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SEX DETERMINATION USING ARCHAEOLOGICAL, ANTHROPOLOGICAL, AND GENETIC METHODS – A COMPARATIVE STUDY ON A MEROVINGIAN POPULATION FROM GOTHA-BOILSTÄDT

ABSTRACT: In bioarchaeological practice, many different approaches and methods of sex estimation in archaeological skeletal specimens are in use. We compare three common approaches from archaeology, physical anthropology, and molecular anthropology to investigate the sex of an individual.

The Merovingian graveyard of Gotha-Boilstädt in Central Germany dates from the 6th to the 8th century C.E. There is archaeological evidence of early Christianity, however, many objects also indicate the existence of other notions towards an afterlife. Altogether, 45 burial features contained human remains, in some cases there were multiple burials or remains of older disturbed burials within one grave pit. In total, 52 individuals from inhumation graves were investigated. Among them, 10 were subadults below 14 years, six were juveniles (14–20 years), and 36 were adults above 20 years. Based on the grave goods, the sex (gender) of 20 individuals could be assessed, identifying 13 females, including one subadult individual, and seven males. Employing the methods of physical anthropology, it was possible to estimate the sex of one subadult individual (rather male), four juveniles (three probable males and one probable female) and 32 adults (12 males, and 20 females). For aDNA investigation, the preservation of six individuals did not allow any sampling and, therefore, these six individuals had to be excluded from further analysis. Among the remaining 46 individuals, the preservation of nuclear aDNA was mostly good, only in six individuals was extraction of aDNA not possible. For one of the subadult individuals, it was not possible to determine the sex, the others were four males and five females. The sex of only one juvenile individual could not be determined by aDNA, bringing the determination up

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to five male and one female juvenile in total. Among the adults, 12 males, 20 females, and 5 individuals with indeterminable sex were identified. Several differences between the archaeological, anthropological, and genetic sex estimations were identified during the investigation. These matches and differences form the topic of this paper.

KEY WORDS: Early Medieval burials – Central Germany – Morphological sex estimation – aDNA investigation – Genetic fingerprinting – Multiplex X/Y-PCR – Archaeological gender evaluation

PREAMBLE

Sex estimation of skeletal remains is one of the main topics of any anthropological analysis. In many cases, the estimation using morphological features of the skeleton, especially the pelvic bone and the skull, is easy and delivers clear results. However, this is not always the case. The methods of sex estimation of subadult skeletons are not uncontroversial and, even in the best case, they only deliver a tentative estimation, not a clear result. Even in adults, commonly, the preservation of the skeleton prevents any sex estimation. In other, less common cases, the morphological secondary sexual features of the skeleton are not distinct enough. In some further, indeed rare cases, the morphology may provide misleading information, unbeknownst to the observer, until other methods are applied for the investigation. Here, the method of choice is, of course, the genetic investigation. The human DNA bears clear answers as to whether an individual is genetically male or female, without any doubt. Of course, there are rare cases of misfit between the genetic determination and the phenotypic pronunciation of biological sex. However, these are not to be confused with endocrine-induced or psychology- or culture-based gender diversity. And, since the beginnings of archaeological science, there have always been efforts to establish a connection between the archaeological characteristics of a burial – grave goods, position of the skeleton, size and shape of the grave pit or other attributes – and the sex. Maybe, in this case of acculturation and perception of the individual, these attributes should rather be considered to represent the gender of the buried individual. These efforts delivered mixed results; however, they were never accepted without doubt and, if possible, always involved crosschecking with biological anthropology. In the present study (this study is based on a full scale investigation of the burial ground, for full results see Nováček in Tannhäuser ed., in print), we have had the uncommon opportunity to combine all three of these approaches. To date, a full-scale morphological investigation of a skeletal series, combined with a complete genetic investigation of all suitable individuals, is still not an everyday occurrence. It is especially rare in early medieval burial grounds, where grave goods are commonly found and their affiliation to both sexes is well-documented, and, therefore, the evaluation of sex (or gender) of the deceased is possible by the means of archaeology. With this paper, we hope to contribute to the current, lively discussion on sex/gender topics in the past. With this contribution, we want to honour the memory of one of the leading researchers on the anthropological sex estimation using the pelvic bone, whose methods involving the incisura ischiadica maior, arc composé, and sulcus praeauricularis became the standard within the canon of the methodological spectrum, Vladimír Novotný.

INTRODUCTION

When working on burial sites, two levels of investigation, the archaeological and the anthropological, have to be considered first. However, only the combination of the results of both allows us to approach the burying group and, further on, the historical population of a particular era and region.

"How does the cemetery date? To which archaeological culture can it be assigned? Is there a recognisable occupation sequence? Are there grave goods? Can social differences between the buried be read from the grave goods?" are some of the basic archaeological questions that need to be answered. First of all, the burial site of a particular individual is examined in order to assess the burial ground or at least its excavated section in a second step. Anthropology focuses on the buried individual first – what was the age-at-death and what sex can be estimated? Are pathological or other features of interest recognisable in the skeleton? – in order to subsequently develop demographic models. In this contribution, we compare three common approaches to sex estimation in

archaeological skeletons, which are based on skeletal morphology, genetic analyses, and the archaeological context.

Physical anthropology conducts morphologic assessment of skeletal features, mainly of the pelvic bones and the skull, although to some degree there are sexual dimorphism in any bone of the skeleton (e.g. in ribs, İşcan 1985), or uses metric analyses of distinct bone dimensions or indices. The pelvic bone differs between males and females for biological reasons: the female pelvic bone is adapted to giving birth (cf. Uhl 2018). The human birth process is among the most difficult ones known, due to a combination of the large neonatal brain and the upright, bipedal stature of the mother (cf. Aiello, Dean 2002). The development of methods on sex estimation from the pelvic bone dates back to the beginnings of anthropology. Already in the 19th century, most of the guidelines were known (cf. Turner 1886). The modern methods are summarised in countless compilations on physical anthropology (e.g. Ferembach *et al.* 1980, Dobisíková 1999, Grupe *et al.* 2015, or Rösing *et al.* 2007), and forensics (e.g. İşcan, Steyn 2013, Krishan *et al.* 2016). They are based on just as countless investigations by countless scholars, such as Acsádi and Nemeskéri (1970), Brooks and Suchey (1990), Brůžek (1991, 2002), Lovejoy *et al.* (1985), Novotný (1979, 1982, 1986) and many others. The strength of the sex estimation from the pelvis is based directly on the connection not only to general biology, but also especially to the functional aspects. The female pelvis is built to give birth, while the male pelvis is not, no matter from which time period or geographic area the investigated humans originate. Nevertheless, at the same time, this constitutes the main weakness of the approach. It is well known (see any of the aforementioned investigations and compilations) that pelvic morphology ranges along a continuous scale between hyper-feminine and hyper-masculine with a rather wide section of overlap. The same can be said about the other, second most valuable skeletal structure in concern of the sex estimation, the skull. Here too, the skull-based sex estimation methods used today are based on investigations dating to the beginnings of anthropology itself (e.g. Broca 1875) and can be found in the aforementioned standards of anthropological investigation methods. Among the scholars who contributed to the development of these methods, not all, not even all the most important ones can be named, but just to mention a few important works, e.g. Acsádi and Nemeskéri (1970), Borovanský (1936), Broca (1875), Howells (1973), and many others. A major difference to the sex estimation based on the pelvis is, however, the non-functional origin of the sexual dimorphism of the skull. This makes the sexual dimorphism of the skull a subject of ethnical or genetic variation, and, thus, makes it less unbiased compared to sexual dimorphism estimation from the pelvic bone. The physiology and biomechanics of human childbirth are always the same, regardless of the ethnicity or other attributes of the mother (cf. Kainer 2021). In case of the skull-based sex estimation, it is necessary to know the morphological variation of the investigated population (cf. Dobisíková 1999) and the estimation is more susceptible to wrong results.

Another major topic of morphological sex estimation is its application to subadult skeletons. Until the development of secondary sexual traits in the skeleton during puberty, there are only few differences, which are controversial. The method by Schutkowski (1993), one of the most commonly utilised ones, shows differing levels of reliability depending on the population (cf. Irurita Olivares and Alemán Aguilera 2016), making it too unreliable to use without a positive control, such as via DNA analysis. Other modern methods (e.g. Luna *et al.* 2020 and Monge Calleja *et al.* 2020) are promising, but still need verification on a large sample of skeletal remains with known sex or with an unassailable proof (DNA) of their validity on different populations.

Genetic investigations analyse the X- and Y-chromosomal sequences in the nuclear DNA of the individual, and, therefore, it is the only way of determining sex in subadult skeletons. However, it is limited by its invasive nature.

Since the invention of the PCR (polymerase chain reaction) by Saiki *et al.* (1985) and Mullis and Faloona (1987), the discovery of ancient DNA (aDNA) in the 1980s (e.g. Pääbo 1985), and the first application of genetic fingerprinting on skeletal material by Jeffreys *et al.* (1992), various methods to investigate the molecular genetic sex have been developed. Initial proof of biological sex was based on the analysis of specific fragments on the Y-chromosome (e.g. Hummel, Herrmann 1991) and repeat structures in the non-coding regions of Y-chromosomal DNA (Butler 2005). Subsequent studies focusing on the molecular sex determination investigated the length polymorphism between the X-chromosomal and the Y-chromosomal amelogenin gene (e.g. Sullivan *et al.* 1993, Stone *et al.* 1996, Lassen *et al.* 2000). However, this method does not allow for a positive sex identification of female

individuals, since the absence of the Y-allele in the amelogenin locus can either be a sign of two X-chromosomes (female) but can also be the result of an allelic dropout event of the Y-chromosome. The latter would subsequently lead to a false sex identification of male individuals. For this reason, it was pointed out shortly after the initial description that amplification of the amelogenin locus alone is not sufficient to perform reliable sex determination and that extensional or alternative methods ought to be developed (Mannucci *et al.* 1994, Santos *et al.* 1998, Brinkmann 2002). Nevertheless, the amelogenin gene is still used for molecular sex determination today in both, anthropological as well as forensic contexts and is included in several commercially available kits for genetic fingerprinting.

Over the years, different authors proposed to combine the amelogenin gene-based method with the investigation of Y-chromosomal short tandem repeats (Y-STRs) (e.g. Santos *et al.* 1998, Steinlechner *et al.* 2002). Some authors such as Honda *et al.* (2000) or Schmidt *et al.* (2003) went one step further. By combining the amelogenin marker with Y-STRs and X-chromosomal STRs (X-STRs), these authors attained a positive sex identification of both, male and female individuals.

The use of next generation sequencing, which not only complements PCR-based analyses but unquestionably greatly enriches them, especially with regard to evolutionary questions, also allows the sex of an individual to be read from the bioinformatically processed data (e.g. Skoglund *et al.* 2013, Mittnik *et al.* 2016). However, if the genetic analysis is aimed exclusively at sex determination, as is the case, for example, in regular anthropological findings for individuals from historic and prehistoric burial sites, the PCR-based approach is still the tool of choice due to its comparatively low complexity.

A new and completely alternative way of molecular sex determination is represented by an analysis strategy from the proteomics field (e.g. Rebay-Salisbury *et al.* 2020, Gasparini *et al.* 2022). A comparative study by Buonasera *et al.* (2020) on sex determination on historical skeletal material seems to suggest that this approach may prove particularly promising.

The archaeological estimation of sex (or rather gender) is usually based on grave goods or other indicators of burial rite, such as the shape or size of the grave pit, or the position of the body in the grave. It requires a knowledge of the culture of the buried population and, of course, the presence of diagnostic grave goods. However, even the most profound knowledge of the material culture cannot unambiguously exclude an exception to the rule. There is an extensive discussion on the role of biology and the biological sex in the lived and felt gender, and, today, the obvious necessity to distinguish between the biological sex and socially and culturally defined gender goes without saying (cf. e.g. Fausto-Sterling 2020) in modern contexts. However, gender archaeology still works on the question, to what degree these concepts are to be adapted in case of archaeological populations. From ethnological studies, a wide variety of cultural notions of gender is known, ranging from hardly any difference in perception of males and females, up to cultures hiding their pregnant women away, as the men are not allowed to know where the babies come from (cf. Corazza, Ropa 2018, Göttner-Abendroth 2022, Mückler 2009 and others). This is an extreme range with any other variations in-between, depending, among others, on religious beliefs, familial and social structures, cultural traditions, economic systems and other aspects (cf. e.g. Beer *et al.* 2017, Loimeier 2021). Most of these are usually either not known, or at least not completely clear in prehistoric populations and from archaeological context, they can be only partly reconstructed. This opens a wide variety of possible misinterpretations. An example: the most likely interpretation of a female grave with weapons would be that this woman did use the weapons herself, either to fight or otherwise, just as anybody else did. The interpretation of weapons as male grave attributes has been repeatedly proven "not as simple as that" (cf. Effros 2000, Gärtner 2017, Simniškytė 2007). But there are further possibilities and interpretations. An excellent example is an old, puzzling find of a morphologically female skeleton with a sword from Suontaka (Finland), who later turned out not to be a female after all, but a male with Klinefelter syndrome (cf. Moilanen *et al.* 2022). In a thorough discussion of this case, the authors bring up many aspects which should be considered in such interpretation, as we do not know the traditions and closer circumstances, beginning with the idea of the "non-binary" person being fully accepted by the society, up to cruel scoff towards a socially excluded male, for instance due to infertility. An expensive evil joke, to put a sword in his grave, surely, but do we really know? Hence, archaeological gender assessment allows us, to some degree, to address the aspect beyond the biological sex, namely the perception of the individual by itself and its society. However, the danger of

