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CURRENT TRENDS IN METHODS FOR ESTIMATING AGE AND SEX FROM THE ADULT HUMAN SKELETON

ABSTRACT: Age-at-death and sex estimation are considered among the essential parameters in osteobiography and represent the first stage of study in bioarchaeology, forensic anthropology and paleoanthropology. This review discusses the accuracy, reliability, and reproducibility of various methodological approaches in estimating important biological profile parameters based on adult skeletal remains. It emphasizes in various aspects the contribution of Vladimír Novotný for the development of current methods for studying the skeleton. The need for accurate and reliable morphological and metric methods is still necessary in the skeletal identification process despite the use of a molecular approach. The results of morphological, metric and other methods complement each other and are equivalent and unbiased if used correctly. The more frequent use of modern imaging techniques and geometric morphometric methods in anthropological research has facilitated the acquisition and revision of available population data. They also allow the development of robust methods applicable to individuals with unknown population affinity. Estimating age after death in adults is still a very difficult part of estimating the biological profile. Current age estimation methods can only estimate age accurately and reliably over very wide age intervals. The fact that these approaches are mostly based on a single skeletal indicator assessed visually or use an inappropriate statistical approach, does not contribute to improving this situation. For estimating sex, the DSP (Diagnose Sexuelle Probabiliste) method is becoming a valid method and widely used technique for estimating sex in terms of accuracy and reliability. Other methods using software programs facilitate the work of biological anthropologists in various areas of research and practice. This article evaluates the current development of age and sex estimation methods and considers possible future directions.

KEY WORDS: Age-at-death estimation - Sex estimation - Biological profile - Morphoscopic - Morphometric - Methods - Accuracy - Reliability - Forensic anthropology - Bioarchaeology - Palaeoanthropology

1. INTRODUCTION

In this paper, only two parameters of biological profile estimation in adults will be addressed. Age-at-death and sex estimation assessment are considered among the essential parameters in the creation of a biological profile in adult individuals and represent the first stage of study in forensic anthropology, bioarchaeology and palaeoanthropology (e.g. Bethard, VanSickle 2020, Franklin 2010, Langley, Tersigni-Tarrant 2017, Messer, Getz 2020). These two topics are by far the most discussed among forensic anthropology-related articles (Lei *et al.* 2019). The rapid development of computer technology at the end of the 20th century and the possibility of using new classification tools have led to an extreme increase in the publication of new methods to estimate age and sex from the skeleton and their modifications (Bethard, Digangi 2019). From 2000 to 2021 alone, Google Scholar provides more than 18,700 links to relevant articles on adult age estimation methods and 17,700 links to articles on adult skeletal sex estimation. Unfortunately, not all the proposed methods for estimating the parameters of an individual's biological profile meet the methodological requirements formulated almost half a century ago by Vladimír Novotný (Novotný 1981) for sex estimation of the hip bone. In general, the requirements he raised (see below) can also be applied to age estimation methods.

According to Novotný (1981), a true method of sex estimation uses sets of mutually uncorrelated variables, where for each there is low variability within each sex and there is a narrow overlapping area of both sexes. A true method must be designed in sufficiently large groups of individuals of known sex from the population in which the method is to be applied. It is also necessary to know the probability of the risk of error when applying the method to unknown cases. The method must also be clearly defined and user-friendly in order to be practical, which means that the method does not require considerable previous user experience (Novotný 1981). Furthermore, it is necessary that the choice of variables in each method represents the evolutionary and functional nature of sexual dimorphism. In current terminology, this means that sex estimation methods must respect the modularity and integration of a given structure (e.g. Esteve Altava 2017a, b, Klingenberg 2008).

Morphological integration means that specific subsets of morphological features tend to overlap strongly with evolution and development, while other subsets are less associated. In current usage, such sets of integrated traits are termed "modules". Modularity

helps us to understand the evolvability and plasticity of organismal forms (Klingenberg 2013). The results of Lewton (Lewton 2012) and Grabowski *et al.* (Grabowski *et al.* 2011) strongly support the coexistence of two modules (ischiopubic and sacroiliac) at the hip bone, with a low level of integration. These modules were called segments by Novotný (Novotný 1986). Modularity and integration also have their place in the methods of estimating sex using the skull and other parts of the skeleton (Bastir 2008, Kolatorowicz 2015). A holistic view from the point of view of variable hierarchization can also be applied to methods of estimating age that use skeletal senescence changes.

2. BIOLOGICAL PROFILE ASSESSMENT AND QUALITY OF METHODS: FOCUS ON AGE AND SEX ESTIMATION

We believe that methods for estimating the biological profile must be as accurate in forensic anthropology as in bioarchaeology and, with some exaggeration, in paleoanthropology. In the latter, the application of true methods is very rare. This is mainly due to the lack of knowledge of the variability and to the damage of skeletal remains. As noted by Vandermeersch, the Neanderthal skull of La Quina 5 has changed its sex at least six times during the half-century since its discovery (Vandermeersch 1981). In the case of incorrect classification, we attribute biological significance to the methodological error (Brůžek 1995). The accuracy of many methods used in bioarchaeology to estimate sex falls well below the level necessary for forensic identification when tested on a different population sample from the one from which the method was derived (Scheuer 2002). The accuracy of the methods should be the same regardless of the area where the method is used, and a 95% limit is required (Brůžek, Murail 2006). Under the influence of the Daubert criteria (Daubert v. Merrell Dow Pharmaceuticals 1993), there has been a considerable change regarding the development of biological profile methods, particularly in the field of forensic science. The Daubert case made, for example, the following claims: the methods and therefore the conclusions made by scientists must be testable and tested, their accuracy and rate of errors must be known, the results should be expressed via probability, and, last but not least, the methods should undergo peer review, be published in renowned journals, and be accepted within the scientific community (Daubert v. Merrell Dow Pharmaceuticals 1993, Grivas, Komar 2008).

Methods from a particular reference sample usually perform poorly when applied to sex estimations in unrelated target samples and produce large sex biases (Walker 2008), that is, differences in accuracy differentiating female and male. A common misunderstanding of DFA (discriminant function analysis) results is that the overall accuracy of sex classification can be applied to every individual in the sample. Every bone measurement and DF (discriminant function) score show an overlap between female and male distributions. The overlapping area represents the "zone of uncertainty," where the skeletal variables of females and males are similar and cannot reliably be distinguished from one another (Galeta, Brůžek 2020). For this reason, some authors have proposed to not necessarily classify the sex in all individuals from the sample in order to avoid misclassifications (e.g. Brůžek *et al.* 2017, Franklin *et al.* 2013, Kranjčič *et al.* 2019, Torimitsu *et al.* 2018). Instead, the probability of the individual belonging to the female or male sex (the classification rule is the posterior probability = 0.5) is computed and the sex estimation is provided only for those for which a probability of greater than 0.95 has been reached, ensuring high classification accuracy on the individual level (Galeta, Brůžek 2020). There is an example in Table 1.

A low number of sex estimation methods (DFA) achieve a classification success rate of more than 95%. Methods with accuracy lower than 70% are almost meaningless for practical use. While methods (DFA) that achieve an accuracy of 70 to 90% with a sectioning point of 0.5 have a high risk of error (15–20%), they do

allow the correct determination of sex with a probability higher than 0.95 in several dozen individuals.

However, the measurement uncertainty is important since it is closely related to accuracy, precision, trueness, and reliability, for which different authors favour different terms to refer to the same concept (Adalian 2020). For example, accuracy is determined in relation to documentation, and reliability reflects intra-observer consistency in repeated trials (Komar, Buikstra 2008). For the methods of sex estimation, the accuracy is the percentage of specimens whose sex is correctly estimated from the total number of sexed specimens or those that reach the classification threshold for posterior probability (Santos *et al.* 2019). Reliability is the success of classification in testing the method on a different independent population (Brůžek, Murail 2006). In the Glossary of the recent manual of statistics in the forensic anthropology (Obertová *et al.* 2020), reliability (of a method/test) is defined as "*the consistency of a method/test in measuring a given variable, that is, a reliable method/test used under the same conditions results in the same outcome (or the ability of a method to differentiate among specimens/ratings).*" Reliability can be assessed by examining the consistency of the results across time or among examiners... Validity (of a method/test) is defined as "*the extent to which a method/test really measures what it is supposed to measure. It can be assessed by examining how well the results correspond with established theories or how it performs against a gold standard.*" (Obertová *et al.* 2020).

In bioarchaeology, 10-year or even 5-year age intervals are often used in age-at-death analyses, which is

TABLE 1: Classification accuracy, error, and the proportion of individuals sexed based on different sex indicators in several reference samples; adopted from (Galeta, Brůžek 2020). PP posterior probability; ^a Individuals with PP(F)>0.50 or PP(M)>0.50.; ^b Individuals with PP(F)<0.50 or PP(M)<0.50.; ^c Individuals with PP(F)>0.95 or PP(M)>0.95.; ^d Individuals with PP(F) or PP(M) between 0.05 and 0.95.; ^e Unpublished data, Pachner collection, Prague, Czech Republic, individuals of known sex, age at death, and stature from lower socioeconomic classes of Bohemia. ^f Pachner collection, Prague and Osteological Collection of the Faculty of Medicine, Brno, Czech Republic; ^g Coimbra, Portugal; ^h Coimbra, Portugal.

