not exemplary of a perfect combination of method and material. One haplogroup usually doesn't describe an entire population, there are instead several haplogroups that are typically assigned for a certain group of individuals. For prehistoric studies the downfall is the number of ancient individuals that would provide a good coverage of an entire population. Usually only a few representatives are found or even just one. Here it is important to remember that the examined individuals do not have to represent the population that once has inhabited the area, where the burial sites are located. It is also problematic to trace the migration events, again, as one individual that carries one mitochondrial haplogroup is not representative for the entire population. Also the mtDNA is a smaller molecule, which does not contain much information about the individual and its heredity. All the above mentioned implies that a population cannot be described using the mitochondrial genetic markers, much due to the lack of the necessary amount of the genetic information.

With the problems lined up, the mitochondrial DNA research is still a good feature of archaeogenetic toolbox. From all the destructive methods of analyses the DNA extraction (and especially mtDNA) does not require large sample sizes. The degradation of nDNA may make it impossible to perform a good statistical study, while the mtDNA (since present in many copies) may be left relatively intact even in the most ancient samples. The information that mtDNA may provide about an ancient individual is sometimes the only information that we can gain, mtDNA is although a good tool for broad studies of population history of human species. This is the reason why I will be using mtDNA as the main material in my study.

2.1.1. DNA as a feature within archaeogenetic research

Mitochondrial DNA (mtDNA) and nuclear DNA (nDNA) analysis comes handy for archaeologists in many ways. Successfully preserved DNA allows gaining an assortment of knowledge about the composition and characteristics of the examined individuals genome and history. With DNA research a variety of biological features might be investigated. DNA studies can be used in search for four main factors: species identification, phenotypes, of which the sex recognition studies are perhaps best known (presence or absence of the Y chromosome), kinship detection (in a broader sense this usually relates to demographic investigations) and traces of diseases (Brown & Brown 2011: 156, 169). Also we can learn more about diet (by tracing markers under selection, such as the 13910T mutation, providing for lactose persistence). Archaeogenetics enables the possibility to prescribe the examined individual the presumable culture-group, lifestyle and social patterns.

Once the mtDNA is successfully extracted and sequenced, there is a test that uncovers its genetic connections. Phylogenetics allows us to investigate evolutionary connections between species, and it can be carried out on a molecular level (McLennan & Brooks 2002: 1058). A phylogenetic tree is a visualization of these connections between examined taxa (usually individuals, species or haplotypes). It allows to place the ancient mtDNA within the human "family tree", that (by choice) can hold both modern and prehistoric human DNA. The “root” of a tree is what individual is placed to be the presumed common ancestor. The construction of a phylogenetic tree is based on differences or similarities between the examined sequences, depending on the method used. Generally there are two types of methods for tree calculation: the distance-methods and the discrete data methods. In distance methods the number of differences between the sequences is calculated to reveal the relationship between them. The discrete data methods (which are usually called parsimony) are more complicated, these are based on similarities, and on the process of finding the tree that requires the least evolutionary steps (mutations) to explain the data. Usually it includes creating several possible phylogenies and thereafter looking for the most suitable evolutionary change to assemble the final tree-diagram. The tree has to be tested once it’s assembled. Bootstrapping is a suitable test of the quality of any phylogenetic tree (Baldauf 2003: 349). The example of a phylogenetic tree
(figure 10) shows an expositive diagram for human mitochondrial haplogroups, with the root-mitochondria marking the presumed most recent common ancestress of African origin.

2.1.2. How different are the mtDNA genomes?

To tell human genomes apart there is the CRS, the so called Cambridge reference sequence, but more recently in a revised version (rCRS). As I mentioned earlier, this was the first human mitochondrial genome sequenced (at Cambridge University) (Anderson et al. 1981). It is used to compare other genomes with, as I also will do in this study. Every new mutation is given a number and a name that represent how it differs from the rCRS sequence.

A haplotype is a unique combination of nucleotides. Several people may share the same mutations and so have the same haplotype. If a group of people of one haplotype share the same ancestor and similar mutations with another haplotype they can be placed in a common haplogroup (Matisoo-Smith & Horsburgh 2012: 35, 36). A haplogroup is made up of similar haplotypes that share a common origin. A phylogenetic tree for human mitochondrial genomes is commonly built on the principle that the rCRS is the “original” genome, a base genome for the comparison with other sequences. Yet it is also a “traditional” genome to use as a reference, constructed in 1981 (Anderson et al.) There isn’t a unified tradition, regarding weather the rCRS should be used as a reference sequence or if it should be placed within the phylogeny into a haplogroup that it belongs to (it belongs to the haplogroup H2a2a1). The conceptually correct sequence is use instead would be the RSRS (Reconstructed Sapience Reference Genome) should be used for comparison and the RNRS (Reconstructed Neanderthal Reference Sequence) as an outgroup for constructing a human phylogeny. This is because the RSRS uses both global sampling of modern humans and the ancient hominids (making it a good reference), thus a suitable root to branch off modern human populations (Behar et al 2012: 675).

![Figure 10](image-url) A tree of human mitochondrial haplogroups, presenting most of the Latin letters that are used as designation for haplogroups. The root is marked by the * symbol, being the most recent common ancestor (Van Oven & Kayser 2009: 388).
2.1.3. Human haplogroups geographical distribution and population history

The mitochondrial haplogroups have different background, and (as several studies show) are restricted to geographical regions. The macro haplogroup L and its sub-haplogroups, L0, L1, L5, L2, L6, L4 and L3 are found in Africa (Gonder et al 2006: 758). The L3 haplogroup is ancestral to the haplogroups M, N and R (out coming from N) which are the mitochondrial lineages found outside of Africa. These are called macro haplogroups, because they are the roots for all the mitochondrial diversity elsewhere. The M group is root for branches C, Z, E, G, Q and D. The N- to I, W, Y, A, S, X and R. The R is a common root for HV, J, T, F, B, P and U. This is only a schematic representation of how the groups are connected to each other. The approximate time for bigger human migration events can be calculated using molecular clock. The haplogroups with L3 background are found to have formed during one successful migration out of Africa, which is placed about 70000 years ago (Soares 2012: 924). The three macro haplogroups have been carried by female representatives to all the continents. The macrohaplogroup M for example have been carried from Africa in an eastern direction. Its descendant haplogroups are found in India and East Asia. The most northern territory that they’ve reached is the North-Eastern Asia where the scions to American haplogroups might have taken shape (Maca-Meyer et al 2001). The N haplogroup was carried north out of Africa, and about 50000 BP expanded into haplogroups that are found in Europe now: H, V, U, K, J, I, T (Richards et al. 1998: 258).