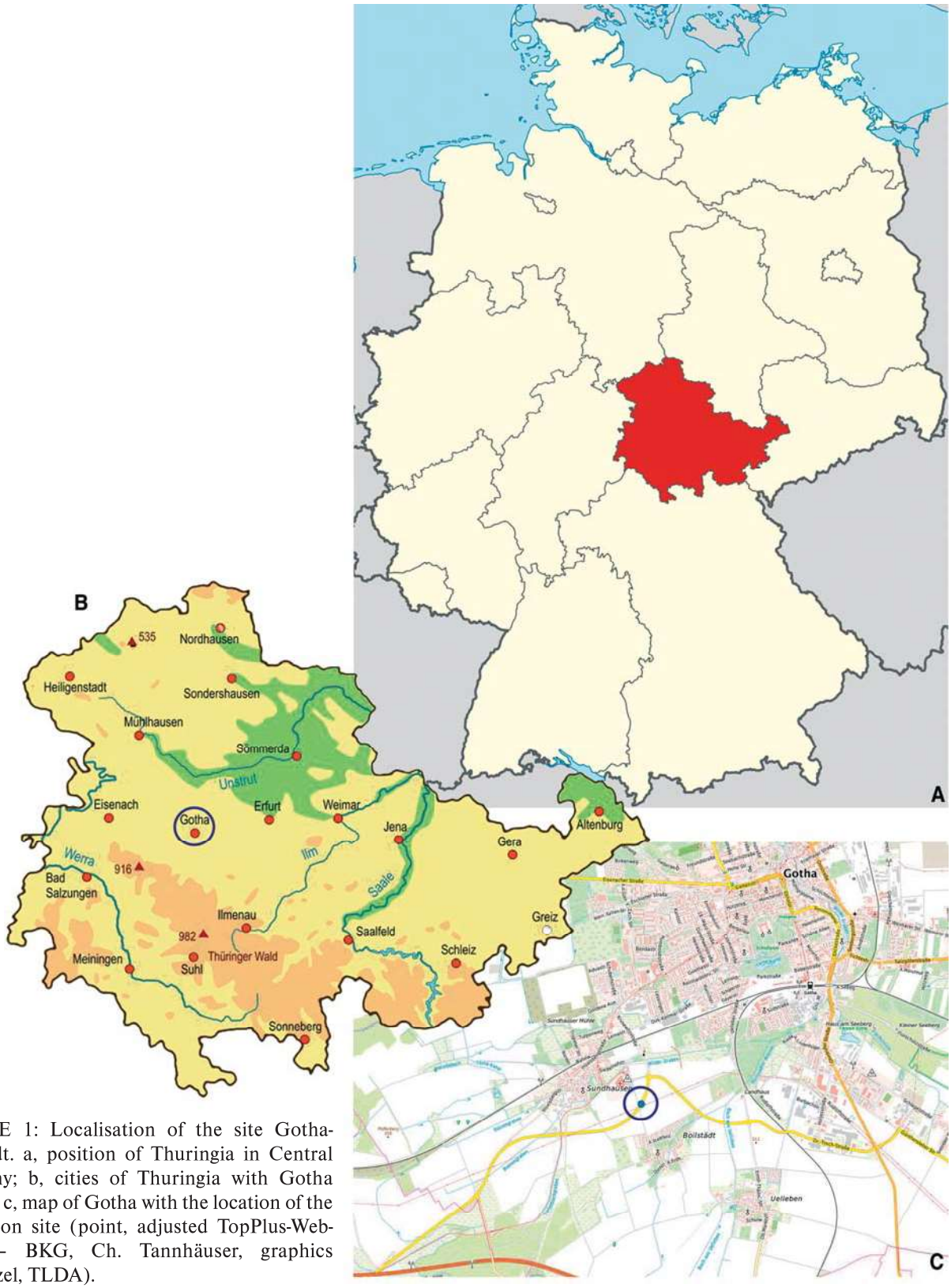


FIGURE 1: Localisation of the site Gotha-Boilstädt. a, position of Thuringia in Central Germany; b, cities of Thuringia with Gotha (circle); c, map of Gotha with the location of the excavation site (point, adjusted TopPlus-Web-Open – BKG, Ch. Tannhäuser, graphics H. Künzel, TLDA).

interpreting our own ideas and cultural imprint into another context is omnipresent and should not be underestimated.

MATERIALS AND METHODS

Prior to the construction of the Gotha-Sundhausen bypass between 2012 and 2013 a full archaeological survey of the construction site was conducted. On a small hillside, called Hammelhög, not far from the Gotha district of Boilstädt (*Figure 1*), a multi-period burial ground was discovered. The majority of the documented grave finds date to the late Merovingian period with an estimated two-thirds of the early medieval cemetery being recorded. The occupation begins with graves from the end of the 5th century, reaches its peak in the 6th and 7th century and ends in the 8th century. The remains of a total of 54 human individuals were documented in the 44 Merovingian burials. Four of the graves were horse burials. The cemetery was used by the inhabitants of a settlement that was likely located nearby. Although excavations along the planned bypass revealed a settlement site about one kilometre away, it only dates up to the 4th/5th century. Settlement features from the late Merovingian period have not been discovered yet in the area. These results make this site one of the large cemeteries of this period, which is of high importance for the history of Thuringia, as it became a part of the Frankish Empire at that time. For this reason, it has been decided to conduct a thorough, interdisciplinary investigation (Tannhäuser, in print). Among the burials were two remains from cremation burials (Feature 1, ind. 2 and Feature 76, ind. 3), which are not considered any further in this study, as these were only incomplete admixtures within other graves, without any sex-specific morphological elements, any grave goods and any aDNA preservation, making them irrelevant for the topic of the present study. The morphological age-at-death and sex estimation was carried out on all the individuals. The completeness of the remains and the preservation of the bone tissue varied, so that in the case of some burials, no skeletal remains were suitable for sampling for genetic analysis. From 48 of the 52 Merovingian individuals from Boilstädt samples could be included for ancient DNA (aDNA) extraction. The equipment of the graves was also variable, from richly furnished burials to burials completely bare of any grave goods, so that an archaeological evaluation of the sex was only possible for 20 individuals.

MORPHOLOGIC INVESTIGATION

The skeletal preservation was recorded according to the standards by Scheelen *et al.* (2015), considering three aspects of preservation: A = the completeness of the skeleton, B = the state of erosion of the surfaces, and C = the state and consistency of the bone tissue. Each category has six degrees, from the best preservation (degree 1) up to extreme incompleteness, surface erosion, and disintegration (degree 6).

For anthropological age-at-death, the recommended standard methods of macroscopic age estimation were used (see Ferembach *et al.* 1980, Grupe *et al.* 2015, İşcan, Steyn 2013, Rösing *et al.* 2007, Stloukal *et al.* 1999, Szilvássy 1988). Age-at-death estimation in subadult and juvenile individuals was based on the scheme of tooth eruption by Ubelaker (1989), the length measurement of long bones according to Stloukal, Hanáková (1978) and Johnston (1962), the measurements of the postcranial skeleton according to Florkowski, Kozlowski (1994), the tables of ossification centres according to Schwartz (1995) and the closure of growth plates according to Brothwell (1981). The evaluation of the development and ossification of the non-adult skeleton was carried out according to the recommendations of Scheuer, Black (2000). In adults, the age-at-death estimation based on the ossification of the palatal sutures was carried out using the methods according to Mann (Mann *et al.* 1987, Mann *et al.* 1991) with consideration of the suggestions in the test of this method according to Ginter (2005). Changes in the facies symphysialis ossis pubis were assessed according to Brooks, Suchey (1990), Nemeskéri *et al.* (1960) and Gilbert, McKern (1973). The morphology of the facies auricularis was assessed according to Lovejoy *et al.* (1985) and Buckberry, Chamberlain (2002). If the sternal rib ends were suitably preserved, the method according to İşcan *et al.* (1984, 1985) was used for age-at-death estimation. The estimated age-at-death data were grouped into standard anthropological age-at-death categories (cf. Herrmann *et al.* 1990) for the statistical analysis: Infans I (0–6.9 years), Infans II (7–13.9 years), Juvenis (14–19.9 years), Adultus (20–39.9 years), Maturus (40–59.9 years), and Senilis (from 60 years on).

For sex estimation of adult skeletons, macroscopic features of the skull and pelvis (if available) were predominantly used (Acsádi, Nemeskéri 1970, Ferembach *et al.* 1980, Phenice 1969, Rösing *et al.* 2007, Stloukal *et al.* 1999). In addition to the morphological assessment, long bone examination

according to Černý, Komenda (1980), length measurements of the os pubis and os ischii according to Herrmann *et al.* (1990), metric examination of the talus according to Steele (1976) and Novotný, Malinovský (1985) as well as metric examination of the femur according to Černý (1971) were used if the pelvis and/or the skull were not sufficiently preserved or informative. Depending on the degree of expression of diagnostic features (Ferembach *et al.* 1980), an individual was estimated to be "male or female" (M/F, more than ± 18 total score), "probably male or female" (M>F/F>M, between ± 11-17 total score), "rather male or female" (M≥F/F≥M, between ± 5–10 total score) or "unidentifiable" (M=F, less than ± 4 total score). Morphological sex estimation on subadult skeletons was carried out using the method based on the mandible and ilium according to Schutkowski (1993). As this assessment does not achieve as high a precision or accuracy, as the sex estimation in adults, the classification was reduced to "probably male or female" (M>F/F>M, both aspects consistent), "rather male or female" (M≥F/F≥M, one aspect male/female, other aspect indifferent) or "unidentifiable" (M=F, both aspects indifferent or contradictory). Unfortunately, there were no suitably preserved individuals <5 years age-at-death for the sex estimation on the facies auricularis ossis ilii using the methods of Luna *et al.* (2020) and Monge Calleja *et al.* (2020). In juveniles, as a "transition" of gradually developing secondary sexual dimorphism, both approaches (Schutkowski 1993 and Ferembach *et al.* 1980) were adapted. Sulcus praeauricularis was assessed according to Novotný (1979) and Brůžek (1991).

All these morphological investigations were conducted by one investigator (JN).

MOLECULAR INVESTIGATION

For the genetic sex determination of the individuals from Boilstädt, a Multiplex X/Y-PCR was used. In order to increase the probability of a positive detection for female individuals (heterozygosity in an X-STR system), the original Multiplex X/Y-PCR (Schmidt *et al.* 2003) was modified and extended. In the optimized form used in this study, three X-STRs as well as three Y-STRs are used along with the amelogenin marker. In addition to the advantage of positive detection of female individuals, the increase of STR-markers leads to an increase in authentication potential in terms of contamination monitoring. The increase in authentication potential is due to the fact that the optimized Multiplex X/Y-PCR is close to STR-based genetic fingerprinting (Butler 2005), i.e. the likelihood that two individuals reveal the same typing result is low. Additionally, all samples from Boilstädt as well as all staff and investigators were genetically fingerprinted with the Heptaplex-PCR (Seidenberg *et al.* 2012) consisting of autosomal STRs.

In the Multiplex X/Y-PCR, female individuals are not only determined by the absence of Y-chromosomal amplification products (amelogenin marker and Y-STRs), but in addition, due to the high heterozygosity of the examined X-STRs, it can be expected that at least one of the three amplified X-STRs shows two alleles demonstrating positively the presence of two X-chromosomes. In male individuals, one single amplification product for each of the X- and Y-STRs in addition to the X- and Y-allele in the amelogenin locus are present. Even in the case of an allelic dropout of the Y-chromosomally localized amelogenin, male individuals can be genetically distinguished from females by the presence of the Y-STRs. Therefore, the Multiplex X/Y-PCR is a powerful and reliable tool for molecular sex identification.

DNA Extraction

After sampling suitable skeletal material from each individual (cf. *Table 4*), possible contaminations were removed by a treatment with a sodium hypochlorite solution (Kemp, Smith 2005). The aDNA was extracted following the protocols described by Frischalowski *et al.* (2015) and Flux *et al.* (2017). Physical breakdown by grinding the skeletal material in a ball triturator (Retsch) was followed by a chemical lysis and digestion. Due to the large amount of humic acids present in the skeletal elements, the lysate was first purified using an organic extraction. Subsequently, the aDNA was extracted using a vacuum- and silica-based system. Multiple independent extracts were generated for each individual.

The detailed protocol for sample preparation and DNA extraction is as follows:

The sampling was performed using a hand drill (Dremel®Multi™) with a diamond saw blade (Horico). All samples were incubated for 10–15 minutes in 6 % sodium hypochlorite solution (Aug. Hedinger GmbH & Co. KG, Stuttgart, Germany) to remove possibly adhering contaminations from the bone surfaces, followed by rinsing with bi-distilled water. Afterwards, the samples were dried over night at 30 °C, then crushed roughly with a steel mortar and milled with

a ball triturator type MM 200 (Retsch) to a fine powder. The following lysis steps were performed under constant rotation of the samples. 250 mg of the powdered sample material was incubated with 3900 µL EDTA (0.5 M, pH 8.0, Invitrogen™) and 100 µL proteinase K solution in Tris/HCl (pH 7.5, 0.01 mol/L, 600 mAnson-U/mL, Merck) for 18 h at 56 °C. Afterwards, an additional lysis step was performed by adding 50 µL sodium dodecyl sulphate (10 mg/mL, Sigma-Aldrich®, incubation for 5 min at 65 °C) to the suspensions. The remaining solid substances were pelleted by centrifugation in a bench-top centrifuge (Type 5430R, Eppendorf) at 3300 rcf for 3 min. Approximately 4000 µL of the supernatant were rotated in approximately 3000 µL of phenol (Carl Roth GmbH + Co. KG) for 6 min at room temperature and afterwards incubated at 56 °C for 10 min for phase separation. The organic phases were removed, then approximately 4500 µL chloroform (Carl Roth GmbH + Co. KG) were added. A rotation was performed for 6 min at room temperature. For phase separation, the samples were incubated at 56 °C for 10 min. 3500 µL of the supernatants were transferred to a 17.5 mL Buffer PB (Qiagen). The subsequent DNA extraction and purification steps were performed with MinElute™ spin columns (Qiagen) via the QIAvac-System (Qiagen) following the manufacturer's protocol. Three washing steps using 700 µL Buffer PE (Qiagen) and a 5 min incubation time each were performed. The MinElute™ spin columns were dry-spun for 1 min at 13000 rpm in a top-bench centrifuge (Type 5415 R) and transferred to a 2 mL collection tube. Three elution steps with 20 µL ultrapure water (RNAse-Free Water, Qiagen) each (5 min incubation at 56 °C) including centrifugations for 1 min at 13000 rpm were performed to obtain a total elution volume of 60 µL. The DNA extracts were stored frozen. All

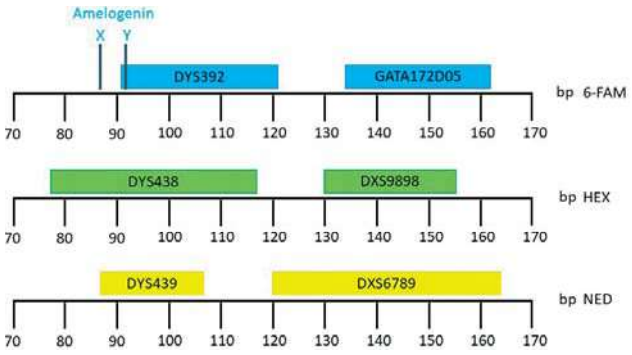


FIGURE 2: Schematic visualization of the allele range of the loci amplified in the Multiplex X/Y-PCR. The length of the coloured bars indicates the fragment length ranges the investigated systems can occupy. The only exception is the amelogenin locus, as only two specific products are generated: 86 bp for the X-chromosomal and 92 bp for the Y-chromosomal amelogenin allele. Furthermore, 6-FAM (blue), HEX (green) and NED (yellow) represent the fluorescent dyes linked to the respective primers of the different systems. (Graphics J. Mazanec).