Discriminating feature	N	Estimation at PP>0.50				Estimation at PP >0.95			
		Accuracy (%)	Error (%)	Total sample sexed (%) ^a	Unclassified ind. (%) ^b	Accuracy (%)	Error (%)	Total sample sexed (%) ^c	Unclassified ind. (%) ^d
Stature ^e	75	74.7	25.3	100.0	0.0	50.0	50.0	4.0	96.0
Patella ^f	183	80.3	19.7	100.0	0.0	96.4	3.6	15.8	84.2
Tibia ^g	95	84.2	15.8	100.0	0.0	92.5	7.5	45.3	54.7
Os coxae ^h	220	96.8	3.2	100.0	0.0	99.5	0.5	85.5	14.5

beneficial because it enables better comparison. However, such narrow intervals are accompanied by great risk of misclassification (Osborne *et al.* 2004). In order for age estimates to be accurate and reliable at the same time, some researchers have stated that estimation into three broad age intervals is the only option (Adserias-Garriga, Wilson-Taylor 2019, Buk *et al.* 2012, Calce 2012). Some examples include: less than 30, 30–60, +60 years (Buk *et al.* 2012); 20–34 years, 35–45 years, and 46+ years (Falys, Lewis 2011); or up to 40 years, 40–65 years, and over 65 years (Adserias-Garriga, Wilson-Taylor 2019). However, the validation studies of, for example, the method of Calce (Calce 2012) do not confirm this (Mays 2014, Navega *et al.* 2018). The difference between the reference and test populations may be to blame. At the same time, regression-based models have been proven to be unsuitable for skeletal aging (Lucy *et al.* 1996, Schmitt *et al.* 2002).

The current trend is abandoning models based on linear regression and leaning towards probabilistic approaches that seem more appropriate (Aykroyd *et al.* 1999, Boldsen *et al.* 2002, Brennaman *et al.* 2017, Buk *et al.* 2012, Kotěrová *et al.* 2018, Lucy *et al.* 1996), especially in the forensic context. The Bayesian approach, which is used the most in contrast to the regression-based model, is less sensitive to the phenomenon of "age mimicry" (Masset 1990), provides more accurate age estimates and smaller confidence intervals.

3. WHAT METHODS ARE USED IN PRACTICE TODAY?

It is an exaggeration to say that there are two worlds of researchers – the world of scholars who design methods and the world of practitioners who use them in practice. While we know about the first group of researchers from their publications for which they propose, modify, and test methods for estimating the biological profile from the skeleton, we have little information about the second group of researchers who use the methods to identify unknown skeletons in forensic anthropology and bioarchaeology.

In 2011, Falys and Lewis analysed data related to adult age estimation methods used in papers published in three anthropological and archaeological journals from 2004 to 2009 (Falys, Lewis 2011). The results indicate that the most frequently used methods were dental attrition and cranial sutures, followed by pubic

symphysis and auricular surface. We have a slightly better idea about the methods used in practice from North American forensic anthropologists. In 2012, there was a survey of age-at-death estimation methods used among 145 members of the American Academy of Forensic Sciences (section of Physical Anthropology) based on a questionnaire. The authors of the review, Garvin and Passalacqua, concluded that the methods based on the use of the pubic symphysis still remain the most highly favoured aging technique (78%); on the contrary, cranial sutures and dental wear were among the least preferred (Garvin, Passalacqua 2012).

Similar to the above survey of age estimation methods, Klales (2020) conducted a survey of sex estimation methods involving 154 forensic anthropology practitioners all over the world. *"Regardless of the level of experience, the pelvis was always the most preferred, followed by skull... However, ease of use and tradition may explain why morphological methods continue to be utilized"* (Klales 2020a). Regarding the particular methods, both in the case of the pelvis and the skull, the most preferred methods are stated in order of importance from the textbooks by Buikstra and Ubelaker (Buikstra, Ubelaker 1994) and Krogman and İşcan (Krogman, İşcan 1986). The specific methods of preference are the Phenice (Phenice 1969) method for the hip bone and the Walker (Walker 2008) method for the skull. The vast majority of respondents stated that their results are presented in research publications (89%), archaeological reports (71%) and forensic case reports (67%). *"What is surprising – and perhaps quite alarming at least from a forensic perspective – is the number of respondents who reported using their own data, own reference collections, unpublished methods and/or invalidated and unreliable methods for sex estimation"* (Klales 2020a). However, we do not have published information about the methods used in the European forensic-anthropological community. It can be said that practitioners in forensic anthropology and bioarchaeology use methods that have worked for them and they probably do not change them much during their professional life.

4. RECOMMENDATIONS FOR STANDARDIZATION OF METHODS

Despite the lack of information on the methods used in practice, there are numerous recommendations for estimating the biological profile and for choosing appropriate methods. Among the best known are "Standards for Data Collection from Human Skeletal

Remains" (Buikstra, Ubelaker 1994) and "Recommendations for Age and Sex Diagnoses of Skeletons" (Ferembach *et al.* 1980), which are based on the previously proposed methods (Acsádi, Nemeskéri 1970). These manuals were also among the most referenced standardised guidelines in the Falys and Lewis survey concerning adult age estimation (Falys, Lewis 2011). However, the methods they include provide a historical framework for biological profile estimation methods. In the post-Daubert era of the last ten or twenty years (Dirkmaat, Cabo 2012, Garvin *et al.* 2012), methods have been increasingly examined to ensure that their results are accurate, replicable, and statistically and legally defensible, with known errors and standards for application (Boyd, Boyd 2018, Lesciotto 2015).

There are several recent recommendations for estimating age and sex, processed primarily in the context of forensic anthropology in English speaking countries (Anonymous 2010, 2013, 2018, 2019, 2020, Mitchell, Brickley 2018). According to these recommendations, sex estimation should be performed with the use of population- and period-specific standards (except when sex is estimated based on the pelvic bone). Forensic anthropology stresses the need to develop "population specific standards" (Franklin, Blau 2020, Spradley *et al.* 2008, Ubelaker 2014) using modern samples, rather than ignoring secular change, and continuing to rely on the nineteenth and early twentieth centuries skeletal collections (Boyd, Boyd 2018). The limited applicability of population-specific standards has been repeatedly pointed out (Franklin, Flavel 2019, Guyomarc'h *et al.* 2016, Kotěrová *et al.* 2016). A key issue facing any practitioner in building a biological profile is choosing the appropriate local or global reference algorithm (Garvin, Klaes 2020, Komar, Buikstra 2008). In the case of bioarchaeology, it is almost impossible to obtain population-specific standards for past populations. Thus, it is simultaneously advantageous and necessary to use methods that are based on multi-population datasets of identified individuals originating from contemporary populations.

According to the SWGANTH and ANZPAA guidelines for North American and Australian forensic anthropologists, respectively, the results of sex estimation expressed as a degree of certainty (probability value) should be indicated, especially when a sex estimation is less than certain. As well as the estimation of other skeletal parameters, sex estimation should be performed, even if samples for DNA analyses are taken. Unaccepted practices with little or no scientific basis in human osteology are not considered reliable and at this

time they are not recommended. This also includes sex estimation of subadult remains with non-fused innominate elements, a statement of sex as "gender" and conclusions based on methods that have not been validated (Anonymous 2010, 2020). For age estimation, suitable skeletal indicators must meet the following criteria and recommendations: observed changes should be gradual and unidirectional with age; and traits should highly correlate with chronological age and occur roughly at the same age in all individuals (at least within a distinguishable sub-group). The aging pattern should also be reliably classifiable and measurable, and finally, the age indicator itself must be resistant to taphonomy (Algee-Hewitt 2017, Milner, Boldsen 2012a). When population-specific standards are not available, multi-population standards that cover greater variance should be used. Factors of the environment and life history of the individual can introduce non-age-related variation in the expression of degenerative traits and thus represent a potential source of error. It is known that for the estimation of age-at-death in certain periods of adult life, some methods are more reliable. According to guidelines, most research suggests that combining multiple age indicators provides more accurate results, as opposed to using single indicators. However, this question has not yet been satisfactorily resolved (see below). Researchers should avoid the reporting of overly precise age estimates without providing the information of the error involved (Anonymous 2013, 2020). Besides the best practices outlined by contemporary guidelines, there is no anthropological standard for the aging of the adult skeleton (Parsons 2017).

Researchers affiliated with FASE (Forensic Anthropology Society of Europe) which is a subsection of IALM (International Academy of Legal Medicine), published two articles related to age-at-death estimation of both human remains and living individuals, and present their recommendation based on their experience (Baccino *et al.* 2013, Cunha *et al.* 2009). These reviews give the reader practical advice on choosing the right strategy and methods for age estimation for different stages and skeletal parts of human remains preservation. They also give us insight into what methods are being used in practice by European anthropologists associated with FASE.