The biggest interest for me lies in the Eurasian and American haplogroups: A, B, C, D, E, F and G. These are the most studied clades within the dilemma of how Americas have gotten peopled. Using the divergence between haplogroups, the first human migration to the Beringia (a region that includes the territories of Alaska, Western Canada and easternmost part of Siberia) have been counted out to have taken place between the 22,414 BP and 29,545 BP (Torroni 1994: 1161). The size of this population have been estimated to less than 80 individuals, which is about 1% of Asian population at that time (however this study didn't include arctic American populations) (Hey 2005: 0971). That first population have developed its own mitochondrial diversity, which is arranged in haplogroups A2, B2, C1 and D1. These haplogroups are also called Pan-American, and their estimated age is about 20000 years (Achilli et al 2008: 5). These mitochondria make up the most of the Amerindian population (with addition of B and X haplogroups) and are also found in the Eurasian populations. This presence is explained with several migration event from the territory of and beyond the Bering Strait.

There is a clear connection between the American and Asian haplogroups, which is expected as indigenous American populations have Asian ancestry. It is supported by an array of genetic studies. An early example of their relation to each other is a common feature, that there is no presence of any western admixtures in them, unlike in the Central Asian populations (Comas et al 2004: 502). The mitochondrial diversity in Eastern Asia is restricted to the non-european haplogroups from M, N and R clades: B, F, R9, R11, R* (from the R clade), A, N9a, Y (from the N haplogroup) and C, D, G, M*, M3, M7, M8, M9, M10; M11, Z (from the M clade).

The most common in North-East area are C and D haplogroups. The mitochondrial diversity of the North-East Asia the best example of recent inter-continental connections between polar population groups (Derenko et al 2007: 8). This can be traced both by the large haplogroup group belonging and by subclades that are found in assorted populations. For example, the Chukchi people (form the most North-East of Russia) have the haplogroup A as the most occurring one. It is a commonly occurring haplogroup in American population, together with the D haplogroup, that is believed to have been brought to the Beringia area about 7000 BP. The precise subclade is the D3, which cannot be found in Siberian populations, but is present in Amerindians (Eskimo-Aleut populations). The D group genealogy has two hypothesis of spreading into America. First it that migration occurred about 15000 BP, as the Beringia became a refuge area during the post glacial period. There the D haplogroups could differentiate.

However, the theory of a migration wave from Siberia more than 6000 years BP, resulting
in the D3 haplogroup spreading to American continent, have more supporters (Derbeneva 2002: 419-420). The most north-eastern populations that inhabit Chukotka carry the A2a, A2b, and D2 haplogroups (Chukchi and Siberian Eskimos). Sometimes C and D are found within one population, like the Yukaghir and Nganasan populations (C2a, Cb2, C3, D4-D9) (Volodko et al 2008: 1094-1097). Also many subclades of haplogroup F1 are found in populations across Central and East Asia (Mongols, Japanese, Kazakhs and Buryats) (Bermisheva et al 2002: 881). It has its spreading from Japan to the South Siberia. The mitochondrial diversity is indeed vast in the North-East Asia and, despite active research, the population history suffers from the lack of ancient samples and complete mtDNA genome studies. Studies of the ancient DNA might help to clarify both the genealogy of the mtDNA and the history of the Asia-American contacts through history.

2.2. Individuals

The sampling was allowed by prof. Stepanov, North Eastern University and Fedoseeva S., Institute of Arctic Archaeology. Both situated in Yakutsk, where I and prof. Stepanov have performed the sampling. The original amount of samples that I could process was eighteen, only five will be discussed in this thesis. All of the 18 samples were subjected to destructive process of DNA extraction. Five out of eighteen showed positive results with full coverage of the mitochondrial genome. These five individuals presumably belonged to various culture-groups, which are thought to have been hunter-gatherer people, populating this near-arctic region. Some of the individuals might be at a stage of transition between different epochs, from Neolithic to Bronze Age, based on the typology of the lithic artefacts, pottery, metal objects and other archaeological findings.

2.1.1. Matta (N2a)

A peculiar, half-ruined burial was found by the Matta River. More precise, 4 km from the mouth of the River Matta, which is the Sinyaja Rivers left tributary, 132 km from the Sinyaja Rivers mouth. The burial is called Matta, inheriting its name from the village, closest to the burial site in Megino-Kangalassky District. It was discovered during an archaeological expedition of Yakutian State University in 1996 (Sentekjaeva 2015).

The skeleton (figures 12-13) was found on 10 to 15 cm depth in sediment accumulated by erosive processes, on the terrace of the Matta Lake. This burial is dated to be 3890+-30 years BC (Lidochen 2001: 206), the Late Neolithic. Burial contained a fragmented skeleton, presumably with individuals head positioned towards the North-North-East (Lidochen 2001: 205). The skeleton wasn't complete, the entire right part of the skeleton was missing. This burial was also badly preserved, likely because of a road positioned close to it. An interesting detail is that instead of the left hand of the individual there were bones from a metatarsi or a metacarpi from a species of the genus Lepus (Sentekjaeva 2015: 17). Osteologically the burial is presumed to be of a female. The fragments taken for the analyses are two bones from left foot (pes sinister): metatarsale 1, os cuneiforme (2). The cuneiforme was used up entirely during the work.
Figure 11 Analysed individuals are marked in colour on a fragment of the map of Yakutia. Map by Stepanov A. 2016 (image by Stepanov A. 2016 and reproduced with his permission, @Stepanov A 2016).
Figure 13 The profile of the Matta burial (image from Pestereva et al. 2016 and reproduced with their permission, © Pestereva et al. 2016).
2.2.2. Onnyos (N5a)

The burial was found in 1978, on the left promontory of the mouth of the River Dzhybadah (left tributary of Amga River). It lays close to the western outskirts of the Onnyos village (figures 14-15).

Onnyos burial is famous for the skeletal remains of animals, found in the grave. The burial contained a complete skeleton, the chest was partially covered with red ochre (Lidochen 2001: 205). The individual was placed in the grave in a stretched position, head oriented to the South, feet to North, arms bend at the elbows with hands placed on the waist (on top of coxae bones) (Lidochen 2001: 206). Traces of violent death were recorded, the individual was found to be male. There were a number of artefacts in the burial, several are highlighted: fishing hooks, made from a long bone of a large animal (Everstov 1988: 65), rolled in a circle a fox was found on top of the feet of the human. Also, between the legs (shins) a skeleton of a sable (Martes zibellina) was discovered (Sentekjaeva 2015: 16). It has been suggested, by the artefacts, that the burial is from Middle Neolithic, the Belkachi culture. A tooth (molar) from the individual was taken for analyses.