TABLE 1: Primer-sequences of the Multiplex X/Y-PCR.

Primer	Primer-sequences (5´ to 3´)	Dye label	allele-range (bp)
Amelogenin, upper Amelogenin, lower	CCTGGGCTCTGTAAAGAATAGTG AGCTGATGGTAGGAACTGTAAAAT	6-FAM	X: 86 Y: 92
DYS392, upper DYS392, lower	CAAGAAGGAAAACAAATTTTTT GGATCATTAAACCTACCAATC	6-FAM	91 - 121
DYS438, upper DYS438, lower	GAATAGTTGAACGGTAAACAGTATATTT GAGTGAAACTCCATTTCAAATAGAA	HEX	77 - 117
DYS439, upper DYS439, lower	GGAGACAGATAGATGATAAATAGAAGAT ACCATCATCTCTTTACTTATACTTTCTATC	NED	87 - 107
DXS6789, upper DXS6789, lower	GTTGGTACTTAATAAACCCTCTTTT GGATCCCTAGAGGGACAGAA	NED	120 - 164
DXS9898, upper DXS9898, lower	CACACCTACAAAAGCTGAGATATA CATCCAGATAGACAGATCAATAGATT	HEX	130 - 155
GATA172D05, upper GATA172D05, lower	CAGGTGGTTAGTGGTGATGGT TCTGGGTTTATACCCCAAATAAT	6-FAM	134 - 162

chemicals and consumables were checked for possible contaminations by the preparation of extraction blanks and negative controls.

PCR Amplification for sex identification

The optimized multiplex analysis system applied for the here presented investigation consisted of the following genetic markers: Amelogenin, DYS392, DYS438, DYS439, DXS6789, DXS9898, and GATA172D05. The arrangement of the STR-Systems and the amelogenin marker, the fluorescence dye labeling for the fragment length analysis, and the expected allele-ranges for each marker in the Multiplex X/Y-PCR are displayed in *Figure* 2. The primer-sequences are shown in *Table 1*.

Amplification parameters

The amplifications were carried out in total volumes of 25 µL. To a mixture of 12.5 µL Qiagen Multiplex PCR Master Mix Plus, we added 2.4 µL primerset (including all upper and lower primers in specific concentrations as follows: 0.4 µM amelogenin, 0.2 µM DYS392, 0.2 µM DYS438, 0.1 µM DYS439, 0.12 µM DXS6789, 0.12 µM DXS9898, and 0.24 µM GATA172D05), and 0.5–10.1 µL DNA extract.

Cycling parameters

For the Multiplex X/Y-PCR a three-step PCR was conducted. After five minutes of initial activation the cycling was performed as follows: first, 10 cycling steps of 1 min denaturation at 94 °C, 1 min of annealing at 55 °C, and 1 min elongation at 72 °C followed by

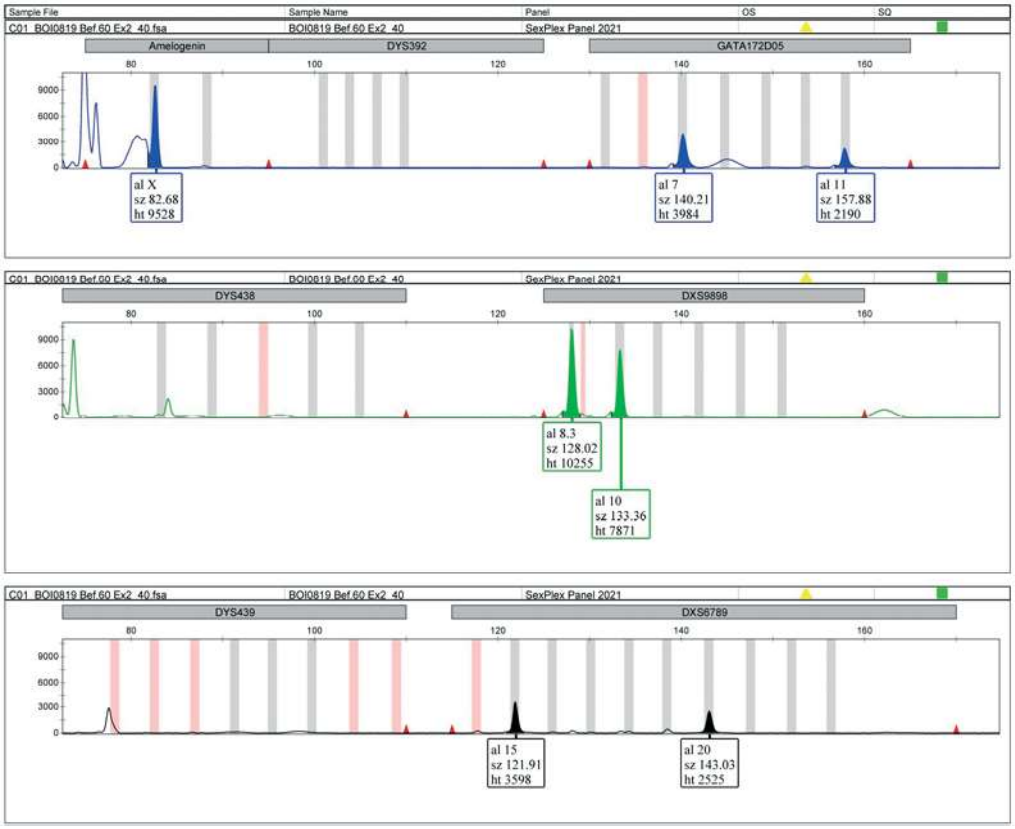


FIGURE 3: Shown is an example of the electropherogram of a female individual (feat. 60). The examined X- and Y-STR systems as well as amelogenin are displayed. The grey/red bars (bins) in every system represent possible allele expressions. Female individuals have two X-chromosomes and therefore show only one peak in the amelogenin locus. Due to a high heterozygosity rate of the DXS systems, two alleles are predominantly present. One allele may indicate homozygosity or DNA degradation-related allelic dropout. In the DYS systems, no signals are detected since no Y-chromosome is present in female individuals. (Graphics J. Mazanec).

second, 30 cycles with 1 min denaturation at 94 °C, 1 min annealing at 50 °C, and 1 min elongation at 72 °C. Afterwards, a final extension for 45 min at 60 °C and a soak for 10 min at 10 °C.

Agarose gel electrophoresis and allele determination

The amplification success was checked through agarose gel electrophoresis. The gel preparation and the setup of the electrophoresis parameters were conducted following laboratory standards for aDNA (Hummel 2003). The allele determination was performed through capillary electrophoresis in a 3500 Series Genetic Analyzer (Applied Biosystems™). For data acquisition and fragment length determination the 3500 Data Collection Software 3.1 as well as GeneMapper® Software 5 (Life Technologies) were used. Results of the fragment length analysis are displayed as electropherograms (*Figures 3* and *4*).

Archaeologic investigation

In the cemeteries of the Merovingian period, where the burials were usually well furnished, research traditionally attributed certain grave goods to sexes or genders (Trenkmann 2021, Paust 2014, Koch 1977, 1990, Paulsen 1967, Christlein 1966, Schmidt 1961).

Jewellery such as pearl necklaces, brooches and earrings as well as keys, belt hangings and spindle whorls are attributed to females' graves. Lighters, awls and shaving utensils, but above all weapons, are regarded as typical components of males' graves. At the same time, there are sex-unspecific grave goods such as clothing components – simple belt buckles, belt tongues, etc. – combs or foodstuffs.

The fact that there are sometimes discrepancies between this cultural gender determination on the basis of the grave inventories and the biological sex of the buried person has already been discussed several times in the past (Gärtner 2017). Therefore, in this paper, we

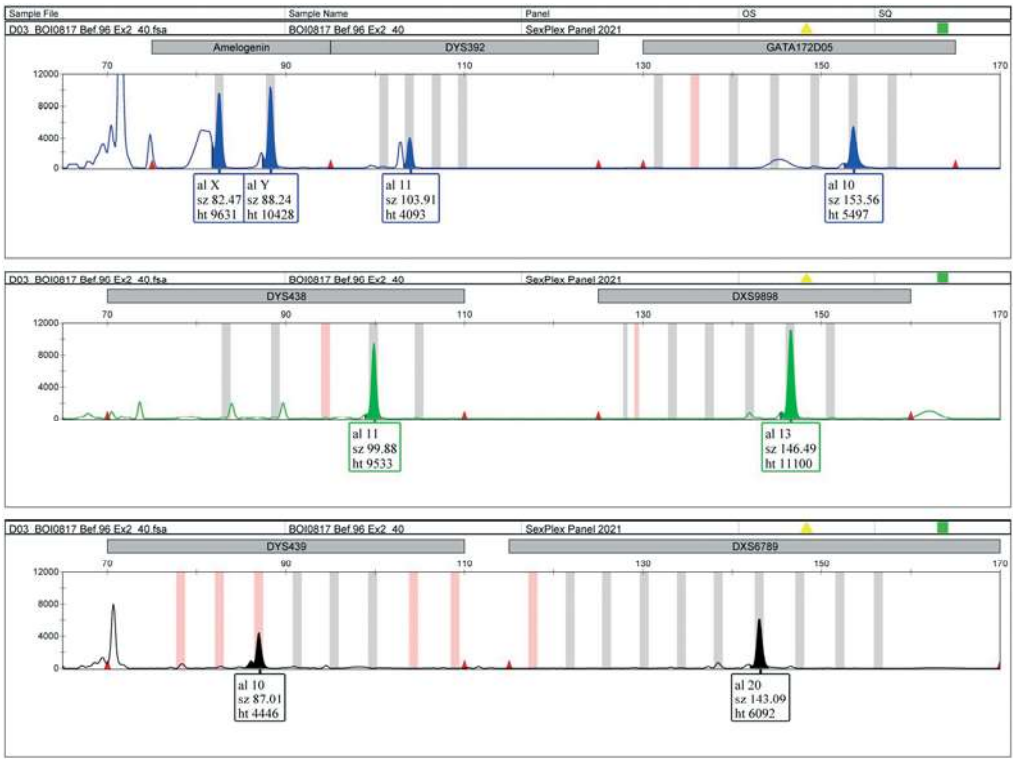
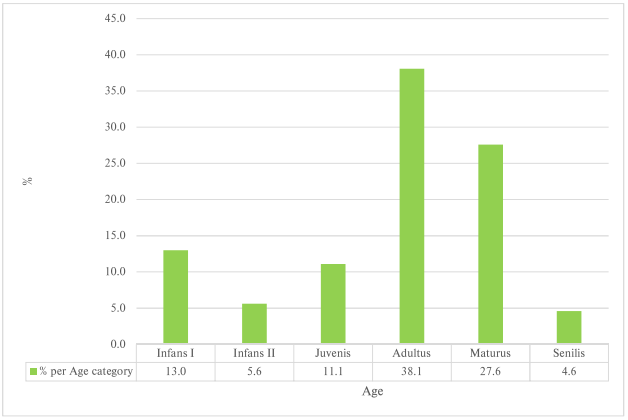


FIGURE 4: Shown is an example of the electropherogram of a male individual (feat. 96). Male individuals have one X- as well as one Y- chromosome and therefore have two peaks in the amelogenin locus. In both, the DXS as well as DYS systems, only one allele is amplified. In system DYS392, the peak located on the grey bar (bin) is an allele, whereas the peak one bp before it is a PCR artefact (split peak). (Graphics J. Mazanec).

TABLE 2: Summary of demographic data – Morphology. Columns M and F include the > (probably male/female) and ≥ (rather male/female) individuals.

Age category	M	F	M=F	Total
Infans I	0	0	7	7
Infans II	1	0	2	3
Juvenis	2.5	1	2.5	6
Adultus	6.4	9.7	2.5	18.6
Maturus	6.1	7.8	1	14.9
Senilis	0	2.5	0	2.5
Total	16	21	15	52



GRAPH 1: Age-at-death distribution in the whole population.

decided to utilise the term "gender" in case of the archaeological evaluation.

RESULTS

Morphologic sex estimation

The investigation of age-at-death and sex showed, individuals of both sexes and all age groups were buried at the cemetery of Boilstädt. There were 10 subadults, 6 juveniles, 18 or 19 adults, 14 or 15 mature and 2 or 3 senile individuals. A summarising presentation of the purely morphological age-at-death and sex estimations of all individuals can be found in table 2 and in graph 1. The proportional numbers of individuals in graph 1 can be explained by the fact that an individual whose age-at-death estimation falls into several age classes is calculated with a corresponding proportion in each of these, so that these proportions add up to 1. For example, a male individual that died at the age-at-death of 18–22 was calculated with 0.5 in each of the age classes Juvenis and Adultus. Another male individual, who died at the age-at-death of 25–40 (45) years, was calculated with 0.9 into the age class Adultus and with 0.1 into the age class Maturus, to express the higher weighting of the age class Adultus, but at the same time to account for the allocation to the first years of the age class Maturus,

FIGURE 5: weakly pronounced sulcus praeauricularis in individual from feat. 113 (morphologically, genetically, and archaeologically female). a, medial view with facies auricularis (photo H. Arnold TLDA); b, axial view from caudal of the incisura ischiadica maior. (Photo J. Nováček).