The aim of this paper is to provide an overview of the evolving approaches to sex and age-at-death estimation from human skeletal remains using both morphological and metric data that can contribute to greater accuracy and reliability in estimating the biological profile of adult individuals from the skeleton.

Emphasis will be placed on methodological aspects, validity testing and practical use, which are facilitated by the use of software. *"In the 21st century, we need to move away from using the term (and practice) of generating assessments and, instead, rely on estimates of sex (and other biological parameters) using valid and reliable methods (either morphological or metric). Our estimates should, in turn, include associated accuracy, probabilities, and error rates, and our methodological research at minimum should include these parameters, as well as tests of statistical assumptions"* (Klaes 2020b).

5. CURRENT STATE OF AGE ESTIMATION METHODS

The methods of age-at-death estimation of the last 20 years have been significantly affected by two milestones. These are, firstly, the aforementioned Daubert criteria and, secondly, the Rostock manifesto (Hoppa, Vaupel 2002). Both turning points have been reflected in the development of age estimation methods in forensic anthropology, as well as in bioarchaeology. The Rostock manifesto aimed to establish theoretical recommendations for paleodemography in reaction to required biostatistical improvements. Its call for the incorporation of biostatistical approaches, i.e. the Bayesian approach, particularly into age-at-death estimation methods (and into other paleodemographic analyses as well) resulted in a very important output – the Transition analysis (TA). This is Boldsen's multifactorial technique, which combines multiple skeletal indicators (Boldsen *et al.* 2002, Milner, Boldsen 2012b). To facilitate the evaluation of indicators, the authors created the ADBOU software, which uses Bayesian statistics and transition analysis, where the transition from one stage into another is computed. As an output, the software provides a multifactorial likelihood estimate and a maximum likelihood estimate for individual skeletal structures with "individualized" confidence intervals (Boldsen *et al.* 2002, Getz 2020). TA is unquestionably a statistical step forward since it helps to overcome some of the limitations that existed at the time of developing this approach (i.e. fixed age intervals, open-ended age categories, and age-mimicry). However, method performance is inconsistent in different populations; numerous tests of TA have shown that the degree of accuracy is low and TA age ranges are often too broad (Getz 2020). Simon and Hubbe, for example, reached a mean absolute error of 11.6 years (Simon, Hubbe 2021); Jooste *et al.* 10.4 years (Jooste

et al. 2016); and Xanthopoulou *et al.* concluded that TA performed worse than traditional methods and provided lower accuracies in their sample (Xanthopoulou *et al.* 2018). Currently, the third version of TA (Getz 2020), which includes many more skeletal indicators (*Figure 1*) that can be scored, was released (at the time of writing this paper in Beta version – <http://statsmachine.net/software/TA3/>).

Aging takes place at all levels, whether at the macroscopic or microscopic level (biochemical and histological changes), in both, bone and dental tissues. Leaving the biochemical methods aside, since they are used only very rarely, methods to assess the intrinsic structure of the tooth have been of great interest to this day. A not entirely new approach that has been on the rise is cementochronology (e.g. Bertrand *et al.* 2019a, b, Couoh 2017). Very strong correlation (0.927) was reported between age estimates and chronological age; however, the readability of incremental lines decreases with age. Moreover, taphonomy can cause serious limitations (Bertrand *et al.* 2019a) that may impact the applicability of the method on archaeological skeletal remains, which has not yet been fully explored (Bertrand *et al.* 2019a, Colard *et al.* 2015). Furthermore, the standardized procedure is required to reduce bias during sample preparation. Even though it is considered a reliable and very promising approach, more research is needed particularly concerning older individuals.

Even though some of the biochemical and histological methods could provide more accurate and reliable estimates (e.g. Giuliani *et al.* 2016, Rösing *et al.* 2007, Zinni, Crowley 2017), they usually require some irreversible intervention into the bone or dental structure, which have the further drawbacks of higher costs, greater time requirements and the need for laboratory equipment (Cunha *et al.* 2009, Lewis, Kasper 2018). Given these limitations, the most commonly applied methods in bioarchaeology and forensic anthropology are still the ones examining gross morphology (i.e. the metamorphosis of articular surfaces). The sternal ends of ribs, the pubic symphysis, the auricular surface of the ilium and, newly, the acetabulum should be highlighted among the most frequently used skeletal indicators (e.g. Langley *et al.* 2017, Nikita 2017). Sternal ends of ribs show a longer period of metamorphosis; however, their usage is limited due to very poor preservation (Falys, Lewis 2011). The pubic symphysis is probably the most traditional skeletal indicator of age and has been used for the longest time. Dozens of new methods and revisions of original methods are proof of this (e.g. Brooks, Suchey 1990,

Schmitt 2004, Stoyanova *et al.* 2017, Todd 1920). However, this skeletal indicator is useful only up to approximately 40 years of age, before degenerative changes become too variable and unpredictable (e.g. Baccino *et al.* 2014, Dudzik, Langley 2015, Márquez-Grant 2015). Beyond this age, very wide age intervals are produced (Brooks, Suchey 1990, Stoyanova *et al.* 2017). At the same time, a poor preservation rate very often prevents pubic symphysis from being used more frequently. The auricular surface is among the more protected skeletal indicators, characterized by metamorphic changes correlating with chronological age (Buckberry, Chamberlain 2002). Lately, attention has been paid to another articular surface: the acetabulum (e.g. Rissech *et al.* 2006, San-Millán *et al.* 2017, 2019). It is also a better preserved skeletal area that is less exposed to taphonomic influences (Calce, Rogers 2011). According to several studies, the acetabulum is a promising indicator for estimating the age of adults older than 60 years (Cunha *et al.* 2009).

The very nature of the visual assessment of age changes in the majority of conventional methods is also a frequently addressed issue for its subjectivity and dependence on the experience of the evaluator. Kotěrová

et al. conducted research in which they applied various mathematical approaches to visually scored data with the aim of reaching more accurate age estimation (Kotěrová *et al.* 2018). However, none of the approaches led to significant improvement, as in previous studies (e.g. Buk *et al.* 2012, Martins *et al.* 2012); the estimate of an adult's age within three wide intervals (which offer accurate and reliable estimates) has not been surpassed. Similarly, Getz found that the statistical approach itself cannot improve age estimates based on visually assessed skeletal indicators in the TA and transition analysis-based approaches (Getz 2020).

In accordance with the Daubert criterion of producing more objective results and with rapid technological advances in 3D scanning and medical imaging, new age estimation options are arising. 3D representations offer the possibility of internal structure examination (e.g. Barrier *et al.* 2009, López-Alcaraz *et al.* 2015) and surface quantification (e.g. Biwasaka *et al.* 2019, Stoyanova *et al.* 2017, Villa *et al.* 2015a). As the usefulness of virtual models like 3D representations of dry bones has demonstrated many times and the fact that they are commonly used for morphological or metric assessment (e.g. Chapman *et al.* 2014, Decker

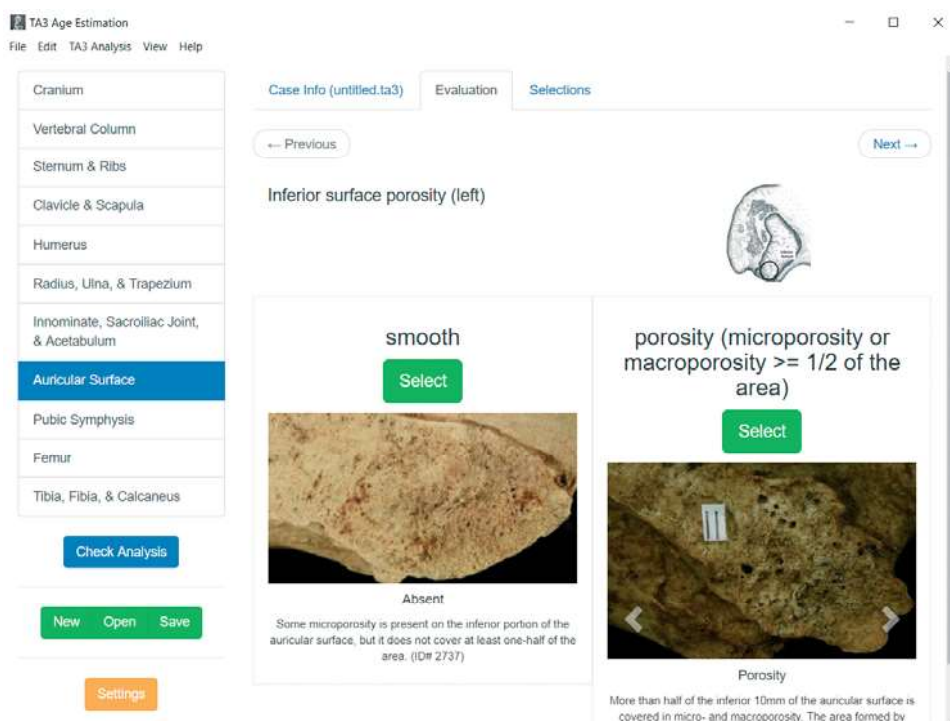


FIGURE 1: Transition analysis 3 (TA3) – graphical user interface.

et al. 2011, Mesteková *et al.* 2015), few researchers raised questions about whether 3D models acquired with different scanners are comparable (Kotěrová *et al.* 2019, Villa *et al.* 2015b), or whether they have an impact on subsequent biological profile analyses: age-at-death and sex estimation. Villa *et al.* reported a comparable overall anatomical shape of two pelvic articular surfaces; however, their curvature values showed systematic bias among the three tested scanners. Nevertheless, the curvature values changed in a similar way with increasing phase or score (Villa *et al.* 2015b). Kotěrová *et al.* reported that the tested scanners differed in how much detailed surface they captured (Figure 2); however, the differences had negligible impact on age-at-death and sex analyses (Kotěrová *et al.* 2019).