Figure 14 The profile of the Onnyos burial (Kozlov 1980:59).

The image is copyright protected and cannot be displayed here in the digital version.

Figure 15 Frontal drawing of the Onnyos burial with fox and sable (Kozlov 1980:59).

2.2.3. Dyupsya (N3a)

The burial was found in the Ust-Aldansky District of Sakha Republic. This is one of the four Early Iron Age burials found in Yakutia. The skeleton is in an “embryo” position, found 5 km North-East of the Dyupsya village (Stepanov 2010: 32). The position of the burial was 2 meters beyond sea level and on 50 centimetres depth. The individual was presumably a male,
his bones bare multiple post-mortem marks from teeth of a carnivore animal (wolverine). Several bones are missing, including a large part of the skull (figures 16-17). The earth around the body was of a lighter shade, this might be explained by the dissolved organic material surrounding the burial (Stepanov 2010: 32). Iron fragments were found in the grave, along with a long knife made out of bone, fragments from a bow and an awl. Dated to between 500 BC and 500 AD, Dyupsya is considered to be an Early Iron Age burial. One tooth was given for the analyses.

Figure 16 Dyupsya burial in profile (image from Stepanov et al. 2010 and reproduced with their permission, @Stepanov et al. 2010).
2.2.4. Kyordyughen 1 (N4b2)

This burial site was discovered in 2004 (Alekseyev et al. 2006: 45). The location is 10 km to the North from Churapcha village, Churapchinsky District of Sakha Republic. It was found on a terrace by a lake with coordinates (WGS-84): N62°04'07"; E132°19'53". This burial intrigued the archaeologists as soon as it was discovered. The first feature that archaeologists came upon before they discovered the burial itself was a plate-setting, assembled into a shield, covering the entire burial (figure 18). Under the plate setting a near-complete skeleton (N4b2) that once belonged to an adult man was found. By the feet of this skeleton a pile of plates were found and interpreted as a plate-armour. The man was buried in a stretched out position, parallel with the lake. The skeleton was very close to surface (20-35cm). A pack of human bones was found above the knee (what would be the lap) of the first individual. These bones are on 10-35 cm depth and are called Kyordyughen 1 (2). The near-complete individual is Kyordyughen 1 (1). The inventory of the grave was rich, by the left leg of individual 1 a number of ornaments out of stone and bone were found. By the right leg remains of what might have been a bow. By the right shoulder of the individual 1 an adze-tool was placed. There is a number of interesting osteological features about this burial. The individual 1 had

Figure 17 Dyupsya burial frontal drawing (image from Stepanov et al. 2010 and reproduced with their permission, ©Stepanov et al. 2010).
lost most of the teeth from the right mandible, and the alveoli recovered completely. The fibula has a fracture that successfully healed. There is a suggestion that the left femur of the individual 1 does not belong to the same individual as the upper part of the skeleton. Under the skull of individual 1 a small collection of coal was found.

This burial is dated to Late Neolithic, Yumyuakhtakh culture, judging from a fragment of pottery as a part of burial inventory. The later dating of the bones gave the following results: individual 1 dated to 2190-1880 BC; individual 2 dated to 2570-2290 BC. Plates of the armour are made out of ivory, unfortunately discussion is still on about what animal it belonged to. One of the plate was dated to be 30900-28240 BC. It might be mammoth or wholly rhino ivory (Stepanov et al. 2012: 54). The artefacts of the burial and the fashion of its assemblage gave rise to a theory, that Kyordyughen is a burial of a high-ranked man that might have had a life of combat. The pile of bones that represent the second individual may be interpreted as a sacrifice (Stepanov et al. 2012: 58). A tooth from individual Kyordyughen 1 (1) was processed.
Figure 12 Kyordyughen "warrior" burial (image from Stepanov et al. 2012: 52 and reproduced with their permission, @Stepanov et al. 2012: 52).
2.2.5. Kyordyughen 2 (N4a1)
This site was discovered in 2012 by archaeologists from the North-Eastern Federal University. It was treated as burial since its discovery, despite the wide area that the bones were scattered over. The position of this burial is in close proximity to the Kyordyughen 1, with coordinates: (WGS-84): N62°04'07"; E132°19'54". The burial is described as fragmented, and the fragments were spread across a wide area between the depth between 10 and 40 centimetres. Among other smaller groups of outmost interest is the concentration of teeth and vertebral fragments. We were allowed to sample one tooth from this site.

2.2.6. General information about the samples
Samples were retrieved from museum and university collections in different state of preservation. The aim was to collect the most compact and intact material, preferably teeth. In the case of N2a, the only intact bone that I could get was from the foot of the individual, as no teeth could be retrieved. In the table I line up the all the anthropological material that was included in the study.

Table 1 General information on the individuals.

<table>
<thead>
<tr>
<th>Sample number</th>
<th>Site</th>
<th>Bone fragment</th>
<th>Tooth</th>
<th>Culture</th>
<th>Earlier dating</th>
</tr>
</thead>
<tbody>
<tr>
<td>N2a</td>
<td>Matta burial</td>
<td>x</td>
<td></td>
<td>Yumyuakhtakh culture</td>
<td>3890+–30 BC Late Neolithic</td>
</tr>
<tr>
<td>N5a</td>
<td>Onnyos burial</td>
<td>x</td>
<td></td>
<td>Belkachi culture</td>
<td>Middle Neolithic</td>
</tr>
<tr>
<td>N3a</td>
<td>Dyupsya burial</td>
<td>x</td>
<td></td>
<td></td>
<td>500 BC - 500 AD Early Iron Age</td>
</tr>
<tr>
<td>N4a1</td>
<td>Kyordyughen 2</td>
<td>x</td>
<td></td>
<td></td>
<td>Late Neolithic</td>
</tr>
<tr>
<td>N4b2</td>
<td>Kyordyughen burial 1, individual 1</td>
<td>x</td>
<td></td>
<td></td>
<td>2570-2290 BC Late Neolithic</td>
</tr>
</tbody>
</table>

2.3. From the sampling and extraction to mtDNA genome assembling

2.3.1. DNA sampling
DNA may be found in any kind of organic materials. For work with ancient DNA the cleanliness of the procedure is crucial, as well as selection of the source material. The second is an important assignment and, although the better preserved material is usually selected, it is still impossible to predict the amount and quality of the DNA in the sample, before the results
of the laboratory work are at hand.