TABLE 3: Overview of the morphological results of sex estimation, birth trauma (according to description by Novotný 1979, 1986, cf. Brůžek 2002), and possible osteoarthritis in sacroiliac joint per individual. Age (years):(possibly at least) probable age-at-death interval (possibly up to); Sex estimation: M/F: male or female, M>F/F>M: probably male or female, M≥F/F≥M: rather male or female, M=F: unidentifiable, according to the description in methods; Pelvis and skull: ± values according to Ferembach *et al.* 1980 in adults, >/=: estimations according to Schutkowski 1993 in subadults; or both previous in juveniles; X: if not applicable due to poor preservation). Birth trauma: yes: clear sulcus, possible: weakly pronounced sulcus; Osteoarthritis with its laterality: R = right joint, L = left joint, B = bilateral.

No.	Feature/ Individual	Age (years)	Sex estimation	Pelvis	Skull	Birth trauma	Osteoarthritis
1	1/1	6–9	M=F	X	X		
2	2/1	(30) 35–45 (50)	F≥M	-6	+1		
3	2/2	(30) 40–50	M≥F	+5	X		
4	7	2–4	M=F	X	X		
5	60	40–50	F	-12	-41	yes	L
6	61/1	25–35	M	+6	+17		
7	61/2	15–17 (18)	M=F	F>M (-6)	M>F (+2)		
8	62	30–55	F≥M	X	-6		
9	63	30–50	M=F	X	X		
10	68	40–50	F>M	-12	X	possible	
11	70	25–35	F	-19	-36	possible	R
12	73/1	35–45 (50)	F	-14	-18	possible	
13	73/2	15–25	M=F	X	X		
14	76/1	50–60	F	-15	-42	yes	L
15	76/2	20–40	F≥M	-6	X		
16	77	20–25	F	-12	-42		
17	86	2–4	M=F	X	M=F		
18	87	30–40	F	-22	-43	yes	B
19	88	25–35 (40)	M	+25	+36		
20	89	16–18	M>F	M>F (+9)	M=F (-4)		
21	90	2–4	M=F	F>M	M>F		
22	91/1	18–22	M	+12	+29		
23	91/2	15–17	M>F	M>F (+16)	M>F (+16)		
24	92	(40) 45–55 (60)	F	-13	-28		
25	95	30–45	M	+9	+10		L
26	96	30–35 (40)	M	+15	+57		
27	97	60+	F≥M	Both skull and pelvis mostly missing, irregular, see discussion			
28	98	(40) 45–55	F	-20	-46	possible	
29	99	30–40	F	-29	-15	possible	
30	112	13–14	M≥F	M>F	M=F		
31	113	25–35	F	-23	-24	possible	
32	114	30–40	M>F	+12	X		
33	115	45–55	M	+28	+33		
34	116/1	25–35	F	-10	-25	possible shortly prior to death	
35	116/2	0–0.25	M=F	X	X		
36	117	9–12	M=F	M>F	F>M		
37	118	35–45 (50)	M	+21	+23		
38	120	40–50 (55)	F	-12	-32	possible	
39	122/1	40–50	M	+17	+6		L

TABLE 3: Continued.

No.	Feature/ Individual	Age (years)	Sex estimation	Pelvis	Skull	Birth trauma	Osteoarthritis
40	122/2	15–20	M=F	X	X		
41	123	0,5–1.5	M=F	X	X		
42	128	14–17	F>M	F>M (-5)	F>M (-23)		
43	129	30–50	M=F	X	X		
44	130	50–70	F	-10	-48	possible	
45	131	35–45	M	+12	+18		
46	133	20–40	M=F	X	X		
47	134	25–35 (40)	F	-32	-41	yes	
48	135	3–4	M=F	F>M	M>F		
49	139	2–3	M=F	F>M	M>F		
50	140	40–50 (55)	M	+26	+22		
51	150	60+	F	-11	-37	yes	L
52	152	45–55	M	+19	+15		L

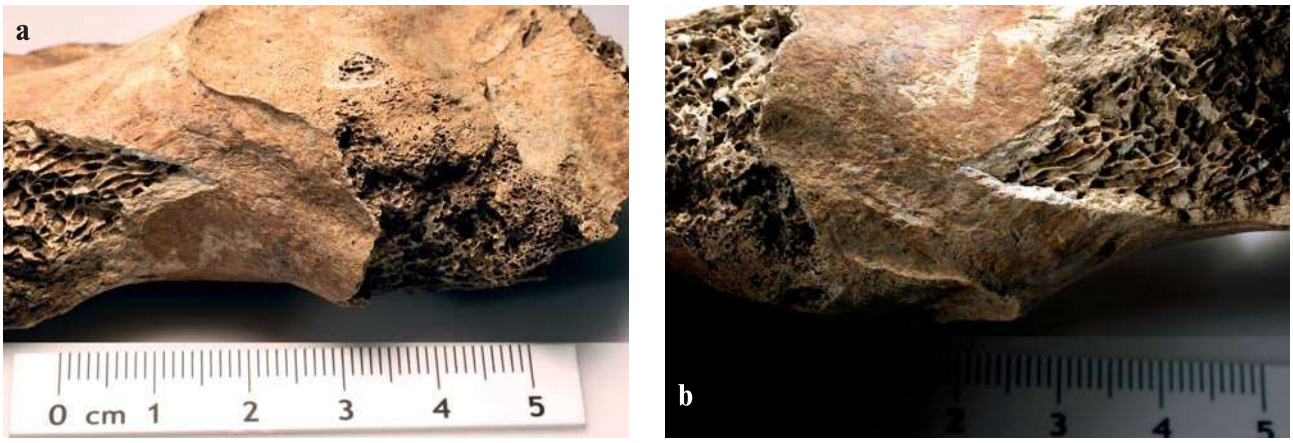


FIGURE 6: two views of a possible, weakly pronounced sulcus praeauricularis in individual 1 from feat. 116 (morphologically, genetically, and archaeologically female). a, view from dorso-caudo-medial, whitish-grey layer of new built bone formation indicates a partly re-modelled haemorrhagical process, which took place no longer that several weeks or some few months prior to death of the individual; b, axial view from caudal, rough surface indicates, the surface of the sulcus was not fully re-modelled. Possibly, the birth trauma happened just shortly before death, and the sulcus was not yet full-blown. (Photo J. Nováček).

which cannot be excluded. This procedure makes the statistics more precise; however, the division of some individuals into several age classes results in data with commas (e.g., "18.6 adult individuals").

Only one child skeleton was suitable for sex estimation: a 13–14 years old male from feature 112. Among the juveniles and adults, 15 individuals were estimated as males, 21 as females, and six were not possible to estimate. For full results of the morphological sex estimation on pelvis and skull, see table 3. In five individuals, clear sulci praeauriculares according to definition and morphological description by Novotný (1979, 1986, cf. Brůžek 1991, and based on personal communication and training by Novotný [1996–1999]) were detected. In another nine individuals, possible, rather weakly pronounced sulci could be found (*Figure 5*). One of those individuals also showed a newly built bone layer in and around the edges of the weakly pronounced sulcus (*Figure 6*). One individual showed weakly

FIGURE 7: Dorsal pitting on the dorsal edge of the right symphysis pubica in individual from feat. 134 (morphologically, genetically, and archaeologically female). (Photo J. Nováček).

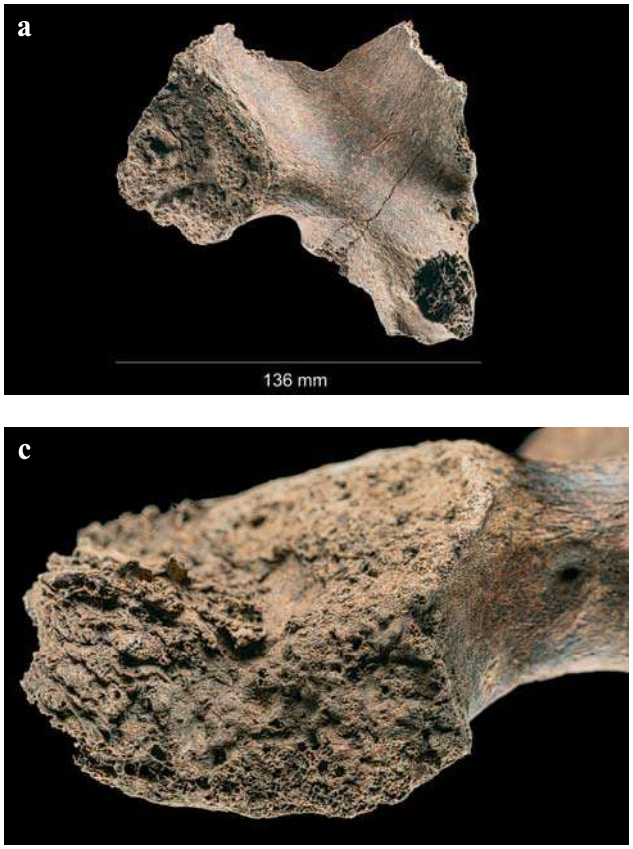


FIGURE 8: three views of a probable trauma of the left pelvis of the individual from feat. 152 (morphologically, genetically, and archaeologically male). a, overview of the pelvic bone; b, detail of the bulgy, fissured, porous, irregular facies auricularis; c, detail of the dorsal-most part of the facies auricularis, which is bent laterally about in 90° from the remaining joint and strongly compressed. (Photo H. Arnold TLDA).

TABLE 4: Individuals, sample materials, investigated X- and Y-chromosomal loci and biological sex. – no signal for the amplified marker due to DNA degradation, ( ) uncertain result due to DNA degradation, ♂ = male sex, ♀ = female sex, (♀) = tendency female, n. d. not determined, XXY = Klinefelter syndrome.

Individual	Sample material	Amelogenin	DYS392	DYS438	DYS439	GATA172D05	DXS9898	DXS6789	Sex determined by molecular analysis
1.1	teeth 26, 55	X/Y	11	10	11	(10)	8.3	(15)	♂
2.1	teeth 37, 43, 44	X/Y	11	10	11	5	8.3	23	♂
7	femur	-	-	-	-	-	-	-	n.d.
60	teeth 46, 47	X/X				7/11	8.3/10	15/20	♀
61.1	teeth 14, 24, 27	X/Y	11	10	11	9	8.3	23	♂
61.2	petrous bone	X/Y	(13)	(13)	13	7	8.3	20	♂
62	teeth 41, 43, 48	-	-	-	-	-	-	-	n.d.
63	femur	-	-	-	-	-	-	-	n.d.
68	tooth 11	-	-	-	-	-	-	-	n.d.
70	teeth 11, 14, 28	X/X				9/10	8.3/13	22/22	♀
73	lumbar vertebrae	X/X				9/-	12/-	15/21	♀
76	teeth 11, 33	X/X				(5)/9	12/14	20/22	♀
77	teeth 15, 26	X/-				7/(9)	12/-	20/-	(♀)
86	petrous bone	X/X				9/10	8.3/12	20/23	♀
87	teeth 35, 41, 46	X/X				5/9	8.3/12	21/22	♀
88	teeth 16, 44	X/Y	11	10	12	10	13	20	♂
89	teeth 15, 26, 43	X/Y	13	12	11	9	8.3/14	15/21	♂ XXY
90	petrous bone	X/Y	13	12	12	11	-	24	♂
91.1	teeth 25, 26	X/Y	(13)	12	11	(9)	(8.3)	(21)	♂
91.2	teeth 14, 28, 35	X/Y	13	12	11	9	(8.3)	21	♂
92	teeth 35, 47	X/X				8/11	8.3/-	20/24	♀
95	petrous bone	X/Y	13	12	12	9	12	21	♂
96	tooth 37	X/Y	11	11	10	10	13	20	♂
97	femur	-	-	-	-	-	-	-	n.d.
98	teeth 21, 26, 36	X/X				5/7	8.3/11	21/22	♀
99	teeth 44, 45, 48	X/X				7/7	11/14	20/23	♀
112	teeth 16, 34,36	X/X				(9)/10	8.3/11	20/-	♀
113	teeth 17, 28, 37	X/X				9/11	11/12	22/22	♀
114	thoracic vertabrae	X/X				7/9	12/13	21/22	♀
115	petrous bone	X/Y	13	12	13	5	13	23	♂
116.1	teeth 35, 43, 47	X/X				9/9	8.3/8.3	15/21	♀
116.2	petrous bone	X/X				7/9	8.3/12	21/21	♀
117	teeth 36, 46	X/X				5/8	8.3/-	21/-	♀
118	teeth 35, 36	X/Y	13	12	12	7	11	23	♂
120	teeth 24, 26	X/X				10/10	11/12	20/20	♀

TABLE 4: Continued.

Individual	Sample material	Amelogenin	DYS392	DYS438	DYS439	GATA172D05	DXS9898	DXS6789	Sex determined by molecular analysis
122.1	cervical vertabrae	X/Y	(13)	12	(11)	9	(11)	(15)	♂
122.2	metatarsal	X/Y	(12)	(10)	-	(11)	(13)	(22)	♂
123	petrous bone	X/Y	(13)	(12)	(12)	-	8.3	(15)	♂
128	teeth 17, 25, 26	-	-	-	-	-	-	-	n.d.
129	humerus	-	-	-	-	-	-	-	n.d.
130	tooth 43	X/X				9/11	8.3/12	19/23	♀
131	tooth 33	X/Y	13	12	12	9	12	20	♂
134	teeth 14, 38, 46	X/X				9/9	8.3/14	15/20	♀
135	petrous bone	X/X				7/10	8.3/12	20/20	♀
139	thoracic vertabrae	X/Y	12	10	11	8	8.3	21	♂
140	teeth 28, 36, 38	X/Y	11	10	11	5	11	21	♂
150	tooth 16	X/X				9/10	8.3/11	20/21	♀
152.2	teeth 34, 43, 48	X/Y	13	10	13	5	13	20	♂

pronounced pitting on the dorsal surface of the symphysis pubica (*Figure 7*). Traces of osteoarthritic changes of the facies auricularis on the pelvis and/or sacrum were found in three males (17.6%) and five females (23.8%), see table 3. One male individual (feat. 152) showed a folded, strongly fissured, bulgy and coarsely porous dorsal third of the left facies auricularis (*Figure 8*), the sacrum and the right half of the pelvis were unfortunately not preserved in this individual.