In the past few years, research has focused on the surface quantification of the given joint area and the surrounding area (mainly on the pubic symphysis and on the auricular surface of the ilium) in order to capture age-related changes more objectively (Biwasaka *et al.* 2013, Slice, Algee-Hewitt 2015, Stoyanova *et al.* 2015, Villa *et al.* 2015a). The research was most developed by the Slice, Algee-Hewitt and Stoyanova team, who proposed the free user-friendly software 'forAGE' (Kim

et al. 2018, Slice, Algee-Hewitt 2015, Stoyanova *et al.* 2015, 2017, 2019). Even though their results are quite comparable to the outputs of traditional methods, they offer us hope of an age estimate that is independent of the observer's experience. Moreover, with the use of data mining methods, they give us the promise of more accurate and reliable age estimates.

There has been and still remains a great deal of discussion about the appropriate number of skeletal identifiers to be used to estimate age. Alongside single indicator-based methods (e.g. Brooks, Suchey 1990, Buckberry, Chamberlain 2002, Calce 2012, Işcan *et al.* 1984), there are multifactorial approaches (e.g. Acsádi, Nemeskéri 1970, Boldsen *et al.* 2002). For a long time, it was assumed and believed that a single skeletal indicator itself could capture the entire period of adult life. Even though some of the methods based on age-related changes of one indicator are used to this day, it is slowly being accepted that various skeletal indicators are more convenient and contribute most to age estimation in different periods of adult life (Adserias-Garriga, Wilson-Taylor 2019, Milner, Boldsen 2012a). In the case of methods that somehow combine more indicators, there are two factors that prevent their wider

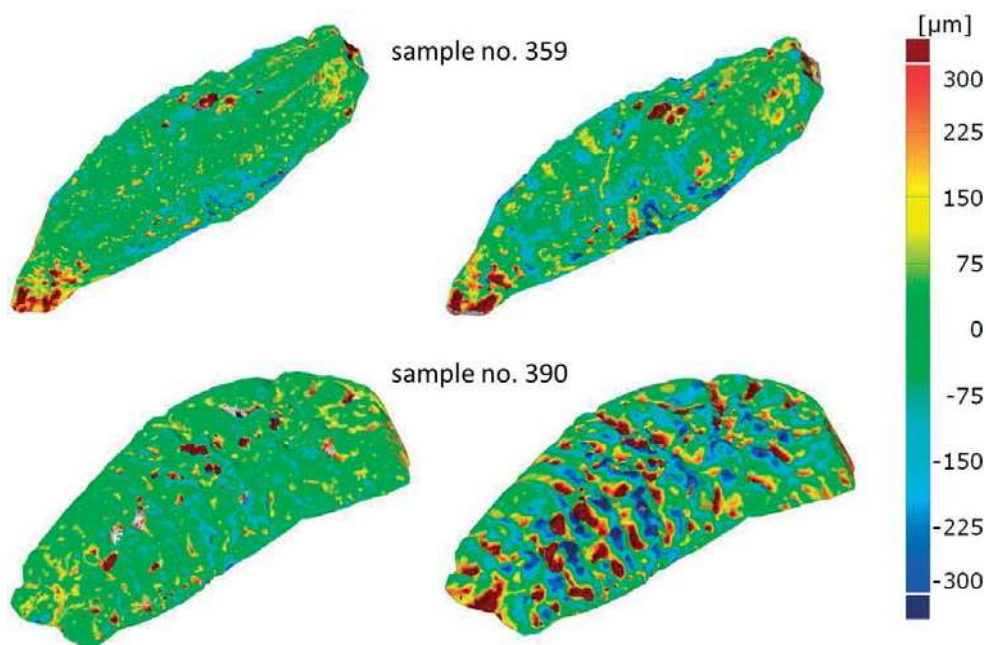


FIGURE 2: An example of a colour-coded map showing deviations between the reference (Redlux Profiler) and the compared surface (left: HP 3D SLS scanner; right NextEngine scanner). Red scale – the compared surface is above the reference surface; Blue scale – the compared surface is below the reference surface (Kotěrová *et al.* 2019).

use: 1) lack of consensus on how to combine them to obtain a single estimate with reasonable confidence intervals and 2) what methods should actually be used (Franklin 2010, Garvin *et al.* 2012). Apart from the skeletal indicator and the aging technique used, the unavailability of population-specific standards also often limits the application of aging methods (e.g. Bassed *et al.* 2011, Mays 2014, Navega *et al.* 2018, Schmitt 2004).

6. CURRENT STATE AND ADVANCES IN SEX ESTIMATION METHODS

The pelvis and skull continue to be the preferred skeletal elements for estimating sex. The description and understanding of sexual differences in the skeleton are the basis for the study of skeletal sexual dimorphism; however, skeletal dimorphism itself could never be considered a method. Despite the multitude of published statistical methods for sex estimation, many of these methods either lack the associated probabilities of sex classification (e.g. Brůžek 2002) and/or, despite high classification probabilities, are not translatable into a method (e.g. Bytheway, Ross 2010) that could be practically applied (Klales *et al.* 2020). Krishan *et al.* (2016) provide a more detailed description of the different statistical approaches, skeletal regions utilized for sex estimation, and sex assessment approaches; they also include specific research papers for each, but that is beyond the scope of this work (Krishan *et al.* 2016).

The recommended error for a sex estimation method is less than 5%, that is, the accuracy of the method needs to be over 95% for the classification of a given individual (Sifakis *et al.* 2020). However, this is not entirely true. The method with a classification accuracy of 95% does not guarantee that the risk of error of less than 5% will be guaranteed for each individual. There is a zone of overlapping values corresponding with a zone of incertitude, where the probability of belonging to a set of females (or males) is in many cases lower (Galeta, Brůžek 2020). It is not the high accuracy of the method, but the high posterior probability (over 0.95) that ensures the reliability of the sex estimation of an individual case (e.g. Franklin *et al.* 2013, Kranioti, Apostol 2014, Murail *et al.* 2005). However, the accuracy level that is only expressed as a percentage of correctly classified cases is the real pitfall for users. There is the incorrect assumption that in a method with an accuracy of, for example, 92%, all individuals are correctly classified with a posterior probability of 0.92. Unfortunately, the extreme majority of researchers make

this assumption and in the evaluation of the performance of methods, they explicitly take into account only the overall accuracy of the method.

6.1 Employing morphological pelvic traits in sex estimation

In principle, we can see two historical methodological lines that lead to the use of morphological features of the pelvic complex in sex estimation. Firstly, it is the North American school, which began with the publication of an article by Phenice (1969), which proposed a method for evaluating the 3 characters *os pubis* (Phenice 1969). This pioneering study was modified in the last decade by Klales *et al.* (e.g. Klales *et al.* 2012). Secondly, the methodological line of the European tradition based on Novotný's research (e.g. Novotný 1975, Novotný, Vávrová 1971) was developed especially by Brůžek (Brůžek 1991, 2002).

From Phenice (1969) to MorphoPASSE by Klales (2018)

Phenice (1969) published the most popular method for morphological sex estimation based on the pelvis, which uses three features of the pubic area (ventral arch, subpubic concavity, and the medial aspect of the ischiopubic ramus). Features are scored according to the predominance of a particular trait in one sex over the other. The Phenice method was able to estimate the sex from adult pubis with an accuracy of 96% (Phenice 1969). In a series of tests, levels of accuracy ranged from 59% to 96% (Kenyhercz *et al.* 2017), so this inconsistency casts doubt on the Phenice (1969) method. This oscillation of results in a number of European samples of identified individuals living in different periods, could be due to the varying degrees of sexual dimorphism of the pubis, which does not reflect the overall sexual dimorphism of the pelvis (Brůžek 1991). A certain shortcoming of the original method is also the absence of statistical decision-making.

A revised method using Phenice's traits and appropriate statistical analysis was published by Klales *et al.* (Klales *et al.* 2012). They also modified the terminology and the subpubic contour and adjusted the description to account for the concavity below the symphyseal face, as well as the shape of the entire ischiopubic ramus, which reflects the subpubic angle. Klales *et al.* (2012) built upon Phenice's binary observations by assigning five-character states with ordinal scores (from hyperfeminine 1 to hypermasculine 5). This scoring method allowed the reliability and

accuracy of the results to be calculated. For the pubic traits, this has included the development of a five-stage visual recording system for all three of the Phenice traits (Klales *et al.* 2012). A spreadsheet for using the functions set by Klales *et al.* is available at <http://nonmetricpelvissexing.weebly.com/> (Mitchell, Brickley 2018).