The laboratory methods require accuracy, due to a high risk of contamination of the ancient material with the scholars modern DNA. Therefore, work with ancient DNA requires a type of facility where the contamination risk is brought down to a minimum.

I worked with the samples at AFL* ancient DNA laboratory facilities. The different steps of work were performed in two of the laboratories, using protective clothes and regularly cleaning the working surfaces. The samples were cleaned with weak bleach solution (1% HCl) and purified water. The samples were also subjected to ultraviolet purification of all the surfaces in a cross linker. The drilling was performed in an open hood, using a dentist drill (Dremel). The amount of bone powder for the samples varied from 50 to 100 milligrams. For all of the following steps of samples processing a blank sample for negative control was included, in order to follow the contamination level if necessary.

2.3.2. Sample processing

The extraction of the DNA was performed based on the Yang protocol (Yang et al 1998). The powder of a sample is a mixture of both organic and inorganic components. The ultimate aim is to dissolve the sample and the reagents are selected accordingly. Carbamide (urea) and ethylenediaminetetraacetic acid (EDTA) are mixed into a Yang-Urea buffer with Proteinase K added separately. Buffer from urea and EDTA was prepared and UV irradiated before the use. The reaction is set for 24 hours at 38°C. The temperature can be regulated if the powder doesn't fully dissolve at the first stage (raised to 55°C). The purification in done using the Amicon® Ultra centrifugal filters and MinElute filters (Qiagen). The buffers used in the purification procedures are QIAGEN buffers: binding buffer (PB), washing buffer (PE) and elution buffer (EB). The product from bone powder is 110µl of DNA extract that is stored at -20°C.

2.3.3. All of the DNA in the sample

The DNA extracts were used to build libraries on, with the help of molecular cloning, using pre-bought kits. The libraries were tested and amplified according to the same protocol, by Mayer (Meyer & Kircher 2010). The steps of the DNA library building are the following: Blunt End Repair, Adapter Ligation and Adapter fill-in. The libraries are a collection of processed DNA fragments, enabling future work (PCR) with the desired fragments of the samples. For every step a separate master mix was prepared, following different incubation programs in a thermal cycler. Between the steps the product was purified, using the MinElutes and buffers (same as in the extraction purification).

For the Blunt End Repair the master mix as the following: Tango Buffer/4µl, dNTPs (25mM)/0.16µl, ATP/0.4µl, T4 PNK/2µl, T4DNA Pol/0.8µl, H2O/12.64µl. The total volume of the mix and DNA is 40µl, incubation in a thermal cycler is for 15 minutes at 25°C, followed by 5 minutes at 12°C. The purification step is done in MinElute tubes, with 200µl of PB buffer to bind the DNA to the membrane (spinning at 13000 rpm for 1 minute). The waste is discarded and the membrane is washed with 600 µl of PE buffer twice, discarding the waste after each step. Then the MinElutes are "dried" through spinning without any washing buffer and MinElutes are transferred into DNA low-bind 1.5 µl tubes, removing the lid. 22 µl of elution buffer is added and incubated at 37°C for 5 minutes. After the tubes are spun at 13000 for 1 minute to receive the purified product. Next step is the Adapter Ligation with the following master mix: H2O/ 10µl, T4 DNA ligase Buffer (10x)/ 4µl, PEG-4000 (50%)/ 4µl, Adapter mix/1 µl (of a 1:10 dilution with 10pmol in final volume), T4 DNA ligase (5 U/µl)/ 1µl. With the addition of the 20 µl of DNA the final volume is 40 µl. Incubation for adapter ligation is 30 minutes at the temperature of 22°C. The product is than purified with MinElute, as described above. The final step of the library building is the Adapter fill in where the master mix is this: H2O/ 14.1 µl, Thermopol buffer 10x/4.0 µl, dNTPs (25uM each)/ 0.4 µl,
Bst polymerase, LF (8 U/μL)/ 1.5 μl. The final volume in this step together with the 20 μl of DNA is also 40 μl and the incubation period is for 20 minutes at 37°C with the following heat kill at 80°C for 20 minutes. The libraries were tested with gel electrophoresis.

Figure 13 Photography of a completed gel. Two big white arrows show the trace of samples, containing DNA. The smaller arrow points at a ladder, a pre-bought base-pair mix used as a ruler to measure the DNA product. Photography by author.

By the length of the ladder an assumption can be drawn about the DNA qualities from the particular sample. With the help of this method, each sample was assigned a certain number of cycles for the PCR (polymerase chain reaction). The PCR is set up for 6 reactions, 5 of which have 3 μl of library DNA and 1 is a PCR blank. They are set up with a following master mix: ddH₂O/15.25 μl, 10X TaqGold Buffer/2.5 μl, 25mM MgCl₂/2.5 μl, 25mM dNTPs/0.25 μl, IS4_short_amp 10uM /0.5 μl, Index primer (1-22) 10 uM/0.5 μl, AmpliTaq Gold/0.5 µl. Each sample was assigned an index. The PCR was ran on a thermal cycler in a different laboratory facility. The program is as follows: initial denaturation at 94°C for 12 minutes, denaturation at 94 °C for 30 minutes, annealing at 60 °C for 30 minutes, elongation at 72°C for 45 minutes, final extension is at 72°C for 10 minutes after which the samples are kept on a 4°C temperature. Each sample is prescribed a separate index. The denaturation, annealing and elongation is repeated a number of cycles, assigned earlier.

The product is then pulled into one tube and purified with Agencourt AMPure XP beads (Beckman Coulter) with the following protocol for the product. 0.5x volume of beads should be added to the product, vortexed and spun down quickly and incubated in room temperature for 5 minutes. Than tubes should be placed on a magnetic rack for 1 minute. The supernatant should be removed and placed in a fresh DNA low-bind tube. To this supernatant 1.8x beads of the volume should be added, vortexed and spun down quickly. The incubation in room temperature is 10 minutes and 3 minutes on the magnetic rack. The supernatant may be removed and the beads that now contain the short fragments from the amplified DNA libraries should be washed three times with 200ul of 70% EtOH. When the ethanol is removed in the last step, the tubes should remain open for 10 minutes to dry out the remaining ethanol. DNA is eluted with 36ul of TET buffer. This is done by vortexing each tube for no less than 20 seconds, spinning it down quickly and incubating in room temperature for 10 minutes. Than tubes should be placed on the magnetic rack for 5 minutes. When all the beads migrate to the rack the product can be pulled and stored in a new DNA low-bind tube at -20°C.