Molecular sex determination

In total, the sex of 41 of the 48 investigated individuals could be determined using the Multiplex X/Y-PCR *(Table 4 and cf. Table 6)*. The sex of only seven individuals could not be determined due to strong DNA degradation. Of the 41 sex-determined individuals, 21 were identified as female and 20 as male. Exemplary electropherograms of a female as well as a male individual are shown in *Figure 3 and Figure 4*, respectively.

Of particular interest is one of the 20 male burials (feature 89), which shows reproducible heterozygous allelic expressions in two of the three X-STRs, in addition to all Y-chromosomal sequences. Contamination by a female processor can be ruled out, suggesting the presence of Klinefelter syndrome (Mazanec 2022, Mazanec *et al.* in prep.).

Archaeologic gender evaluation

For the cemetery of Boilstädt, the sex/gender of the buried individuals was initially assessed based on grave goods. Since the burials were very variably equipped and only a few contained a gender-specific ensemble of grave goods, of the total 52 individuals only about one third – 13 women and 7 men – could be distinguished by gender based on the objects found in their graves (see table 5). As an example of a convolute of male grave goods, the burial from the feature 131 (*Figure 9*, a grave with full weapon equipment), and as an example of typically female, rather rich equipped burial from feature 113 (*Figure 10*) can be considered.

Sum-up of results of all methods

The full results of age-at-death as well as all three approaches of sex estimation or gender evaluation are presented in *Table 6*.

Table 7 and graph 2 show the summarised results of the morphological age determination and the combined evaluation of the morphological and metric sex determination with the aDNA examination.

TABLE 5: Archeologic sex (gender) estimation according to the deposited grave goods.

		jewellery			weapons						food and drink depositions						
feature	sex / gender	earrings	glass beads	spindle whorls	spatha	sax	spear	shield	arrowhead	riding equipment	animal bones	egg-shells	ceramic vessel	knives	combs	belt-buckles	miscellaneous
1														•		•	iron ring
2	male								•		•				•	•	
7																	
60	female		10, necklace	•							•			•		•	
61	male		1			•	•		•		•	•	•	•		•	fire steel
62	female		10, necklace	•											•	•	
63															•	•	
68	female		22, necklace											•		•	almandin fragments
70	female		57, necklace	•											•	•	Latène glass arm ring, iron stud, iron needle
73												•		•	•	•	iron ring
76																	
86																	
87																	
88																	
89																	
90																	
91																•	
92													•	•		•	
95														•		•	
96	male		13		•	•	•	•		•	•	•		•	•	•	bronze oil lamp, gold coin, glass gaming stone
97	male								•					•			
98														•			
99	female																bronze fingerring

TABLE 5: Continued.

		jewellery			weapons						food and drink depositions						
feature	sex / gender	earrings	glass beads	spindle whorls	spatha	sax	spear	shield	arrowhead	riding equipment	animal bones	egg-shells	ceramic vessel	knives	combs	belt-buckles	miscellaneous
112																	
113	female		1	•							•		•	•	•	•	
114											•	•			•	•	calve tie
115														•		•	
116	female		9, necklace	•													
117	female	•	19, necklace														iron bracelet
118														•			
120	female			•										•	•	•	
122											•	•				•	
123													•				
128														•			
129																	
130	female	•	12, necklace	•									•	•	•	•	bronze ring
131	male				•	•	•	•		•	•			•	•	•	shaving utensils, fire steel
133	female		9, necklace											•		•	
134	female	•		•										•		•	
135																	
139																	
140	male						•				•				•	•	bronze tweezers
150	female	•												•			
152	male						•				•				•		

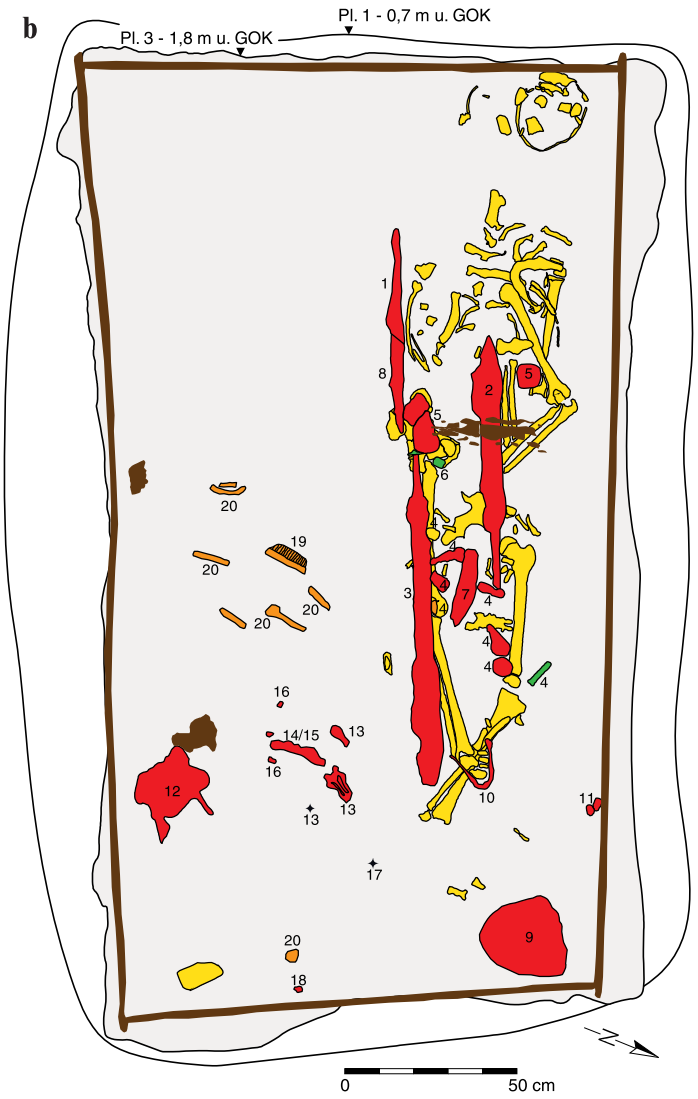


FIGURE 9: feat. 131 (morphologically, genetically, and archaeologically male) – burial with typical male gravegoods. a, photo after the finished uncovering of the block recovery in the restoration department of TLDA; b, 1 – iron lance tip; 2 – iron sax; 3 – iron spatha; 4 – weir hanger, eight objects belonging to the carrying strap of the spatha; button, buckles, strap end, fitting; 5 – waist belt, three objects belonging to the sax's sling; buckle, fittings; 7 – toilet cutlery, two corroded iron objects; knife, scissors; 8 – iron knife; 9 – iron shield boss and iron shield shackle as components of a round shield; 10 – iron spur, fragmented; 11 – two iron strap ends; 12 – six iron objects that are part of the bridle for a horse; toggle snaffle, buckle, strap end, rivets; 13 – a bag, three iron objects, belonging to the bag; buckle, iron fittings or strap ends; 14 – two iron tools, belonging to the contents of the bag; awl, a metal fragment; 15 – lighter, belonging to the contents of the bag; iron fire steel, firestone; 16 – two iron rivets, probably belonging to the bridle; 17 – two iron rivets and fragments of a tinplate, probably belonging to the bridle; 18 – iron rivet, probably belonging to the bridle; 19 – single row three-layer comb, bone/antler; 20 – animal bones; two fragments and a rib of a sheep-sized animal; 1 coxa, 2 femora of a juvenile pig – sus domesticus. (A: photo H. Arnold TLDA, b: graphics H. Künzel TLDA).

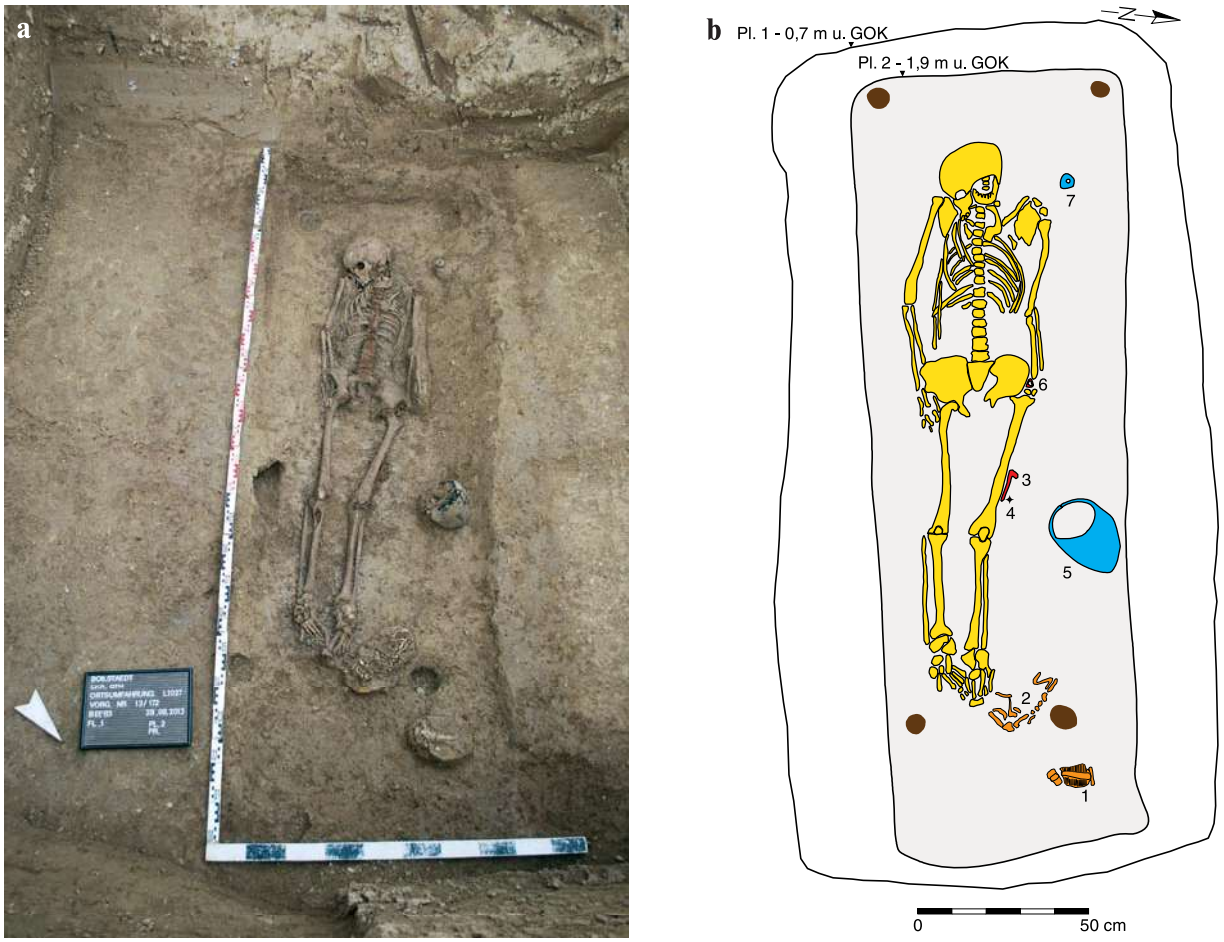


FIGURE 10: feat. 113 (morphologically, genetically, and archaeologically female) – burial with typical female gravegoods. a: photo in situ; b: 1 – double row three-layer comb, bone/antler; 2 – animal skeleton, cricetus cricetus; 3 – one knife and six rivets, corroded together, rivets probably belong to the knife sheath; 4 – cylindrical glass bead; 5 hand-made pottery; 6 – iron belt buckle; 7 – double conical spindle whorl made of clay. (A: photo A. Mayer TLDA, b: graphics H. Künzel TLDA).

The combination of the morphological and the genetic sex determination results in a total of 21 male and 26 female individuals of all age classes. In another 5 individuals, the sex could not be determined even with combined methods.

DISCUSSION

Comparison of the methods

As expected, most of the discrepancies between morphological sex estimation and genetic sex determination were found in the skeletons of children and adolescents. The only individual in which the sex could be assessed morphologically with the help of the methodology according to Schutkowski (1993) was an individual aged 13–14 years (feat. 112). The morphology indicated slightly male or indifferent characteristics on the pelvis and mandible (cf. Schutkowski 1993), so that the morphological evaluation was "rather male" (M≥F), but the genetic examination was able to determine that it was in fact a female. In none of the other individuals, regardless of infancy age category, could the sex be estimated morphologically. Except for three individuals, this circumstance was due to the incomplete preservation of the diagnostic skeletal elements; the remaining three did not show sufficient expression of sex-relevant characteristics to allow any estimation to be made. In view of this minimal sample of one false estimate and

TABLE 6: Overview of the results per individual. M male, F female, > probable M/F, ≥ rather M/F, M=F undeterminable, n.d. not determined due to DNA degradation, n.i. not investigated, – no assessable grave goods.