Subsequently, another study (Kenyhercz *et al.* 2017) noted the impact of population variation of sex estimation from the pelvis. Directly using the method of Klales *et al.* (2012), sex was estimated for a large meta-population sample of American, South African, Thai, and Hispanic individuals. The authors noted that populations from the USA and South Africa produced the highest sex classification accuracies (90.7%–95.6%), while the Thai and Hispanic populations presented the lowest sex classification accuracies (approximately 88%) (Kenyhercz *et al.* 2017). However, the recalibration of the global equation that takes into account many geographical areas improves classification accuracy and reduces sex bias; thus it could be used for sex estimation in diverse, worldwide populations (Kenyhercz *et al.* 2017). We agree with Klales that the utility of morphological features and methods in forensic contexts must include statistical estimates of error. Although this is not yet the case in bioarchaeological or paleoanthropological contexts, in the interest of "good science" the same principle should be applied here as well (Klales 2020c).

The quest for user convenience was completed by the creation of MorphoPASSE software (<https://www.morphopasse.com/>). It is a free, interactive program designed to estimate sex using the morphological traits of the pelvis and skull. The database contains ordinal data collected from over 2,500 individuals from 15 contemporary and historical skeletal collections that represent five broad geographical ancestral backgrounds (Klales, Cole 2018). With this application, it is easier to estimate the probability of the biological sex of the skeleton, as some publications suggest (e.g. Zejdlik *et al.* 2021, Zoeller *et al.* 2021). The importance of this software is diminished by the fact that the preservation of the pubic bone rarely exceeds 30% in archaeological samples (Waldron 1987), which limits the use of the MorphoPASSE software (Klales 2018).

From Novotný (1975) to the R package PELVIS by Santos *et al.* (2019)

Long-term research of the sexual dimorphism of the pelvis in primates and humans (Novotný 1968, 1971) has resulted in an analysis of the discriminant power of

14 morphoscopic features of hip bone frequently used in sex determination. From this list of features, Novotný recommended the use of 3 (the preauricular surface, the sciatic notch, and the inferior aspect of the hip bone) to check the results of discriminant functions. He proposed his own evaluation procedure for their assessment (Novotný 1981). Novotný also replaced a descriptive or ordinal evaluation of features (e.g. small, wide, shallow) with trichotomous scoring: ("yes" or "no") or an intermediate category reserved for cases of hesitation or observational uncertainty ("I cannot decide"). This approach reduces the risk of misclassification (Brůžek 1991). Brůžek (2002) included three composite features evaluated according to Novotný (1981) with two features generally used for sex estimation in the proposal of his method. He included these features in his study of 402 adults from collections in France and Portugal (Brůžek 2002). A combination of five features resulted in the accuracy being close to 98%. The features were the preauricular surface, the greater sciatic notch, the composite arch (from sacroiliac pelvic module), the inferior margin of the hip bone, and the ischiopubic proportions (from the ischiopubic pelvic module). However, there were some general criticisms of the standard qualitative methods the lack of associated probabilities of sex classification, greater subjectivity, and reliance on experience. Conversely, these techniques are quick and easy to apply (Klales 2020b).

To overcome the disadvantages of the absence of a statistical decision, Santos *et al.* used the data of 592 hip bones from adults of known sex (Coimbra, Paris, Spitalfields, Hamann-Todd and the Terry collections) to build a reference sample (Santos *et al.* 2019). Two other samples (composed, respectively, of 518 hip bones from the same skeletal collections and 99 CT-scan images) were both used for validation purposes. The authors used the same 5 features as Brůžek (2002), which they divided into 11 simple traits, and used the trichotomous evaluation of Novotný (1981). To sex the human hip bone, the R-Shiny application "PELVIS" was developed, implementing a sexing method based on logistic regression. It is freely available (<https://cran.r-project.org/web/packages/PELVIS/index.html>). This new statistical method (Santos *et al.* 2019) has a slightly better accuracy rate (99.2%) than the former method of Brůžek (2002), but a higher rate of indeterminate individuals (12.9% vs. 3% for complete bones). The result is presented by the probability values of being male or female (*Figure 3*).

The benefit of this approach is the reduction of the error rate; however, at the cost of more undetermined

PELVIS — A visual method for sexing the human os coxae based on Bruzek's nonmetric traits.

Data input: manual editing | Data input: from text file

Name of the unknown individual:

Strategy for variable selection: ☐ None ☐ AIC ☒ BIC

Confidence level for a sex estimate: ☐ 90 % ☒ 95 %

Preauricular surface (PrSu)

Development of negative relief on preauricular surface (PrSu1):

Aspects of grooves or pitting (PrSu2):

Development of positive relief on preauricular surface (PrSu3):

Great sciatic notch (GrSN)

Proportion of length of sciatic chords (GrSN1):

Form of contour notch chords (GrSN2):

Contour of posterior notch chord relative to line from point A to sciatic notch breadth (GrSN3):

Composite arch (CArc)

Relation between outline of sciatic notch and outline of auricular surface: ☒ Could not be observed ☐ F, Double curve ☐ 0, Intermediate form ☐ M, Single curve

Inferior pelvis (InP)

Characterization of margo inferior ossis coxae (InP1):

Phallic ridge (InP2):

Ischio-pubic ramus aspect (InP3):

Ischiopubic proportion (IsPu)

Relation between pubis and ischium lengths: ☐ Could not be observed ☐ F, Pubis longer than ischium ☒ 0, Intermediate form ☐ M, Ischium longer than pubis

Compute sex estimate

Results

Posterior probabilities are rounded to the third decimal place.

Show: 10 entries

	PrSu1	PrSu2	PrSu3	GrSN1	GrSN2	GrSN3	GrSN	CArc	InP1	InP2	InP3	InP	IsPu	Sex estimate (Bruzek 2002)	Statistical sex estimate (2019)	Prob(F)	Prob(M)	Selected predictors in LR model	10-fold CV accuracy (%)	Indet. rate in CV (%)
Indiv01	f	NA	f	F	f	f	F	F	f	f	f	F	0	F	F	0.992	0.008	PrSu1, GrSN1, CArc, InP3, IsPu	99.17	15.73

Showing 1 to 1 of 1 entries

[Download the complete table \(CSV file\)](#)

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FIGURE 3: Illustration from the graphic user interface of the R-shiny application PELVIS (Santos *et al.* 2019). An example of sex estimation of an individual using 10 out of 11 possible traits.

individuals. The error rate was constantly lower than 1.5%, resulting in the sex estimates being particularly reliable (Santos *et al.* 2019).

6.2 Sex estimation and pelvic morphometric variables

From Novotný (1975) to the DSP method (Brůžek *et al.* 2017)

Although the discriminant functions (DF) of pelvic dimensions are highly accurate and reliable (e.g. Brůžek 1991, Novotný 1981), their limitation is a fixed number of dimensions, which does not allow their flexible response when used in different taphonomic conditions.

As noted by Santos *et al.* (Santos *et al.* 2020), the theoretical framework for the study of pelvic sexual dimorphism and the design of the sex estimation tool Diagnose Sexuelle Probabiliste or DSP and DSP2 (Brůžek *et al.* 2017, Murail *et al.* 2005) were greatly influenced by the work of Novotný (Novotný 1981, 1986). Novotný conducted extensive literature research and selected almost 40 linear dimensions of the hip bone, already used by authors from the beginning of the 20th century, to describe the sex differences of the pelvis and those in sex estimation (Novotný 1981). He

chose their reduced number in terms of the magnitude of sex differences and the smallest zone of overlapping values; he used such variables to calculate DFA, which provides very high accuracy in the range of 95 to 100% (Novotný 1981, 1986). Brůžek (Brůžek 1984, 1991) studied Novotný's recommended dimensions in addition to some variables from the literature: a total of 32 variables were analysed in two samples of hip bones of known sex from the Coimbra and Paris collections. The testing of their intra- and inter-observational erroring in another sample (Brůžek *et al.* 1994) led to the selection of 17 dimensions for future research. The selection and final decision on the variables for the DSP involved a compromise between the taphonomic criteria and the statistical parameters of the selected variables (Santos *et al.* 2020).

Diagnose Sexuelle Probabiliste (DSP and DSP2) is a tool for assessing the sex of an unknown individual using up to 10 metric pelvic measurements. The reference datasets were based on 2,040 hip bones from a worldwide sample covering 12 different populations originating in Europe, Africa, North America, and Asia (Brůžek *et al.* 2017, Murail *et al.* 2005, Santos *et al.* 2020). The first version of DSP (Murail *et al.* 2005) raised some doubts

about the method itself (e.g. Baumgarten *et al.* 2015). The authors expressed some concerns about the replication of landmarks and considered that DSP did not use a typical logistic regression (i.e. with a classification rule). According to Baumgarten and Kenyon-Flatt (Baumgarten, Kenyon-Flatt 2020), almost all the criticisms of the DSP were resolved and controversial details were clarified in the second version DSP2 (Brůžek *et al.* 2017, Santos *et al.* 2020).