After the bead-cleaning the amplified libraries were tested using BioAnalyzer at NHRM**. The traits from BioAnalyzer show the amount and the length of different fragments in the amplified libraries. This helps to make a final decision about sending the product for sequencing. Also the information about the concentration of the DNA in each sample will be necessary for calculation of the needed concentration of each samples as they will be placed in one tube for sequencing. After the calculation to balance out samples of different quality samples are pulled in one tube. Since each sample was prescribed an individual index, after sequencing we will be able to tell the samples apart. The samples were shotgun sequenced. As a result of sequencing we receive all the DNA from sample from human, bacteria, microorganisms. Since my interest is mitochondria, I’ve received the mitochondrial DNA sequences and performed further analyses with them both online and offline using software
MEGA (Tamura et al. 2011) and DnaSP (Rozas et al. 2010).

2.3.4. Working with sequences. Phylogenetics.
Within an ancient mitochondrial genome it is easy to miss out of some the important mutations, if one doesn’t do a careful analyses. Accepting the mistakes (such as gaps and misplaced base pairs) would lead to multiplying them in the further work. I have gone through the complete mitochondrial genome sequences of each ancient individual, to make out weather some of the least obvious nucleotide positions are mutations or DNA damaged sites. I’ve corrected the sequences, inserting the most probable nucleotides where needed, based on the sum of all the cloned variants of the position.

2.3.5. Identification of the haplotype
The mitochondrial haplogroups were identified using an online-tool HAPLOFIND (Vianello et. al. 2013). The precise mutations were traced using PhyloTree (van Oven & Kayser 2009). Each change in the sequence is of importance for assigning the correct haplogroup.

2.3.6. Production of phylogenetic tree
I aligned the sequences in MEGA software. In order to build a comparative phylogeny the five genomes have to be put within a group of sequences. The sequences are in free access at the website of NCBI**** (National Center for Biotechnology Information). I've decided that the modern genomes used to compare the ancient individuals with should be representatives of populations, which are presently living in Northern Asia, and might be connected to the ancient individuals territorially. The table of the individuals and the sequences is attached in the appendix of the thesis. I've also added the RSRS, the Neanderthal and the Denisovan mitochondrial genomes to the phylogeny, which all can serve as out-branches for all other sequences. After putting the sequences together I created a phylogenetic tree, using bootstrapping as testing method, to see were on the branches the ancient genomes find themselves. A tree diagram is the best way to show the results.

2.3.7. Dating
For the 5 working sequences it was decided to re-check the dates. I sampled the roots of the teeth for this purpose. From the 2a sample I drilled out powder in the clean laboratory. The samples were sent off to BETA***.
3. Results

3.1. Sex

The sequenced DNA allowed to make sex estimations of the individuals, based on the presence or absence of Y chromosome. In the DNA from sample N2a no Y chromosome was found, which means that it is a female. The remaining four individuals (N3a, N4a1, N4b2 and N5a) all showed traces of Y chromosome, making them male.

3.2. MtDNA

3.2.1. N2a Matta

The mitochondrial genome of this individual was highly fragmented, yet it was still possible to assign it to the F1 subclade. The assignment is based on the following mutations: C3970T-G13928c-T16304C (R9). T6392C-G10310A (F). G6962A-T10609C-G12406A-C12882T (F1). T16189C (F1 subclade). T146C!-C1734T and T5628C-C15402T. The haplogroup F belongs to the R macro haplogroup, which is defined by two mutations T12705C and T16223C. These are present in the sequence from N2a.

3.2.2. N5a

The genome of the N5a individual contains a high number of poorly traced C bases. It can be assigned to the D4 haplogroup. By mistake it was first placed into D4b1c haplogroup, based on the following mutations: C5178a-T16362C (D). G3010A-C8414T-C14668T (D4). G8020A (D4b). C10181T-T15440C-A15951G-G16319A (D4b1). T239C-A297G-G951A (D4b1c). However, uncovering of one more mutation (4023C) finalises the result: C722T-T4023C-T6374C-C9785T (D3). The mitochondrial genome of this individual belongs to the D3 haplogroup.

3.2.3. N3a

This mitochondrial genome belongs to C4b3 haplogroup, which lies within the macro haplogroup M. The defining mutations for this mitochondrion are the following: T489C-C10400T-T14783C-G15043A (M). A4715G-C7196a-G8584A-A15487t-T16298C (M8). A249d (CZ). T3552a-A9545G-G11914A!-A13263G-T16298C-C16319A (C4). G6026A-G11969A-T15204C-C4. 2232.1A (C4a'b'c'). A3816G (C4b). C16291T (C4b3).

3.2.4. N4b2 and N4a1

N4b2 individual was subjected to mtDNA analyses before, and was assigned the A4 haplogroup, using HVR1 polymorphisms 16223, 16290, 16319 and 16362. I had the entire mitochondrial genome to work with, which allowed me to reassess the previous results. The mutation on the position 16223 wasn't observed. The 16223 and 16278 positions allow to assign the A12a haplogroup, calculating the differences between the haplogroups to the RSRS. The authors of the previous study use rCRS instead, and mean that the mutation at 16223 is found in haplogroup G2a and D. It actually belongs to haplogroups Y, X2h and R. The 16278 if found in the haplogroups N10b and X. So the mutations from the same individuals belong to different haplogroups. This reflects how problematic it is to distinguish a haplogroup of mtDNA while working with incomplete mitochondrial sequence.

The N4a1 individual has analogical polymorphisms as the N4b2 individual. I would like to elaborate on how similar the mitochondrial genomes of these individuals are. The reads of nucleotides for uncertain positions for both of the mitochondria were checked and it turned out that most of the dissimilarities are C to T and G to A mutations. The origin of these mutations may be connected to damage, typically found in ancient DNA. The amount of mutations is also unconvincing, in relation to the rest of the genome that is identical between these two individuals. These two individuals undoubtedly share a common maternal ancestry, yet I have no proof of them coming from the same family-group.

Table 1 Dating and haplogroup identification results.