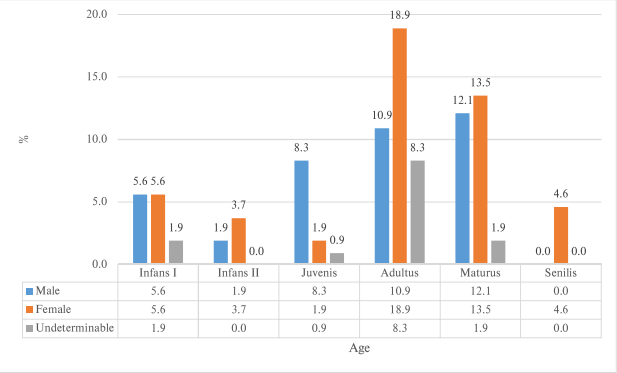
No.	Feature/Individual	Age (years)	Sex – Morphology	Sex – Molecular genetic analysis	Gender – Archaeology
1	1/1	6–9	M=F	M	–
2	2/1	(30) 35–45 (50)	F≥M	M	M?
3	2/2	(30) 40–50	M≥F	n.i.	–
4	7	2–4	M=F	n.d.	–
5	60	40–50	F	F	F
6	61/1	25–35	M	M	M
7	61/2	15–17 (18)	M=F	M	–
8	62	30–55	F≥M	n.d.	F
9	63	30–50	M=F	n.d.	–
10	68	40–50	F>M	n.d.	F
11	70	25–35	F	F	F
12	73/1	35–45 (50)	F	F	–
13	73/2	15–25	M=F	n.i.	–
14	76/1	50–60	F	F	–
15	76/2	20–40	F≥M	n.i.	–
16	77	20–25	F	F	–
17	86	2–4	M=F	F	–
18	87	30–40	F	F	F
19	88	25–35 (40)	M	M	–
20	89	16–18	M>F	M	–
21	90	2–4	M=F	M	–
22	91/1	18–22	M	M	–
23	91/2	15–17	M>F	M	–
24	92	(40) 45–55 (60)	F	F	–
25	95	30–45	M	M	–
26	96	30–35 (40)	M	M	M
27	97	60+	F≥M	n.d.	M
28	98	(40) 45–55	F	F	–
29	99	30–40	F	F	–
30	112	13–14	M≥F	F	–
31	113	25–35	F	F	F
32	114	30–40	M>F	F	–
33	115	45–55	M	M	–
34	116/1	25–35	F	F	F
35	116/2	0–0.25	M=F	F	–
36	117	9–12	M=F	F	F

TABLE 6: Continued.

No.	Feature/Individual	Age (years)	Sex – Morphology	Sex – Molecular genetic analysis	Gender – Archaeology
37	118	35–45 (50)	M	M	–
38	120	40–50 (55)	F	F	F
39	122/1	40–50	M	M	–
40	122/2	15–20	M=F	M	–
41	123	0.5–1.5	M=F	M	–
42	128	14–17	F>M	n.d.	–
43	129	30–50	M=F	n.d.	–
44	130	50–70	F	F	F
45	131	35–45	M	M	M
46	133	20–40	M=F	n.i.	F
47	134	25–35 (40)	F	F	F
48	135	3–4	M=F	F	–
49	139	2–3	M=F	M	–
50	140	40–50 (55)	M	M	M
51	150	60+	F	F	F
52	152	45–55	M	M	M

three impossible sex estimates, no reliable assessment can be postulated as to what extent the method according to Schutkowski (1993) is applicable to the individuals from Gotha-Boilstädt.

Even in juvenile individuals, the sex-relevant characteristics are often insufficiently or incompletely expressed, so that sex estimation must be carried out and evaluated with caution (cf. Dobisíková 1999). Thus, the higher proportion of indeterminable individuals by means of morphology, which could be specified with the help of the genetic examination, is not surprising. All juveniles and one late juvenile-early adult individual that were morphologically estimated as males were genetically confirmed as males. In addition, two further individuals, which could not be estimated morphologically, were determined as genetically male. Another individual, morphologically estimated as female (feat. 128), unfortunately did not



GRAPH 2: Age-at-death distribution by sex, combined results.

TABLE 7: Summary of demographic data – Morphology and DNA combined.

Age category	M	F	M=F	Total
Infans I	3	3	1	7
Infans II	1	2	0	3
Juvenis	4.5	1	0.5	6
Adultus	5.9	10.2	2.5	18.6
Maturus	6.6	7.3	1	14.9
Senilis	0	2.5	0	2.5
Total	21	26	5	52

provide sufficient DNA for the genetic examination, therefore this morphological estimation remains unverified. Only one late juvenile-early adult individual remained undeterminable. This individual showed a highly incomplete preservation without the possibility of morphological or genetic examination. Therefore, for subadult individuals, the genetic examination represents a decisive improvement of the overall results.

Among the adults, the morphological sex estimation could be confirmed by the genetic examination in most cases. For 16 adult females and 10 adult males, the results of the morphological and genetic sex determination agreed. In another three female and one male adult, the morphologically estimated sex could not be genetically verified due to insufficient preservation of DNA or the lack of suitable sampling possibilities. Only in the case of two individuals were there discrepancies between the morphological and genetic results. In the case of one individual (feat. 2, ind. 1), the sex was estimated morphologically as "rather female" (F≥M). Except for the mandible with indifferent sex characteristics (mentum rather male, ramus mandibulae/margo inferius rather female, angulus mandibulae indifferent, total value +1), the individual had no skull and only fragments of the pelvis were preserved, with only a few evaluable sex-relevant characteristics that tended towards the female direction (total value -6). The metric values of the skeleton also yielded rather indifferent to contradictory results (e.g. diameter of the caput femoris dx. was with 46 mm rather male, whereas the max. length of the femur dx. was only 430 mm). During the genetic examination, the very well-preserved DNA proved beyond doubt to be male in view of the presence of a Y-chromosome. The other individual (feat. 114) was morphologically estimated as "probably male" (M>F). The skeleton from the disturbed grave had no skull and the well-preserved pelvis showed predominantly male characteristics (total value +12), also the metric values rather indicated a male estimation (e.g. diameter of the caput femoris dx. 46 mm, max. length femur dx. 471 mm). The very well-preserved DNA provided an undoubted determination as female.

These results show that in atypical forms of human sexual dimorphism (smaller men with rather broad pelvises, large, robust women with narrow pelvises), morphological sex estimation reaches its limits and sometimes even provides misinterpretations. The specification or verification of the morphological examination through genetic examination is therefore sensible and advisable, especially in the case of incomplete skeletons and skeletons with ambiguous or contradictory sexual characteristics.

Regarding the archaeological gender evaluation, an estimate could be achieved for only 13 women and 7 men, as most graves did not contain any gender-specific goods, or had been robbed. This unsatisfactory result of the sex determination was considerably improved by the subsequent morphological and genetic examination of the skeletons. The comparison of the results was mostly consistent with the sex determination of the individuals. In 15 cases, 5 males and 10 females, the results of archaeology, morphology and genetics match. In two additional cases, the archaeology either matched the morphological estimation or the genetic determination, while the other method, respectively, was not possible to conduct (feat. 68 and 117). In one case (feat. 2, ind. 1), the archaeology, a male evaluation, fitted to the genetics, and supported this result, while the morphology delivered an apparently wrong result (see last paragraph). One individual (feat. 133, poorly preserved remains of skull vault, teeth crowns and few diaphyses) did not deliver any results in morphology or genetics, but the archaeological evaluation suggested a female. In the light of the overall results, this assumption will probably be correct. Nevertheless, it was not included into the overall interpretation of the biological results. Only one individual (feat. 97) delivered contradictory results. The poorly preserved bones of this individual were extremely small and gracile, so that they were even misclassified as subadult during the first preliminary examination. Preserved were only few non-diagnostic fragments of the skull and pelvis, as well as diaphyses of large long bones without any epiphyses. However, this was an adult: the 40× magnification of compact bone tissue revealed typical traits of adult bone tissue with beginning impact of age-induced osteoporosis, cf. Nováček 2012. It was an extremely gracile individual on the borderline of dwarfism, but with completely normal proportions. Due to secondary aspects, such as the morphology of the proximal femur, the morphological estimation tended towards a designation as "rather female" (F≥M), however, this is the case only under the premise that the reduced size of the bones did not have any further impact on their morphology, which is possible and likely to apply, but it is not verifiable. Meanwhile, the archaeological gender was evaluated as male, as the individual have had an arrowhead in its grave, which is considered among typical male grave goods. Unfortunately, due to its

preservation, the aDNA investigation did not deliver any result, and, therefore, this thrilling case will remain unsolved. The pearl necklace from the male grave (feat. 96), usually an attribute for female burials, was not located in the chest or neck area, the original wearing situation. It was found embedded in the conglomerate of a completely degraded organic substance on the right hip of the skeleton. Presumably, the man wore a pouch on his right hip in which, among other objects, the aforementioned glass bead necklace was stored. These objects undoubtedly had a special meaning for the individual. A comparable finding was documented in the cemetery of Schleitheim-Hebsack (Burzler *et al.* 2002: tab. 72). In the case of the morphologically male-like individual from feat. 114, which, however, turned out to be of female genetics (see last paragraph), unfortunately, the robbed, disturbed grave did not contain any gender-specific grave goods.

Sulcus praeauricularis and changes to facies auricularis as parameters in sex estimation

A sulcus praeauricularis could be determined in five individuals. In another nine individuals the expression of the possible sulcus praeauricularis was not sufficient to interpret it, beyond any doubt, as a sulcus according to the definition of Novotný (1979), but could only be classified as a "suspicion of" one. All these 14 individuals were genetically identified as female. It can thus be postulated that the presence of a sulcus praeauricularis, or even the suspicion of its presence, is to be interpreted as a convincing indication of female sex, without wanting to clarify the question of whether it is a feature of childbirth or not (cf. Brůžek *et al.* 2022). In this context, it should be noted that only one of the individuals with a sulcus praeauricularis, also exhibited traces of a possible parturition trauma on the dorsal margins of the symphysis pubica (pit formation, see *Figure 7*, cf. Houghton 1975, Stewart 1957). Even if it is a reliable feature of parturition trauma, its presence is many times less common than the presence of the sulcus praeauricularis. Thus, the interpretation of the sulcus praeauricularis in the definition according to Novotný (1979, 1986, cf. Brůžek 1991) as a clearly female feature proved to be reliable in the individuals from Gotha-Boilstädt.

Whether or not the sulcus praeauricularis is actually a consequence of childbirth (trauma) is not undisputed (cf. Cox 2000, Ubelaker, De La Paz 2012), as a similar structure has been demonstrated in nulliparous females (Perréard Lopreno *et al.* 2022), or even in male individuals (Maass, Friedling 2016). Hopefully, this question will be resolved by future studies (cf. Pany-Kucera *et al.* 2021). Furthermore, it should be noted that the presence of the sulcus praeauricularis is by no means indicative of the total number of pregnancies, even though recent studies suggest that the expression and severity of the sulcus increases with the number of births survived (Igarashi *et al.* 2020).

De facto, the sulcus praeauricularis is a particular ligamentopathy, possibly attributable to a specific process, birth trauma, an injury to the ligaments and the joint capsule of the female pelvis during childbirth with an associated haemorrhage into the joint capsule. Any other interpretation is hard to plausibly establish. Nevertheless, it cannot be ruled out that trauma other than parturition trauma could also trigger an injury to the connections of the sacroiliac joint. It should be noted, however, that the ligamentous apparatus that stabilises the sacroiliac joint would presumably prevent any injury that was not severe enough to not be accompanied by a fracture of the pelvis, sacrum and/or spine without the hormonally induced loosening effect in the run-up to childbirth (cf. Uhl 2018). Examples of such severe injuries would include falling off a horse at full gallop onto the rump, being run over by a cart over the back, or similar. However, such injuries would probably be detectable on the skeleton and the interpretation of a sulcus praeauricularis would have to be adjusted accordingly. In the absence of such features of further, severe trauma, this interpretation can be assumed as unlikely and the sulcus praeauricularis assumed to be the probable result of birth trauma. In the present case of a male individual with a clear, severe pelvic trauma (see *Figure 8*, feat. 152, probably compression fracture, such as due to falling off a horse), arthritic changes did form in the region of the sacroiliac joint, but none that could be interpreted as typically resulting from parturition trauma. The individual showed no trace of a sulcus praeauricularis.

The question of osteoarthritic changes to the sacroiliac joint should be taken into account to a certain extent in the morphological sex estimation. The sacroiliac joint forms a transition from the spine to the pelvic girdle, and thus bears the entire weight of the upper body and is often the cause of back complaints due to tension, blockages or wedging (cf. Grifka, Krämer 2013). The pathological changes, which can also occur prematurely due to stress, should be considered during age-at-death estimation based on the facies auricularis (cf. Buckberry, Chamberlain 2002, Lovejoy *et al.* 1985). The predominantly sedentary lifestyle of modern society favours the incorrect loading

of this joint and leads to the fact that the sacro-iliac complaints and changes have become much more frequent than appears to have been the case in past populations who carried out less sedentary activities (cf. Resnick, Niwayama 1981). Everything said so far applies equally to males and females, which was also confirmed in the present study. In total, only eight individuals (true prevalence 18.2 %) showed traces of arthritic changes to this region. Among them were three males (17.6 %) and five females (23.8 %), a non-significant difference due to the small sample size (Fisher's exact test 1.000). It should be noted, however, that all of these five females also showed signs of birth trauma, which makes this diagnostic aspect relevant to the question of possible birth trauma. This is in line with the observations of Pany-Kucera *et al.* (2019), who count a rim formation at the ventral edge of the facies auricularis (sacral preauricular extension or sacral preauricular notch, Pany-Kucera *et al.* 2019) among the birth-indicating features of the skeleton. Structures that could be defined as such an extension or notch were not observed in any males of the skeletal series.

CONCLUSIONS

For the 52 individuals of the Gotha-Boilstädt cemetery, sex estimation was carried out using three different approaches. The results of the two anthropological methods, i.e. morphological estimation and molecular genetic sex determination, were directly compared. In addition, the gender evaluation based on the archaeological classification of grave goods was available for about half of the individuals.

In principle, it must be considered that only molecular genetic analysis can determine the biological sex beyond doubt by analysing X- and Y-chromosomal sequences. The morphological sex indicators in adult skeletons ideally represent the biological sex, but due to the variability of characteristics, this does not necessarily always lead to unambiguous and accurate results. Only in populations with a high degree of sexual dimorphism, high congruence of the two anthropological approaches to sex estimation (determination) can be expected. This in turn depends on the chronology and biogeographical origin of the individuals to be assessed. If there is no clear sexual dimorphism, misidentification is possible or even to be expected. The same applies to the sex estimation of individuals in the prepubertal growth phases, i.e. children and adolescents. Only in the rarest cases is it possible to estimate the sex of subadult individuals via morphological methods. If osteometric methods are used, sex estimations can be achieved, but these are subject to inaccuracies and higher error rates.