To validate the reliability of DSP2, samples of adult hip bones of known sex were used. The first series was

composed of 120 hip bones from the Maxwell Museum Documented Collection, University of New Mexico. The second series consisted of 503 hip bones of both sexes from the Simon identified collection housed at the Department of Anthropology, University of Geneva, Switzerland (Brůžek *et al.* 2017).

At least 4 out of 10 variables are required to estimate sex using DSP, which is supposed to be the minimum number of variables required to capture a reasonable amount of information on pelvic shape. In the usual framework of linear discriminant analysis, an individual

Read me first DSP V2 Measurements Range variation About DSP V2														
Obs	Pum	Spu	Dcox	limt	lsmm	Scox	Ss	Sa	Sis	Veac	PF	PM	SEX	Status
specimen A		26.00	192.00		96.00	142.00	64.00	80.00	34.00	47.00	0.997	0.003	Female	✓ Computation made
specimen B		28.00	212.00	38.00	112.00						0.02	0.98	Male	✓ Computation made
specimen C		27.00	204.00	42.50	108.00		73.50	74.50			0.308	0.692	N/A	✓ Computation made
spec. D (1)	73.00	25.00	194.00			150.00					0.999	0.001	Female	✓ Computation made
spec. D (2)	69.00	25.00	194.00			150.00					0.984	0.016	Female	✓ Computation made
1A-M	75.20	30.70	227.00	48.00	121.30	164.00	75.00	80.20	39.50	59.40	0	1	Male	✓ Computation made
2A-F	74.00	25.00	191.00	52.00	99.00	142.00	64.00	68.00	35.00	49.00	1	0	Female	✓ Computation made
3A-F	79.00	23.00	210.00	43.00	103.00	161.00	68.00	76.00	33.00	52.00	1	0	Female	✓ Computation made
4A-F	75.30	23.20	202.00	43.00	104.10	156.00	70.20	73.90	36.10	51.30	1	0	Female	✓ Computation made
5A-M	70.50	30.00	206.00	40.00	110.20	142.00	75.70	68.20	37.80	54.10	0	1	Male	✓ Computation made
6A-M	80.10	29.90	226.00	40.50	123.30	171.00	85.20	85.20	45.30	58.60	0.001	0.999	Male	✓ Computation made
7A-F	69.20	24.40	192.00	38.50	99.30	148.00	69.30	68.20	37.00	50.80	0.991	0.009	Female	✓ Computation made
1B-M	80.20	30.70	227.00	48.00	121.30	164.00	75.00	80.20	39.50	59.40	0.012	0.988	Male	✓ Computation made
2B-F	69.00	25.00	191.00	52.00	99.00	142.00	64.00	68.00	35.00	49.00	1	0	Female	✓ Computation made
3B-F	74.00	23.00	210.00	43.00	103.00	161.00	68.00	76.00	33.00	52.00	0.999	0.001	Female	✓ Computation made
4B-F	70.30	23.20	202.00	43.00	104.10	156.00	70.20	73.90	36.10	51.30	0.989	0.011	Female	✓ Computation made
5B-M	75.50	30.00	206.00	40.00	110.20	142.00	75.70	68.20	37.80	54.10	0.023	0.977	Male	✓ Computation made
6B-M	85.10	29.90	226.00	40.50	123.30	171.00	85.20	85.20	45.30	58.60	0.074	0.926	N/A	✓ Computation made
7B-F	64.20	24.40	192.00	38.50	99.30	148.00	69.30	68.20	37.00	50.80	0.675	0.325	N/A	✓ Computation made

☒ Auto refresh

FIGURE 4: DSP2 graphical user interface – simulation of the method's robustness. The first five observations are the native examples given when opening the software (Brůžek *et al.* 2017). The first seven red lines correspond to seven individuals (1A – 7A) randomly extracted from the Suppl. Material file (Brůžek *et al.* 2017). The second seven red lines (1B – 7B) correspond to the same individuals and simulate the robustness of the method: Pum variable was increased by 5 mm in males (feminizing effect) and decreased by 5 mm in females (masculinizing effect). Obs – observation; 1 – male 14_340-Geneva, 2 – female 1_20_g-Paris, 3 – female 2_50_g-Coimbra, 4 – female 5_30_g-Johannesbourg, 5 – male 8_100_g-Cleveland, 6 – male 10_180_d-Washington, 7 – female 12_60_g-Thai. Pum, Spu, Dcox, limt, lsmm, Scox, Ss, Sa, Sis, Veac – measurements (see definitions in Brůžek *et al.* 2017). PF – probability of being female; PM – probability of being male; SEX – assigned sex.

is assigned to the group for which (s)he obtains the maximal posterior probability, i.e. an individual would be identified as male if $p(\text{Male}) > 0.5$, or otherwise female. However, for more reliable sex estimations, DSP2 conforms to the conservative decision rule adopted in osteological studies: a posterior probability of 0.95 is considered a safe classification threshold (Kranioti, Apostol 2014). Any individual who does not reach this value will remain indeterminate. DSP2 can be freely downloaded from the following website: <http://projets.pacea.u-bordeaux.fr/logiciel/DSP2/dsp2.html>, and needs no registration.

The graphical user interface of DSP2 is presented in Figures 4 and 5. Figure 4 shows the importance of the dimensions for a successful classification.

At the same time, it is shown that the DSP2 method is robust, because decreasing (increasing) the value of pubis length (Pum) by 5 mm does not change the resulting classification (Figure 5). A variation of 5 mm for the most important dimension, which is the length of the pubis, is significantly higher than any measurement error. The removal of the 4 most

Read me first		DSP V2		Measurements		Range variation		About DSP V2						
Obs	Pum	Spu	Dcox	limt	Ismm	Scox	Ss	Sa	Sis	Veac	PF	PM	SEX	Status
specimen A		26.00	192.00		96.00	142.00	64.00	80.00	34.00	47.00	0.997	0.003	Female	✔ Computation made
specimen B		28.00	212.00	38.00	112.00						0.02	0.98	Male	✔ Computation made
specimen C		27.00	204.00	42.50	108.00		73.50	74.50			0.308	0.692	N/A	✔ Computation made
spec. D (1)	73.00	25.00	194.00			150.00					0.999	0.001	Female	✔ Computation made
spec. D (2)	69.00	25.00	194.00			150.00					0.984	0.016	Female	✔ Computation made
1C-M					121.30	164.00	75.00	80.20	39.50	59.40	0.004	0.996	Male	✔ Computation made
2C-F					99.00	142.00	64.00	68.00	35.00	49.00	0.91	0.09	N/A	✔ Computation made
3C-F					103.00	161.00	68.00	76.00	33.00	52.00	0.994	0.006	Female	✔ Computation made
4C-F					104.10	156.00	70.20	73.90	36.10	51.30	0.901	0.099	N/A	✔ Computation made
5C-M					110.20	142.00	75.70	68.20	37.80	54.10	0.001	0.999	Male	✔ Computation made
6C-M					123.30	171.00	85.20	85.20	45.30	58.60	0.001	0.999	Male	✔ Computation made
7C-F					99.30	148.00	69.30	68.20	37.00	50.80	0.831	0.169	N/A	✔ Computation made
1D-M		30.70			121.30		75.00		39.50	59.40	0.005	0.995	Male	✔ Computation made
2D-F		25.00			99.00		64.00		35.00	49.00	0.944	0.056	N/A	✔ Computation made
3D-F		23.00			103.00		68.00		33.00	52.00	0.931	0.069	N/A	✔ Computation made
4D-F		23.20			104.10		70.20		36.10	51.30	0.944	0.056	N/A	✔ Computation made
5D-M		30.00			110.20		75.70		37.80	54.10	0.111	0.889	N/A	✔ Computation made
6D-M		29.90			123.30		85.20		45.30	58.60	0.016	0.984	Male	✔ Computation made
7D-F		24.40			99.30		69.30		37.00	50.80	0.961	0.039	Female	✔ Computation made

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FIGURE 5: DSP2 graphical user interface – importance of the dimensions for a successful classification. The first five observations are the native examples given when opening the software (Brůžek *et al.* 2017). The first seven red lines correspond to seven individuals (1C – 7C) randomly extracted from the Suppl. Material file (Brůžek *et al.* 2017), where the first four most important variables were removed (Spu, Dcox, limt). The second seven red lines (1D – 7D) correspond to the same individuals and simulate the robustness of the method: half of the 10 variables were removed (Dcox, Scox, Pum - susceptible to damage, limt, Sa). Obs – observation; 1 – male 14_340-Geneva, 2 – female 1_20_g-Paris, 3 – female 2_50_g-Coimbra, 4 – female 5_30_g-Johannesbourg, 5 – male 8_100_g-Cleveland, 6 – male 10_180_d-Washington, 7 – female 12_60_g-Thai. Pum, Spu, Dcox, limt, Ismm, Scox, Ss, Sa, Sis, Veac – measurements (see definitions in Brůžek *et al.* 2017). PF – probability of being female; PM – probability of being male; SEX – assigned sex.

important dimensions (*Figure 5*) resulted in three females out of seven individuals not being assigned a sex, but not being assigned the opposite sex.