<table>
<thead>
<tr>
<th>Sample number</th>
<th>Site</th>
<th>Dating</th>
<th>Calibrated Age BC</th>
<th>Haplogroup</th>
</tr>
</thead>
<tbody>
<tr>
<td>N2a</td>
<td>Matta burial</td>
<td>5940 ± 30 BP</td>
<td>Cal BC 4895 to 4865 and Cal BC 4850 to 4725</td>
<td>F1d</td>
</tr>
<tr>
<td>N5a</td>
<td>Onnyos burial</td>
<td>5420 ± 30 BP</td>
<td>Cal BC 4340 to 4235</td>
<td>D3</td>
</tr>
<tr>
<td>N3a</td>
<td>Dyupsya burial</td>
<td>2520 ± 30 BP</td>
<td>Cal BC 790 to 730, Cal BC 690 to 660 and Cal BC 650 to 540</td>
<td>C4b3</td>
</tr>
<tr>
<td>N4a1</td>
<td>Kyordyughen 2</td>
<td>3910 ± 30 BP</td>
<td>Cal BC 2475 to 2295</td>
<td>A12a</td>
</tr>
<tr>
<td>N4b2</td>
<td>Kyordyughen burial 1, individual 1</td>
<td>4040 ± 30 BP</td>
<td>Cal BC 2830 to 2820 and Cal BC 2625 to 2475</td>
<td>A12a</td>
</tr>
</tbody>
</table>

3.2. The ancient mitochondrial genomes within a phylogeny.
The phylogeny, presented in a form of a tree, contains mitochondria from the five ancient siberian individuals, Neanderthal mitochondrion, RSRS, rCRS and a number of modern mitochondria, most of which are of the same haplogroups as the ancient sequences, or close positioned branches. From start I had 60 sequences within the phylogeny. This work was done in MEGA5 [3]. The evolutionary history was built by the Maximum Parsimony method, using the Subtree-Pruning-Regrafting (SPR) algorithm (Nei & Kumar (2000: 126) with search level 1, in which the initial trees were obtained by random sequences. The bootstrap consensus tree inferred from 300 replicates is representing evolutionary history of the data analysed (Felsenstein 1985). The branches of the tree that didn’t correspond in partitions reproduced in at least 50% bootstrap replicates were excluded from the phylogeny. The positions analysed were 1st, 2nd, 3rd and “noncoding”. All the positions that contained gaps in the data were eliminated. The total number of positons analysed in the final dataset is 14283.

Figure 2014 Five ancient individuals mitochondria, placed together with modern mitochondria within a phylogenetic tree. By author.
4. Discussion

Below I will summarise and discuss the main findings of this study, divided in two parts first the implications of the new dates for the broad understanding of prehistoric cultures. In the second part of this chapter I will summarise in more detail the genetic findings and relate this discussion to the broader debates of population dynamics over time. In the last part of the chapter I will discuss the results in relation to the overall archaeology of the region.

4.1. How new dating changes time-borders for prehistoric cultures

The most ancient culture-group within this study is believed to be Belkachi culture, represented by the Middle Neolithic Onnyos burial. Thus, the dating of the individual provides a chronological anchor-point for the Belkachi culture and it is cal 4340 BC to cal 4235 BC.

The second most ancient culture entity is the Neolithic Yumyuakhtakh culture, represented by the fragmented Matta burial. The new date for the Matta individual is about a thousand years older than the previously established date, which places her outside the chronology of the Yumyuakhtakh culture. The calibrated dating for this individual is cal 4895 BC to cal 4865 BC and cal 4850 BC to cal 4725 BC. This chronologically altered the Matta woman significantly, making her the most ancient individual in this study. This highlights the archaeological periodization of Neolithic culture-groups of Sakha Republic, which I will come back to.

The chronological deviation of the Kyordyughen individuals is smaller, but still notable. It differs with some hundreds of years from the earlier received dates. Interestingly the difference in dates between the individuals still remains. The calibrated dates are the following: for N4b2 2475 BC to 2295 BC, for N4a1 2830 BC to 2820 BC and 2625 BC to 2475 BC. These burials are Late Neolithic, and the dating confirms a time frame for the Neolithic Age in Yakutia, with a minimal range from cal 4895 BC to cal 2295 BC.

The Dyupsya burial is an Early Iron Age site, the time frames for the span of Early Iron Age were set to be between 500 BC and 500 AD. The new date is older than expected, it falls between 790 BC cal and 540 BC cal, thus pushing the limits of the Iron Age a few years back.

An interesting observation is that all these five individuals were ascribed to an underestimated chronology. They were all dated to be younger previously. Five samples makes it statistically significant, and show that there was a tendency to ascribe human remains to younger strata than they really are from. This may be especially important for the Dyupsya burial (N3a). This site clearly is an Iron Age one, as it contains iron fragments. Thus, the boundary for the early Iron Age is pushed back with the dating of this burial, at a minimum with a few decades. Interestingly there are lithic and bone artefacts found in the Dyupsya burial, such artefacts are typical for as early as Neolithic and Bronze Age contexts. This might point towards a certain continuity of some of the tools from Neolithic to Iron Age. This also means that the new dating of this individual is also the dating of the very border between the Iron Age and previous periods, based on results from a different method than typology.

In case of Yumyuakhtakh culture-group it undermines the established understanding of this cultures time-span. Both the Kyordyughen burials (N4a1, N4b2) and the Matta burial were thought to be representatives for the Yumyuakhtakh culture. From the dating results I would argue against the Matta burial belonging to the Yumyuakhtakh culture. First, because there is about 2000 years difference between the two archaeological sites. Second, because a
distinguished feature of Yumyuakhtakh culture is a special type of pottery. The ceramic remains from Yumyuakhtakh vessels are found in Kyordyughen burial, while lacking in the Matta burial. Third, the Matta burial is much closer in time to the established chronology for the Belkachi culture, which is exemplified by the Onnyos burial. The only artefact from the Matta burial is the hare’s foot, placed where the left hand of the individual was ought to be. The Onnyos burial also contained animal bones, ordered in a special way within the grave. This fact, together with the dating, presents a strong incitement to place Matta in the same culture group with the Onnyos burial— the Belkachi culture. Such correction also excludes the possibility of two different culture-groups coexisting simultaneously in the area of central Yakutia during the discussed part of the Neolithic Age.

4.2. Maternal genetic ancestry of the representatives of the prehistoric culture-groups

4.2.1. Middle Neolithic
The Matta individual belongs to haplogroup F1d, which is common in East Asia while absent in Europe and Americas. The Onnyos individual belongs to haplogroup D3, which is present in a much lower frequency in Asian populations, instead it is common in the Eskimo-Aleut populations. Thus, these two individuals from Neolithic Age present two different mitochondrial lineages; one restricted to Asia and other connected to the Amerindian populations.

4.2.2. Late Neolithic
Both of the individuals from the Kyordyughen site have the mitochondrial haplogroup A12a. The A haplogroup is common in North-East Asia and North America. It is branching from A2, one of the Pan-American haplogroups. Having two individuals, buried at the same site, with difference in a couple hundred years, sharing this haplotype indicates that it was present with some frequency in this area at this age.