Unlike the biological characteristics to be assessed anthropologically, the grave goods represent a social construct, which ultimately means that the archaeological evaluation leads to findings about the social sex of an individual (gender). If biological sex and gender are congruent, it should be possible to achieve high levels of agreement between anthropological, especially molecular genetic, and archaeological approaches with regard to sex determination.

All these considerations about the different available approaches to sex estimation (determination) are followed by the data obtained for the Gotha-Boilstädt skeletal series. Starting with the subadult individuals, it can be stated that sex estimation on children was morphologically impossible, on juveniles only in isolated cases. Molecular sex determination, on the other hand, was successful for almost all non-adults and is thus practically unavoidable if sex determination is desired in these age classes.

The situation is different in the adult age classes. Here, generally good agreements between morphological estimation and molecular sex determination have been achieved, albeit with exceptions. It is important to note here that the quality of the morphological estimation is particularly dependent on the experience of the anthropological expert as well as the completeness and the preservation of the skeletons. The latter in particular also has an impact on aDNA analysis, but if the bones and subsequently the DNA are poorly preserved, it is more likely to lead to a failure of the analysis than to an incorrect determination of the sex.

The comparison between the archaeologically assessed gender on the basis of grave goods and the sex determined by molecular genetics was particularly interesting, as there was a complete match between both approaches. The only questionable case of a possible contradiction occurred between an insecure morphologic estimation and the archaeologic evaluation. Unfortunately, this individual was too poorly preserved for a genetic determination. This means that there seems to be no evidence of gender identities deviating from the biological sex in the archaeologically investigated individuals from the Gotha-Boilstädt cemetery. This also highlights the benefit of combining these three methods for historical gender research.

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REFERENCES

ACSÁDI G., NEMESKÉRI J., 1970: *History of human life span and mortality*. Akadémiai Kiadó, Budapest.

AIELLO L., DEAN C., 2002: *An introduction to human evolutionary anatomy*. Elsevier Academic Press, London.

BEER B., FISCHER H., PAULI J., (Eds.) 2017: *Ethnologie. Einführung in die Erforschung kultureller Vielfalt*. Dietrich Reimer Verlag, Berlin.

BOROVANSKÝ L., 1936: *Pohlavní rozdíly na lebce člověka* [Sex differences on the human skull]. Česká akademie věd a umění, Prague.

BRINKMANN B., 2002: Is the amelogenin sex test valid? *Int. J. Leg. Med.* 116, 2: 63.

BROCA P. P., 1875: Instructions craniologiques et craniométriques. *Mém. Soc. Anthrop. Paris* 2: 1–103.

BROOKS S., SUCHEY J. M., 1990: Skeletal age determination based on the os pubis: A comparison of the Acsádi-Nemeskéri and Suchey-Brooks methods. *Hum. Evol.* 5, 3: 227–238.

BROTHWELL D. R., 1981: *Digging up bones. The excavation, treatment, and study of human skeletal remains*. 3rd ed. Ithaca, N. Y, Cornell University Press; British Museum, London.

BRŮŽEK J., 1991: *Fiabilité des procédés de détermination du sexe à partir de l'os coxal. Implications à l'Ètude du dimorphisme sexuel de l'Homme fossile*. Thèse de Doctorat, Museum National d'Histoire Naturelle, Institut de Paléontologie Humaine. Paris.

BRŮŽEK J., 2002: A Method for Visual Determination of Sex, Using the Human Hip Bone. *Am. J. Phys. Anthropol.* 117: 157–168.

BRŮŽEK J., SANTOS F., PERRÉARD LOPRENO G., 2022: Grooves in the preauricular area of the human ilium as indicator of sex or traces of parturition: historical background with a test of Novotný's method. *Anthr. Anz. - J. Biol. Clin. Anthropol.* 79, 2: 157–167. DOI: 10.1127/anthranz/2021/1397

BUCKBERRY J. L., CHAMBERLAIN A. T., 2002: Age estimation from the auricular surface of the ilium: A revised method. *Am. J. Phys. Anthropol.* 119, 3: 231–239.

BUONASERA T., EERKENS J., DE FLAMINGH A., ENGBRING L., YIP J., LI H., HAAS R., DIGIUSEPPE D., GRANT D., SALEMI M., NIJMEH C., ARELLANO M., LEVENTHAL A., PHINNEY B., BYRD B. F., MALHI R. S., PARKER G., 2020: A comparison of proteomic, genomic, and osteological methods of archaeological sex estimation. *Sci Rep.* 10: 11897. https://doi.org/10.1038/s41598-020-68550-w

BURZLER A., HÖNEISEN M., LEICHT J., RUCKSTUHL B., 2002: Das frühmittelalterliche Schleitheim – Siedlung, Gräberfeld und Kirche, *Schaffhausener Archäologie* Bd. 5. Schaffhausen.

BUTLER J.M., 2005: *Forensic DNA Typing: Biology, Technology, and Genetics of STR Markers*. Elsevier Academic Press, Burlington London.

CHRISTLEIN R., 1966: Das alamannische Gräberfeld von Marktoberdorf im Allgäu. *Materialhefte zur bayrischen Vorgeschichte* Heft 21. Kallmünz/Opf.

ČERNÝ M., 1971: Určování pohlaví podle postkraniálního skeletu. [Sex estimation by means of postcranial skeleton] In: E. Vlček (Ed.): *Sympozium o určování stáří a pohlaví jedince podle kostry*. Pp. 46–62. National museum Prague.

ČERNÝ M., KOMENDA S., 1980: Sexual Diagnosis by the Measurements of Humerus and Femur. *Sborník prací Ped. F. UP Olomouc - Biologie*: 147–167.

CORAZZA J., ROPA G., 2018: *Verborgene Völker: Die letzten Hüter alter Kulturen. Ein Bildband über die letzten ursprünglichen Völker der Erde und ihre faszinierenden Kulturen. Eine spannende Reise durch die Ethnologie*. Frederking & Thaler, Munich.

COX M., 2000: Assessment of parturition. In: M. Cox, S. Mays (Eds.): *Human Osteology in Archaeology and Forensic Science*. Pp. 131–144. Greenwich Medical Media, London.

DOBISÍKOVÁ M., 1999: Určování pohlaví [Sex estimation]. In: Stloukal M., Dobisíková M., Kuželka V., Stránská P., Velemínský P., Vyhnánek L., Zvára K. (Eds.): *Antropologie. Příručka pro studium kostry*. Pp. 168–234. National museum Prague.

EFFROS B., 2000: Skeletal Sex and Gender in Merovingian Mortuary Archaeology. *Antiquity* 74: 632–39.

FAUSTO-STERLING A., 2020: *Sexing the Body, Gender Politics and the Construction of Sexuality*. Updated edition. Basic Books, New York.

FEREMBACH D., SCHWIDETZKY I., STLOUKAL M., 1980: Recommendations for age and sex diagnosis of skeletons. *J. Hum. Evol.* 9: 517–549.

FLORKOWSKI A., KOZLOWSKI T., 1994: Ocena wieku szkieletowego dziecina podstawie weilkosci kosci. *Przeglad Antropologiczny* 57, 1–2: 71–86.

FLUX A. L., MAZANEC J., STROMMENGER B., HUMMEL S., 2017: Staphylococcus aureus Sequences from Osteomyelitic Specimens of a Pathological Bone Collection from Pre-Antibiotic Times. *Diversity* 9, 4: 43. https://doi.org/10.3390/d9040043

FRISCHALOWSKI M., SEIDENBERG V., GROSSKOPF B., WULF F. W., HUMMEL S., 2015: Molekulargenetische Untersuchung des Verwandtschaftsverhältnisses von möglichen Mutter-Kind-Bestattungen aus dem frühneuzeitlichen Eldagsen. *Nachrichten aus Niedersachsens Urgeschichte* 84: 193–206.

GÄRTNER T., 2017: Female warriors and 'cross-dressing' in early medieval Bavaria? Some critical comments – Kriegerinnen und "Cross-dressing" im frühmittelalterlichen Bayern? einige kritische Bemerkungen. *Archeologické rozhledy* 96: 109–120.

GASPARINI A., LUGLI F., SILVESTRINI S., PIETROBELLI A., MARCHETTA I., BENAZZI S., BELCASTRO M. G., 2022: Biological sex VS. Archaeological Gender: Enamel peptide analysis of the horsemen of the Early Middle age necropolises of Campochiaro (Molise, Italy). *J. Arch. Sci.: Reports* 41: 103337. https://doi.org/10.1016/j.jasrep.2021.103337

GILBERT B. M., MCKERN T. W., 1973: A Method for Aging the Female Os Pubis. *Am. J. Phys. Anthropol.* 38, 1: 31–38.

GINTER J. K., 2005: A test of the effectiveness of the revised maxillary suture obliteration method in estimating adult age at death. *J. For. Sci.* 50, 6: 1303–1309.

GÖTTNER-ABENDROTH H., 2022: *Matriarchale Gesellschaften der Gegenwart: Band II: Amerika, Indien, Afrika*. W. Kohlhammer GmbH, Stuttgart.

GRUPE G., HARBECK M., MCGLYNN G. C., 2015: *Prähistorische Anthropologie*. Springer, Berlin Heidelberg.

HERRMANN B., GRUPE G., HUMMEL S., PIEPENBRINK H., SCHUTKOWSKI H., 1990: *Prähistorische Anthropologie*. Leitfaden der Feld- und Labormethoden. Springer, Berlin, New York.

HONDA K., TUN Z., YOUNG D., TERAO T., 2000: Examination of Y-STR mutations in sex chromosomal abnormality in forensic cases. *For. Sci. Int.* 118, 2–3: 136–140.

HOUGHTON P., 1975: The bony imprint of pregnancy. *Bull. N.Y. Acad. Med.* 51, 5: 655–661.

HOWELLS W. W., 1973: Cranial variation in Man. A study by multivariate analysis of pattern of difference among recent human populations. *Papers of the Peabody Museum of Archaeology and Ethnology, Harvard Univ.* 67, Cambridge.

HUMMEL S., 2003: *Ancient DNA Typing- Methods, Strategies and Applications*. Springer-Verlag, Berlin Heidelberg.

HUMMEL S., HERRMANN B., 1991: Y-Chromosome-Specific DNA Amplified in Ancient Humane Bone. *Naturwissenschaften* 78: 266–267.

IGARASHI Y., SHIMIZU K., MIZUTAKA S., KAGAWA K., 2020: Pregnancy parturition scars in the preauricular area and the association with the total number of pregnancies and parturitions. *Am. J. Phys. Anthropol.* 171, 2: 260–274. DOI: 10.1002/ajpa.23961

IRURITA OLIVARES J., ALEMÁN AGUILERA I., 2016: Validation of the sex estimation method elaborated by Schutkowski in the Granada Osteological Collection of identified infant and young children: Analysis of the controversy between the different ways of analyzing and interpreting the results. *Int. J. Legal Med.* 130, 6: 1623–1632. DOI: 10.1007/s00414-016-1354-z

İŞCAN M. Y., 1985: Osteometric Analysis of Sexual Dimorphism in the Sternal End of the Rib. *J. For. Sci.* 30, 4: 1090–1099.

İŞCAN M. Y., LOTH S. R., WRIGHT R. K., 1984: Age estimation from the rib by phase analysis: white males. *J. For. Sci.* 29, 4: 1094–1104.

İŞCAN M. Y. LOTH S. R., WRIGHT R. K., 1985: Age estimation from the rib by phase analysis: white females. *J. For. Sci.* 30, 3: 853–863.

İŞCAN M. Y., STEYN M., 2013: *The Human Skeleton in Forensic Medicine*. 3. edition. Charles C. Thomas, Springfield, IL.

JEFFREYS A. J., ALLEN M. J., HAGELBERG E., SONNBERG A., 1992: Identification of the skeletal remains of Josef Mengele by DNA analysis. *For. Sci. Int.* 56, 1: 65–76.

JOHNSTON F. E., 1962: Growth of the long bones of infant and young children at Indian Knoll. *Hum. Biol.* 23: 66–81.

KAINER F., 2021: *Facharztwissen Geburtsmedizin*. 4. Auflage. Urban & Fischer Verlag/Elsevier GmbH, Munich.

KEMP B.M., SMITH D.G., 2005: Use of bleach to eliminate contaminating DNA from the surface of bones and teeth. *For. Sci. Int.* 154, 1: 53–61.

KOCH U., 1977: Das Reihengräberfeld bei Schretzheim. *Germanische Denkmäler der Völkerwanderungszeit* Serie A, Bd. XIII. Berlin.

KOCH U., 1990: Das fränkische Gräberfeld von Klepsau im Hohenlohekreis. *Forschungen und Berichte zur Vor- und Frühgeschichte in Baden-Würtemberg* Bd. 38. Stuttgart.

KRISHAN K., CHATTERJEE P. M., KANCHAN T., KAUR S.; BARYAH N., SINGH R. K., 2016: A review of sex estimation techniques during examination of skeletal remains in forensic anthropology casework. *For. Sci. Int.* 261, 4: 165.e1–165.e8. DOI: 10.1016/j.forsciint.2016.02.007

LASSEN C., HUMMEL S., HERRMANN B., 2000: Molecular sex identification of stillborn and neonate individuals ("Traufkinder") from the burial site Aegerten. *Anthr. Anz.* 58, 1–9.

LOIMEIER R., 2021: *Ethnologie: Biographie einer Kulturwissenschaft*. Dietrich Reimer Verlag, Berlin.

LOVEJOY C. O., MEINDL R. S., PRZYBEK T. R., 1985: Chronological Metamorphosis of the Auricular Surface of the Ilium: A new Method for the Determination of Adult Skeletal Age at Death. *Am. J. Phys. Anthropol.* 68, 1: 15–28.