The posterior probability was less than 0.95, but still indicated female sex. We see a similar result if we keep only half the dimensions. The number of unclassified individuals increases because their posterior probability does not exceed the selected probability level of 0.95.

The DSP2 has high reliability of the sex estimation of an individual with a posterior probability equal to or higher than 0.95; however, this comes at the cost of reducing the number of correctly assigned individuals. The least successful combination of 4 dimensions has a sex estimation success rate of roughly 40%, but an extremely low risk of errors – below 2%. Using all 10 measurements guarantees sex estimates in approximately 90% of individuals with an error of less than 1%. Many authors have successfully tested the validity of the DSP method in various populations (e.g. de Almeida *et al.* 2020, Chapman *et al.* 2020, Kranioti *et al.* 2019). The DSP2 method is recommended for creating reference data for sex in ancient populations (Jerković *et al.* 2018). Recently, Kuchař *et al.* introduced a method that allows us to automatically compute the position of the anthropological landmarks used in the DSP2 based on a large amount of CT data (Kuchař *et al.* 2021). Their results showed that DSP2 is a very robust method. In a sample of 100 CT scans of males and 100 CT scans of females, they successfully classified 95 males and 99 females using 9 variables (IIMT removed). Five males were indeterminate and one female was misclassified.

To conclude, if pelvic bones are well preserved, the DSP2 is considered the method of choice. This metric method is population independent and returns reliable results: the accuracy of the sex classification under defined conditions reaches 100% with over 95% posterior probability of correct classification (Nikita *et al.* 2020). It is a reliable technique for sexing the hip bone in forensic anthropology, as well as in bioarchaeology, and conforms to the Daubert standards in forensic anthropology (Brůžek *et al.* 2017, Santos *et al.* 2020).

6.3 Pelvic (parturition) scar and sex estimation

Estimation of sex is very often associated with the evaluation of the parturition or pelvic scars (Praxmarer *et al.* 2020). The presence of a preauricular groove in the preauricular area of the iliac bone has often been considered since the beginning of the 20th century as a sign of childbirth in females (Ullrich 1975). There are still many ambiguities regarding the causes and

significance of this trait, as well as its terminology, methods of evaluation and application in biological anthropology (Igarashi *et al.* 2020, Karsten 2018, Maass, Friedling 2016, McFadden 2020, Ubelaker, De La Paz 2012). The lack of material of known sex with an obstetric history of females prevents the finding of a solution. Recently, McFadden and Oxenham have reported the predictive power of the preauricular groove to be 66% for sex and 52% for parity (McFadden, Oxenham 2018). The problem itself was the correct evaluation of bone relief. Novotný (1979, 1981) proposed a method for the unambiguous distinguishing of male and female morphology of the preauricular area of the pelvis. His technique was adopted by Brůžek (2002) and subsequently by Perréard Lopreno *et al.* (Perréard Lopreno *et al.* 2022). This study shows that in the sample of females with known obstetrical history, the preauricular groove, as defined by Novotný (1981) and Brůžek (2002), is present in significant proportions in both, nulliparous females (73.8%) and females who gave birth (79.8%); therefore, it cannot be considered an indicator of parity from skeletal remains (Perréard Lopreno *et al.* 2022). However, the presence of a preauricular groove is a suitable feature of sex estimation, which is diagnosed in Novotný's method (1981) and, verified by Brůžek (2002) and Mikešová (Mikešová 2008), because it is absent in the pelvis of males.

6.4 Estimation of sex from cranial visual traits

When the postcranial remains of a skeleton are missing or badly damaged, sex is often determined through the visual assessment of sexually dimorphic traits on the skull (e.g. Novotný *et al.* 1993, Shearer *et al.* 2012). However, leaving aside the traditional morphological features on the skull that show sexual dimorphism, which is often recognizable by the untrained eye, sex estimation becomes more difficult without the pairwise comparison and with the presence of ambiguous trait expressions, as well as the effects of population variation (Garvin 2020).

In addition to the method proposed by Ascádi and Nemeskéri (Ascádi, Nemeskéri 1970), codified by the "European Recommendations" (Ferembach *et al.* 1980), and Buikstra and Ubelaker (Buikstra, Ubelaker 1994), there is a more detailed but similar method published by Walker (Walker 2008), which is based on 5 visual traits of the skull. Walker provides score frequencies for males and females and sex probabilities associated with each univariate score. He also presents logistic discriminant functions, allowing practitioners to input their trait scores into one of the provided equations to

achieve the final sex estimate more objectively. This increases the statistical rigor, although some subjectivity in the scoring method remains. The exclusion of some variables from the equations and variable coefficient weights suggests that certain traits are more useful than others (Garvin 2020).

Many authors have validated the five-degree method for evaluating the morphological features of the skull (e.g. Garvin *et al.* 2014, Walrath *et al.* 2004), as well as Walker's discriminant functions (e.g. Lewis, Garvin 2016) and other classification methods that use this scoring system (e.g. Garvin, Klales 2018, Langley *et al.* 2018, Stevenson *et al.* 2009). The overview of validation publications and detailed analyses are given in Garvin (Garvin 2020). It should be noted that the method, which uses the evaluation of 5 visual features of the skull, achieves an accuracy that is generally lower than the one reported by Walker (2008), in the range of 60 to 93%, which may depend on the population in which the method is used.

The practical application is facilitated by the MorphoPASSE program and an accompanying manual that can be found at <https://www.morphopasse.com/>. The database itself is accessible via R Studio www.shinyapps.io/MorphoPASSE. The five Walker (2008) traits should be scored using the MorphoPASSE manual (Klales, Cole 2018), not using the original publications, because modifications were made to the traits (Klales 2020d). MorphoPASSE allows the researcher to select the binary logistic regression equations provided in the original publications for sex classification and provides calculation of posterior probabilities of sex affiliation. The software also includes random forest modelling that is the recommended approach in MorphoPASSE and is a flexible machine learning algorithm that creates a series of decision trees using bootstrap aggregating of random training subsets. The MorphoPASSE is a free program that provides a user-friendly tool for using the Walker traits (2008) for reliable and valid sex estimation (Klales 2020d).

6.5 Use of cranial morphometrics in sex estimation

Any skeletal element, provided that a reference population is available, can be used to estimate sex metrically. However, the accuracy of the sex classification is usually lower than that achieved by the hip bone (Nikita *et al.* 2020). In contrast to the pelvic bone, the main drawback of the cranium is that its expression of sexual dimorphism is population specific (e.g. Bertsatos *et al.* 2020, Garvin, Klales 2020, Kotěrová *et al.* 2016, Oikonomopoulou *et al.* 2017).

Any attempts to develop standards for sex estimation of human skeletal remains must take into account that the pattern of sexual dimorphism varies among human populations. Therefore, it is important to avoid the application of metric standards proposed in different populations and time periods than those of the studied sample. Furthermore, as several studies have shown, body size has changed over generations in the population as a consequence of secular trends. These changes are especially related to body height, which directly affects adult morphology, including the stature, long bone lengths and cranial size and shape (Langley, Jantz 2020).

According to Garvin and Klales (2020), global equations derived from a multi-population database may be suitable for paleoanthropological or large intercultural analyses. However, although global standards may seem an adequate solution, they are less representative at the individual level and provide less information of sex classification and less accuracy in forensic anthropology (Garvin, Klales 2020). They argue that global equations are not the right answer when the goal is individual identification. The authors also realize that the need for population-specific sex estimation methods presents several unrealistic and impracticable problems. They see the solution in the building of global databases of skeletal data that would be accessible to everyone. Before this idea can be realized, it is necessary to use the tools we have at our disposal.

Methods for quantitative sex estimation were consolidated into the computer program Fordisc in 1993 (Jantz, Ousley 2005). The Fordisc® 3.1 (current version) computer program was developed to assist in the identification of the sex, stature and ancestry of skeletal remains and calculate discriminant functions using a maximum of 34 cranial and 39 postcranial measurements. The Fordisc reference database includes samples from the Forensic Anthropology Data Bank (FDB) and populations from the Howells world-wide craniometrics dataset. More details about the Fordisc software and its application can be found in (Jantz, Ousley 2020, Manthey, Jantz 2020). Although widely used, this tool has recently been criticized for its low accuracy of sex estimation in a population that is not included in its reference samples (e.g. Guyomarc'h, Brůžek 2011, Ramsthaler *et al.* 2007, Urbanová *et al.* 2014). For these reasons, we do not anticipate its use to estimate sex in bioarchaeology.

A certain extension of possible tools for sex estimation in forensic anthropology is the use of geometric morphometry. 3D-ID (<https://www.3d-id.org/>)

home) is a good example of the application of geometric morphometrics in the forensic context (Ross *et al.* 2012). It is a program that is designed to estimate the sex and ancestry of an unknown individual using geometric morphometrics within forensically relevant groups (Manthey, Ousley 2020). The accuracy of the 3D-ID method regarding sex estimation in two European samples ranged from 74% to 87% for cases with unknown ancestry (Bertsatos *et al.* 2019). Urbanová *et al.* compared the software programs Fordisc and 3D-ID to assess sex and ancestry in a Brazilian population (Urbanová *et al.* 2014). For the sex estimation, they achieved accuracies ranging from 60% to 70%. The lower accuracy of the 3D-ID method was also confirmed by Lottering (Lottering 2020).