4.2.3. Early Iron Age
The Dyupsya individual harbours haplogroup C4b3. Macro haplogroup C is associated with modern population of the North-East Asia.

4.2.4. Conclusion
The mitochondrial assemblage of all the studied ancient individuals reflects the modern mtDNA variation of the North-Eastern Asia and North America: F1d, D3, C4b3 and A12a. The Matta and Onnyos haplogroups shows some derivative from two different geographical areas, from the perspective of modern distribution of their haplogroups. Thus the Belkachi culture-group, and the individuals who were a part of it, might have harboured genetic components from at least two groups, suggesting population influx from two directions. The first from the South (the F1d haplogroup from Matta) and about 500 years later there is indications pointing towards the North-East and potentially North America (the D3 haplogroup from Onnyos). Based on the individuals from the Late Neolithic Yumyuakhtakh culture-group (the Kyordyughen burial site with the A12a haplogroup), suggests that a flow of haplotypes from North America could have continued for thousands of years until the end of the Neolithic period. The direction of the flow is not obvious, because the Inuit populations (as far as in Greenland) are limited to two groups with the discussed
haplogroups which are A (A2a and A2b) and D3. The Inuit groups are also connected to the North-Eastern groups in Russia. There might have been an expansion of groups that populated the polar regions of Bering Strait and North America, or a proto-Inuit group was formed as early as 6200 BP in central Yakutia. Finally, the Early Iron Age population (haplogroup C4b3 from Dyupsya site) may have its origin in the South Siberia. Of course, these hypotheses will be properly tested with nuclear DNA as we continue to sequence DNA from these and other individuals.

The proposed origin of the culture-groups is based on solely five ancient individuals, and their maternal ancestry. The phylogenetic tree (figure 20) shows that the position of the ancient individuals is within the groups of modern North-Eastern Asian populations, such as: Ket, Mansi, Nganasan, Ulchi, Koryak and Yakut. All of the ancient individuals seem to have been a part of the genetic continuation resulting in the modern indigenous groups of the North-East Asia. Actually, the fact that all of these individuals were found on a relatively small territory in Central Yakutia supports the hypothesis of all of them belonging to one proto-group with diverse maternal background but also with a long continuity.

4.3. Archaeological discussion

4.3.1. Dating

Both the dating and the genetic information provided some insight into the population- and culture-history of the prehistoric population of Sakha Republic.

The Middle Neolithic is represented by two individuals. The Matta woman and the Onnyos man. Unfortunately, the Matta burial was destroyed during a road-construction and we only have the remains of an incomplete skeleton and one artefact- a metatarsi or a metacarpi from a hare. This artefact is often present at archaeological sites, and also in Stone Age contexts. For example at the site of Ajvide, burial 7, an individual was buried with a collection of arctic hare’s feet (Mannermaa 2008: 207). The second Middle Neolithic burial of the Onnyos man contained more information. The chest of this individual was covered with red ochre and several objects were placed in the burial. As in the Matta burial, remains of animals were present in the Onnyos burial: skulls from a fox and a sable (which are known as high quality fur animals). Both of the individuals were buried in a stretched out position and oriented towards different directions. The woman’s head was placed towards the North-East while the man’s to the South (Lidochen 2001: 205). The fact that makes the differences between these burials even more protruding is that the individuals’ mitochondrial background also point in opposite geographical directions. The man’s maternal background might reach as far out as North America, while the woman’s genetic background is directed towards the south. The burial rite might have been affected by the gender perspective or the burials position within the landscape. However, there might be an awareness of the origin of the individuals and that may have been the factor that directed the position of the burials.

The two individuals from the Late Neolithic have a north-eastern mitochondrial background. Both men from the Kyordyughen site were buried with a time interval spanning a century. This indicates that the Kyordyughen burial site was used at least through this time period. Both of the individuals have the same haplogroup. I do not have enough genetic information to conclude how close they are related, however there is a striking resemblance between their mitochondrial genome, and (as it is not possible to exclude degradation-artefacts for the differing positions within the genome) the individuals may actually be sharing the same haplotype. It is interesting to discuss a possible family-group at this burial site. The burials of the two men are in a very different state. The well-preserved burial 1 (N4b2) has outstanding artefacts, as the individual was completely covered by a plate-shield. Dating of the ivory of the shield (Pleistocene) implicates that the source of the ivory might have been animal such as mammoth or woolly rhino. The individual in this burial was a man,
who had a broken leg and experienced the loss of several teeth. All of these injuries however healed completely during his lifetime. The burial, completely covered by the shield, contained a bow, a set of arrows and a piece of ornamented pottery among other artefacts.

Figure 15 Figure 16 Arrowheads from the Kyordyughen burial 1 (image from Stepanov et al. 2012: 57 and reproduced with their permission, @Stepanov et al. 2012: 57).

The burial 2 (N4a1) consisted of bones of an individual scattered across a wide area. There are a number of ideas about why this burial was destroyed, however a simple factor to be considered would be that he wasn’t buried deep enough. The season of the burial is uncertain, but the permafrost thaws during the summer season only. If the burial wasn’t well covered - predators would have access to the shallow grave. The probability for this course of events is high, unlike the probability for a grave-robbery case, as there are some remains of artefacts found together with the scattered bones, exclude the theory of grave-robbery.

The character of the mitochondrial make-up of the neolithic individuals does not support the theory of each culture-group being represented by a separate genetically distinct population, as both Middle Neolithic and Late Neolithic individuals share a similar genetic background. Consequently, there is no proof for the support of the theory of “culture-replacement”, as being driven by one population replacing another. But, note, that I am relying on mtDNA from a relatively small number of individuals, and we are preparing a larger study on more DNA specifically addressing this issue.

So far I concluded that the early neolithic population of Yakutia was a mix between groups of different origin, arriving from different territories. I also have argued that there was no replacement of one population by another population. This would mean that the Neolithic culture could develop for millennia, independently of any significant migration events and without a significant amount of gene-flow.
Interestingly, the representative of an Early Iron Age culture has a north-eastern maternal background (the man from the Dyupsya burial). Earlier I have stressed that the Early Iron Age culture sometimes is given name of “Post-Yumyuakhtakh” culture, due to similarities of lithic and other types of artefacts. The norther-eastern haplogroup of the Dyupsya man (C4b3) and the throw-back of the Early Iron Age culture into similarities with the Neolithic Yumyuakhtakh suggests that the culture-group of Yumyuakhtakh represents a continuity: The haplotype may be regional or global, but at least not any possible gene-flow does not seem to alter the material culture of Yumyuakhtakh population in any drastic fashion.