LUNA L. H., ARANDA C. M., MONGE CALLEJA Á. M., SANTOS A. L., 2020: Test of the auricular surface sex estimation method in fetuses and non-adults under 5 years old from the Lisbon and Granada Reference Collections. *Int. J. Legal. Med.* 135, 3: 993–1003. https://doi.org/10.1007/s00414-020-02431-9

MAASS P., FRIEDLING L. J., 2016: Scars of Parturition? Influences Beyond Parity. *Int. J. Osteoarchaeol.* 26: 121–131. https://doi.org/10.1002/oa.2402

MANN R. W., SYMES S. A., BASS W. M., 1987: Maxillary suture obliteration: aging the human skeleton based on intact or fragmentary maxilla. *J. For. Sci.* 32, 1: 148–157.

MANN R. W., JANTZ R. L., BASS W. M., WILLEY, P. S., 1991: Maxillary suture obliteration: a visual method for estimating skeletal age. *J. For. Sci.* 36, 3: 781–791.

MANNUCCI A., SULLIVAN K. M., IVANOV P. L., GILL P., 1994: Forensic application of a rapid and quantitative DNA sex test by amplification of the X-Y homologous gene amelogenin. *Int. J. Leg. Med.* 106, 4: 190–193.

MAZANEC J., 2022: Identifikation – Von der Speziesbestimmung bis zur Identitätsfeststellung mit Hilfe von *ancient* DNA-Analysen. *Diss. Univ. Göttingen*.

MAZANEC J., FLUX A. L., HUMMEL S., in print: Molekulargenetische Untersuchungen der Bestattungen des merowingerzeitlichen Gräberfeldes von Gotha-Boilstädt. In: C. Tannhäuser (ed.): Das merowingerzeitliche Gräberfeld

von Gotha-Boilstädt. Das Frühmittelalter in Thüringen 1. *Weimarer Monographie zur Ur- und Frühgeschichte* 46, Weimar.

MAZANEC J., NOVÁČEK J., HUMMEL S., in prep: Proof of the Klinefelter Syndrome in a Merovingian burial (working title).

MITTNIK A., WANG C. C., SVOBODA J., KRAUSE J., 2016: A Molecular Approach to the Sexing of the Triple Burial at the Upper Paleolithic Site of Dolní Věstonice. *PLoS ONE* 11,10: e0163019. https://doi.org/10.1371/journal.pone.0163019

MOILANEN U., KIRKINEN T., SAARI N.-J., ROHRLACH A. B., KRAUSE J., ONKAMO P., SALMELA E., 2022: A Woman with a Sword? – Weapon Grave at Suontaka Vesitorninmäki, Finland. *Europ. J. Archaeol.* 25, 1: 42–60. DOI: https://doi.org/10.1017/eaa.2021.30

MONGE CALLEJA, Á. M., ARANDA, C. M., SANTOS, A. L., LUNA, L. H., 2020: Evaluation of the auricular surface method for non-adult sex estimation on the Lisbon documented collection. *Am. J. Phys. Anthropol.* 172, 3: 500–510. DOI: 10.1002/ajpa.24012

MULLIS K. B., FALOONA F. A., 1987: Specific synthesis of DNA in vitro via a polymerase-catalyzed chain reaction. *Methods in enzymology 155*: 335–350.

MÜCKLER H., 2009: *Einführung in die Ethnologie Ozeaniens: Kulturgeschichte Ozeaniens, Band 1: Kulturgeschichte Ozeaniens 1.* facultas.wuv Universitätsverlag, Vienna.

NEMESKÉRI J., HARSANYI L., ACSÁDI G., 1960: Methoden zur Diagnose des Lebensalters von Skelettfunden. *Anth. Anz.* 24: 70–95.

NOVÁČEK J., 2012: Möglichkeiten und Grenzen der mikroskopischen Leichenbranduntersuchung. Diss. University of Hildesheim, published online.

NOVÁČEK J., in print: Anthropologische Untersuchung der Bestattungen des merowingerzeitlichen Gräberfeldes von Gotha-Boilstädt. In: C. Tannhäuser (Ed.): Das merowingerzeitliche Gräberfeld von Gotha-Boilstädt. Das Frühmittelalter in Thüringen 1. *Weimarer Monographie zur Ur- und Frühgeschichte* 46, Weimar.

NOVOTNÝ V., 1979: Nové hodnocení sulcus praeauricularis jako nejhodnotnějšího morfoskopického znaku pánevní kosti k rozlišení pohlaví [New assessment of the sulcus praeauricularis as the most valuable morphological trait for sex estimation on pelvis]. *Scripta Medica Brno* 52: 500–502.

NOVOTNÝ V., 1982: *Pohlavní rozdíly a identifikace pánevní kosti* [Sex differences and identification oft he pelvic bone]. Diss. University Brno.

NOVOTNÝ V., 1986: Sex Determination of the Pelvis Bone: A Systems Approach. *Anthropologie* (Brno) 24, 2–3: 197–206.

NOVOTNÝ V., MALINOVSKÝ L., jr. 1985: Determination of sex from the talus and calcaneus. *Scripta Medica Brno* 58: 437.

PÄÄBO S., 1985: Molecular cloning of Ancient Egyptian mummy DNA. *Nature* 314, 6012: 644–645.

PANY KUCERA D., SPANNAGL STEINER M., ARGENY S., MAURER GESEK B., WENINGER W. J., REBAY-SALISBURY K., 2019: Sacral preauricular extensions, notches, and corresponding iliac changes: New terms and the proposal of a recording system. *Int. J. Osteoarchaeol.* 29: 1013–1021. https://doi.org/10.1002/oa.2814

PANY KUCERA D., SPANNAGL STEINER M., ARGENY S., MAURER GESEK B., WENINGER W. J., REBAY-SALISBURY K., 2021: Sacral preauricular extensions and notches as parts of a 'Pelvic Pattern' may provide information on past pregnancies and parturitions. *Anthr. Anz. – J. Biol. Clin. Anthropol.* 79, 2: 183–198. DOI: 10.1127/anthranz/2021/1455

PAULSEN P., 1967: Alamannische Adelsgräber von Niederstotzingen, Kreis Heidenheim. *Veröffentlichungen des staatlichen Amtes für Denkmalpflege Stuttgart*. Reihe A, H. 12. Stuttgart.

PAUST E., 2014: Untersuchungen zu den Bestattungssitten in der Zeit des Thüringer Königreiches. *Diss. Univ. Jena*. < http://nbn-resolving.de/urn:nbn:de:gbv:27-dbt-20161130-1443305

PERRÉARD LOPRENO G., SANTOS F., BRŮŽEK J., 2022: Is the preauricular groove, as defined by Bruzek (2002), an indicator of parity? A test using the SIMON identified skeletal collection (Vaud, Switzerland). *Anthr. Anz. – J. Biol. Clin. Anthropol.* 79, 2: 169–182. DOI: 10.1127/anthranz/2021/1400

PHENICE T. W., 1969: A Newly Developed Visual Method Of Sexing The Os Pubis. *Am. J. Phys. Anthropol.* 30: 297–302.

REBAY-SALISBURY K., JANKER L., PANY-KUCERA D., SCHUSTER D., SPANNAGL-STEINER M., WALTENBERGER L., SALISBURY R., B., KANZ F., 2020: Child murder in the Early Bronze Age: proteomic sex identification of a cold case from Schleinbach, Austria. *Archaeol. Anthropol. Sci.* 12, 11: 265. DOI: 10.1007/s12520-020-01199-8

RÖSING F. W., GRAW M., MARRÉ B., RITZ-TIMME S., ROTHSCHILD M. A., RÖTZSCHER K., SCHMELING A., SCHRÖDER I., GESERICK G., 2007: Recommendations for the forensic diagnosis of sex and age from skeletons. *HOMO – J. Compar. Hum. Biol.* 58, 1: 75–89.

SAIKI R. K., SCHARF S., FALOONA F., MULLIS K. B., HORN G. T., ERLICH H. A., ARNHEIM N., 1985: Enzymatic amplification of beta-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. *Science (New York, N. Y.)* 230, 4732: 1350–1354.

SANTOS F. R., PANDYA A., TYLER-SMITH C., 1998: Reliability of DNA-based sex tests. *Nature genetics* 18, 2: 103.

SCHEELEN K., NOVÁČEK J., SCHULTZ M., 2015: Anthropologische und paläopathologische Untersuchung menschlicher Skeletüberreste aus dem Friedhof um die Türbe. In: S. Ladstätter (Ed.): *Die Türbe im Artemision. Ein frühosmanischer Grabbau in Ayasuluk/Selçuk und sein kulturhistorisches Umfeld*. Sonderschriften des Österreichischen Archäologischen Instituts 53, ÖAI, Wien, 377–487.

SCHEUER L., BLACK S., 2000: *Developmental Juvenile Osteology*. Academic Press, San Diego.

SCHMIDT B., 1961: Die späte Völkerwanderungszeit in Mitteldeutschland. *Veröffentlichungen des Landesmuseums für Vorgeschichte in Halle* 18. Halle a. d. Saale.

SCHMIDT D., 2004: Entwicklung neuer Markersysteme für die ancient DNA-Analyse Erweiterung des molekulargenetischen

Zugangs zu kultur- und sozialgeschichtlichen Fragestellungen der Prähistorischen Anthropologie. *Diss. Univ. Göttingen*.

SCHMIDT D., HUMMEL S., HERRMANN B., 2003: Brief communication: multiplex X/Y-PCR improves sex identification in aDNA analysis. *Am. J. Phys. Anthropol.* 121, 4: 337–341.

SCHUTKOWSKI H., 1993: Sex Determination of Infant and Juvenile Skeletons: I. Morphognostic Features. *Am. J. Phys. Anthropol.* 90: 199–205.

SCHWARTZ J. H., 1995: *Skeleton keys: an introduction to human skeletal morphology, development, and analysis*. Oxford University Press, Oxford, New York.

SEIDENBERG V., SCHILZ F., PFISTER D., GEORGES L., FEHREN-SCHMITZ L., HUMMEL S., 2012: A new miniSTR heptaplex system for genetic fingerprinting of ancient DNA from archaeological human bone. *J. Arch. Sci.* 39, 10: 3224–3229. https://doi.org/10.1016/j.jas.2012.05.019

SIMNIŠKYTĖ A., 2007: Weapons in Iron Age Women's Graves. In: A. Bliujienė (Ed.): Weapons, Weaponry, and Man: In Memoriam Vytautas Kazakevicius. *Archaeologia Baltica* 8. pp. 283–91. Klaipėda University Press, Klaipėda.

SJØVOLD T., 1988: Geschlechtsdiagnose am Skelett. In: R. Knussmann (Ed.): *Anthropologie: Handbuch der vergleichenden Biologie des Menschen* I.1. Wesen und Methoden der Anthropologie. Pp. 444–478. Gustav Fischer, Stuttgart.

SKOGLUND P., STORÅ J., GÖTHERSTÖM A., JAKOBSSON M., 2013: Accurate sex identification of ancient human remains using DNA shotgun sequencing. *J. Arch. Sci.* 40: 4477–4482. https://doi.org/10.1016/j.jas.2013.07.004

STEELE D. G., 1976: The Estimation of Sex on the Basis of Talus and Calcaneus. *Am. J. Phys. Anthropol.* 45: 581–588.

STEINLECHNER M., BERGER B., SCHEITHAUER R., PARSON W., 2001: Population genetics of ten STR loci (AmpFlSTR SGM plus) in Austria. *Int. J. Leg. Med.* 114, 4–5: 288–290.

STEWART T. D., 1957: Distortion of the pubic symphyseal face in females and its effect on age determination. *Am. J. Phys. Anthropol.* 15: 9–18.

STLOUKAL M., HANÁKOVÁ H., 1978: Die Länge der Längsknochen altslawischer Bevölkerungen unter besonderer Berücksichtigung von Wachstumsfragen. *Homo* 29, 1: 53–69.

STLOUKAL M., DOBISÍKOVÁ M., KUŽELKA V., STRÁNSKÁ P., VELEMÍNSKÝ P., VYHNÁNEK L., ZVÁRA K., 1999: *Antropologie. Příručka pro studium kostry*. National museum Prague.

STONE A.C., MILNER G.R., PÄÄBO S., STONEKING M., 1996: Sex determination of ancient human skeletons using DNA. *Am. J. Phys. Anthropol.* 99, 2: 231–238.

SULLIVAN K. M., MANNUCCI A., KIMPTON C. P., GILL P., 1993: A rapid and quantitative DNA sex test: fluorescence-based PCR analysis of X-Y homologous gene amelogenin. *BioTechniques* 15, 4: 636–641.

SZILVÁSSY J., 1988: Altersdiagnose am Skelett. In: R. Knussmann (Ed.): *Anthropologie: Handbuch der vergleichenden Biologie des Menschen* I.1. Wesen und Methoden der Anthropologie. Pp. 421–443. Gustav Fischer, Stuttgart.

TANNHÄUSER C., ed., in print: Das merowingerzeitliche Gräberfeld von Gotha-Boilstädt. Das Frühmittelalter in Thüringen 1. mit Beiträgen von David Bitter, Anna Lena Flux, Gisela Gruppe, Susanne Hummel, Marcus Jung, Janine Mazanec, Oliver Mecking, Roland Müller, Jan Nováček, Astrid Pasch, Kerstin Pasda, Tim Schüler, Sebastian Steinbach, Maria Tomuschat und Grit Zimmermann. *Weimarer Monographie zur Ur- und Frühgeschichte* 46, Weimar.

TRENKMANN U., 2021: Thüringen im Merowingerreich. Zur chronologischen und kulturgeschichtlichen Aussagekraft von Gräberfeldern des 6.–8. Jahrhunderts. *Bonner Beiträge zur vor- und frühgeschichtlichen Archäologie* Bd. 25. Bonn.

TURNER W., 1886: The index of the pelvic brim as a basis of classification. J. Anat. and Physiol. 20: 125–143.

UBELAKER D. A., 1989: *Human skeletal remains. Excavation, analysis, interpretation*. 2. edition. Taraxacum, Washington.

UBELAKER D. H., DE LA PAZ J. S., 2012: Skeletal indicators of pregnancy and parturition: A historical review. *J. For. Sci.* 57, 4: 866–872. DOI: 10.1111/j.1556-4029.2012.02102.x

UHL B., 2018: *Gynäkologie und Geburtshilfe compact. Alles für Station, Praxis und Facharztprüfung*. Thieme Verlag, Stuttgart.

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