4.3.2. Settlement

Disregarding the population-history of the prehistoric society of Yakutia, the fact remains that people chose to settle in a permafrost-covered area already in the Stone Age. The lack of settlement finds is replenished by the amount of typical neolithic artefacts found equally distributed in both male and female burials: fragments of ceramic vessels, fine lithic and bone tools, arrowheads, harpoons, scrapers. The most ancient ceramic remains from Russia were discovered in Yakutia, and in Far East. These ceramic fragments date back to 13600-14000 BC in Far East and 10900 BC in Siberia (both calibrated dates). This adds complexity to our understanding of the Neolitization (Derevianko et al. 2004: 735-739). Still, the economic aspect of the Neolithic population is rarely questioned. There general idea is that there was one from of occupation- hunting, particularly large game. Such explanation of the economy of the neolithic population erases the possibility of over-producing the goods and overall concept of “neolithic” use of the environment. The neolithic economy in the discussed environment must have had different forms from what are commonly considered.

Two environmental factors are important in the discussion of neolithic economy of Yakutia: first, the permafrost does not exclude pastoralism; and second, the territory of Sakha Republic was the last refuge-area for several of the mega-faunal species of the Eurasian continent. The neolitization of Yakutia is as of now an incomplete story of the adaptation of human species to near-arctic permafrost imbedded regions. The study of these people, who chose the polar-night environment, might give us insight into how far a prehistoric human capacity of controlling the environment actually stretched.

4.3.3. Further research

There is a lot of possibilities for further research concerning this study. First of all, a full-genome study of the five ancient individuals would allow to work further on how they relate to each other, to published ancient individuals and modern populations. For example we could tell how the Kyordyughen individuals are related, if their genome is sequenced enough. It is interesting to compare to this group of near-arctic individuals to other neolithic and bronze are individuals in western Eurasia. Was there an intercontinental contact? With whole-genome data we could also look closer on inherited diseases and the phenotypical variation of these people. There is a potential also in studying the taphonomy of the burials. It would be interesting to compare the osteological material to the information in the sources, which unfortunately are scarce.

Another interesting aspect of research is the ceramic artefacts. A trial of lipid analysis and isotopes could give us the insight of what these vessels used for and thus let us speculate about the economy of the ancient society.

An outstanding feature of the Yakutian archaeology is also the metal artefacts. What is the composition of the material that the bronze ornaments are made of? Is it possible that it is local or were the contact network stretching far from the settlement sites? Where and how did the Bronze Age start in Yakutia? In summary, there is an array of possibilities of developing this study into a research project that can help us to understand the near-arctic Siberian
society.
5. Conclusion

The purpose of the study was to study the ancient population of Yakutia. I wanted to add new dimensions to the material and start a discussion. I have to mention that the studied individuals, being the only representatives of the ancient populations, do not necessarily reflect the entire variation of the ancient population and should be regarded as a group of case-studies.

Firstly, new dates of bone collagen from anthropological material has altered the chronology and thus the periodization of pre-historical culture-groups of central Yakutia. I studied individuals from two different culture groups, one of which is present in the middle stage of the Neolithic Age and another one in the late stage. These are the Belkachi and Yumyuakhtakh culture-groups. I shuffled the placing of the Matta woman from Yumyuakhtakh culture to Belkachi culture-group, together with the Onnyos man as the earliest representatives for the Middle Neolithic population of Yakutia. Next are the two Yumyuakhtakh individuals from Late Neolithic Age, which come from the same burial site and have similar dating. The last individual sets the border for Early Iron Age. The time line is as follows: Neolithic Age spans from at least 4895 BC to 2295 BC. The Bronze Age took place between circa 2295 BC and 790 BC. The Early Iron Age starts at the earliest 790 BC. 

Conclusive results are that the mitochondrial genomes of the ancient individuals reflect modern day mtDNA variation in the region (North-Eastern Asia and North America). This data is enough to open a discussion of the possibility of migration of these individuals to and from the area of central Yakutia, but not enough of data to make any conclusive remarks. There are two possibilities: the first on being that there was a constant migration from North America and close-by territories into central Yakutia; the second is that the Neolithic population of Yakutia is a proto-group for modern North East Asian and North American populations. The phylogeny of the modern populations of these regions and the ancient individuals support this theory. The biological sex of the individuals is of importance for a population genetic study at a different level than my research, yet it is still an interesting fact within the archaeological discussion. All of the individuals were found to be males, besides one- the Matta burial. I would also like to mention that there is more variation in the Middle Neolithic population than during the Late Neolithic. There is, however, not enough variation to support overwhelming migration events, which could alter the indigenous population substantially. Instead there is a genetic continuity from Middle Neolithic to Early Iron Age, which is also supported by how little the material culture was altered through this period.

The absence of solid proof for a migration event that could have triggered a “culture-replacement” challenges our understanding of the neolithization process, specifically in this area. Both the climate and permafrost bearing ground would seem as undesired factors for a neolithic economy. Despite this central Yakutia has proven to be a place of interest for people in prehistoric times since the Middle Neolithic Age. The fact that Yakutia was a refuge area for mega-faunal species is too far back in time and cannot directly be connected to the neolithization process or the mere fact that humans chose to settle in the area long after the mega-faunal extinction.

I think that an interdisciplinary approach is necessary for further studies of these prehistoric events, population history and archaeology of Yakutia on a closer level.
6. References.


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**Figure Index**

The tables in thesis were made by author.
All the figures were modified by the author.

Figure 17. Figure modified by author, original is taken from website althistory.wikia.com. Available at: http://althistory.wikia.com/wiki/File:Map_of_Russia_(Russian_America).png.

Figure 18. After Mochanov Yu. A. 2010. 50 years of stone age of Sibiria. Book 2 Agenstvo CIB NBR Sakha.

Figure 19. By author.

Figure 20. By author.

Figure 21. After Mochanov Yu. A. 2010. 50 years of stone age of Sibiria. Book 1 Agenstvo CIB NBR Sakha)

Figure 22. Modified from photo taken from www.yakutskhistory.net. (https://www.yakutskhistory.net/%D0%BE-%D0%BF%D1%80%D0%BE%D0%B8%D1%81%D1%85%D0%BE%D0%B6%D0%B4%


Figure 261. Map provided by Stepanov A. D. 2016.


Figure 19. By author.

Figure 20. By author.


**List of abbreviations**


**Appendix**

This is the table of the modern published mitochondria genomes that I used. All of this data is in open access and can be found online on NCBI website: http://www.ncbi.nlm.nih.gov/nuccore/AY882405?report=GenBank/.
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