6.6 Sex estimation in bioarchaeology

There are relatively few morphometric methods for sex estimation from the postcranial skeleton (Stock 2020); those that exist should be used with caution due to the population specificity and secular trend (Kotěrová *et al.* 2016). Surprisingly, with the exception of publications on sex differences in metric variables, indices and discriminant function analyses, there is no appropriate morphometric method to fill this gap that could be used especially in bioarchaeology. How do we avoid the fact that morphometric methods are strongly population-specific? How do we cope with the fact that bone fragmentation leads to unpredictable results? These questions are also related to the problems of standardization of methods, as suggested by Garvin and Klaes (Garvin, Klaes 2020). While it is possible to propose the design of population standards for recent populations based on CT scans from medical imaging, this approach is impossible for past human populations in bioarchaeology.

This gap was filled by Murail *et al.* (Murail *et al.* 1999) starting from a former idea by Van Vark (Van Vark 1971). They proposed a population-specific approach for sex estimation when the hip bone cannot be used for all the individuals of the sample under study. Their approach includes two steps. In the first step, a reliable sex estimation method is used on a subsample of individuals with sufficiently preserved hip bones. This step is called the "primary diagnosis of sex". The second step includes several extrapelvic measurements taken in sexed individuals in the first step and a calculation of population-specific discriminant functions and their application to individuals from the same skeletal assemblage without hip bones. The second step is called "secondary sexual diagnosis". These two practical

adjustments ("primary and secondary sex diagnoses") were successfully tested in a sample of known sex and lead to a classification with 94.5% accuracy (Murail *et al.* 1999). This approach significantly improves sex determination in archaeological samples (e. g. (Dentz *et al.* 2020, Saliba-Serre, Schmitt 2012, Sosna *et al.* 2008, Thomas 2014). The use of population specific discriminant functions for various parts of the skeleton have been used in numerous studies where individuals estimated by primary sex diagnosis were used as a training sample to generate population-specific discriminant functions for other bones (e.g. Brůžek, Velemínský 2006, Dittrick, Suchey 1986, Stojanowski 2003, Wrobel *et al.* 2002).

The computational use of the approach proposed by Murail *et al.* (1999) can be cumbersome when working with highly fragmented material and implies many difficult repetitive tasks when performed manually (Santos 2021). He designed a R package "rdss" with a graphical user interface that facilitates all the steps of the "secondary sex diagnosis" and allows easy assessment of the quality and relevance of the statistical models built and used during this process. The required steps for installing the package are detailed in its GitLab repository (<https://gitlab.com/f-santos/rdss>).

6.7 Applications of sex estimation methods in paleoanthropology

Many paleoanthropological studies attempt to reconstruct the life of past populations and understand their anatomical variability (e.g. Estalrich, Rosas 2015, Frayer 1980, Sládek *et al.* 2016, Villotte *et al.* 2010). As such, sex is an important factor that needs to be considered; therefore, reliable sex diagnosis is important in the paleoanthropological domain as well.

Earlier studies have often postulated the sex of a fossil individual without any detailed description of criteria that had led an author to such a conclusion (Brůžek *et al.* 2005). This often led to a situation when a fossil individual was a male for some researchers, but a female for others. Sex was predominantly derived from the skull or the overall size and robusticity (Novotný 1983). However, as we have already indicated, skull and overall size are very population specific. Recent populations are structurally different (Velemínská *et al.* 2008) and more gracile (Šefčáková *et al.* 2011), which led to the predominant classification of males among fossil humans (Brůžek *et al.* 2004, Genovés 1954). For example, regarding elevated body stature, an individual from the Upper Paleolithic site Barma del Caviglione had been considered a male since its discovery in 1876

until the pelvic remains revealed that the individual was a very tall female (Brůžek *et al.* 2016). Furthermore, fossil remains are objects of many interpretations and in some cases, sex was also derived from the burial context and speculations about its meaning. A typical example is the triple burial from Dolní Věstonice. Here the middle individual was considered a female with regard to the orche in the pubic area of this individual being interpreted to mean an unsuccessful birth, as well as the significance of the hands of the right-side male reaching this region (Alt *et al.* 1997, Bahn 1988). A thorough analysis revealed that the middle individual was a pathological female (Formicola *et al.* 2001) while the pelvis indicated male sex (Brůžek *et al.* 2006), which was subsequently confirmed genetically (Mittnik *et al.* 2016). This example shows that the burial context should be considered very cautiously, as it is strongly influenced by the researcher's perception, especially in prehistoric cases. At the same time, genetic analyses confirmed in this and many other cases (Fu *et al.* 2016, Hansen *et al.* 2017) that sexing methods based on the hip bone are universal and applicable even on fossil modern humans. Therefore, many studies use modern sex estimation methods from the hip bone on paleoanthropological specimens from the end of the Paleolithic (Gambier *et al.* 2006, Guyomarc'h *et al.* 2017, Hansen *et al.* 2017, Henry-Gambier *et al.* 2002, Samsel *et al.* 2016, Villotte *et al.* 2017).

Despite the applicability of modern sexing methods on the fossil modern human specimens, the fragmentary state of the pelvic remains often limits their use. When the hip bone cannot be used, it is possible to attempt a secondary sex diagnosis (Murail *et al.* 1999). This approach was further developed in the Upper Paleolithic and Mesolithic sample by Villotte *et al.* (Villotte 2009, Villotte *et al.* 2007, 2010, 2011). Specimens with a preserved hip bone were used for primary sex diagnosis and to develop population specific equations that were used to sex other individuals. From the forensic perspective, this approach may seem imperfect, as the reference sample may cover thousands of years and different geographical locations; however, with regard to the scarcity of fossil material, this is currently the best available approach. Furthermore, it was demonstrated that population specific equations may achieve high accuracy in the reference sample of Paleolithic specimens; thus, the use of such secondary sex diagnosis can be considered reliable (Rmoutilová *et al.* 2018).

Modern sexing methods from the pelvis can be reliably applied to fossil modern humans. However,

older fossils and other hominins still pose a problem in the estimation of sex. The main question is when the modern type of pelvic sexual dimorphism appeared. Answering this would give limits to the use of modern sexing methods. Unfortunately, the older the fossil material, the more fragmentary it is, resulting in less known variability and thus greater uncertainty about the kind of sexual dimorphism.

Two key aspects in the assessment of pelvic sexual dimorphism are locomotion and feto-pelvic dimensions. Early hominins, with low feto-pelvic disproportion, may not show strong pelvic sexual dimorphism, so other methods based on body size dimorphism may be more relevant to assess their sex (Brůžek *et al.* 2005). The delivery of large-brained offspring may have been a significant factor by the early Pleistocene, resulting in considerable pelvic sexual dimorphism (Simpson *et al.* 2008). However, high feto-pelvic disproportion does not necessarily lead to similar sexual dimorphism as in modern humans. For example, Neanderthals certainly had difficult childbirth (Franciscus 2009), but their pelvis is structurally different from that of modern humans. The most significant difference is in the length of their pubis, which is very long in both females and males (Rak, Arensburg 1987, Rosenberg 1988). Based on modern human sexual dimorphism, all Neanderthal specimens with preserved pubis would be classified as females (Novotný 1983). Therefore, the sex of Neanderthals is usually assessed by the visual description of sexually dimorphic traits excluding the pubic region (Bonmatí, Arsuaga 2007, Trinkaus 1980, 2016).

The call to use real tested methods of sex estimation in paleoanthropology raised by Novotný (Novotný 1983) has not been fully addressed yet. Fossil modern humans can be reliably sexed by modern pelvic methods or secondary sex diagnosis. On the other hand, to develop a sexing method suitable for non-modern fossil species is not an easy task because the real sex of fossil specimens is not known with certainty except for individual cases when it was possible to make a genetic analysis (Skoglund *et al.* 2013). Genetically sexed individuals may be useful in verifying the suitability of morphological sexing methods; however, DNA analysis cannot be considered the only or even the main manner of sexing fossils, as it is a destructive method and the fossil material is scarce. In order to assess sex in fossil hominins morphologically, it is important to understand the evolutionary history. Therefore, it is preferred to use modern tested methods while every case is assessed individually and caution is taken in non-modern species.

7. CONCLUSIVE NOTES AND RESEARCH PERSPECTIVES

In conclusion, we would like to emphasize three problem areas that should be addressed by future research. The first area concerns the real risk of errors in methods of biological profile estimation. How accurate must reliable methods of biological profiling be? An accuracy limit of 85% has been proposed for sex estimation methods and is considered acceptable (Digangi, Moore 2013); however, it appears to be too low and not in accordance with the Daubert rule, as it allows a 15% risk of error. Given that many osteological methods used in biological profile determination are population specific, the validation studies of these techniques are essential. Very importantly, the potential error rate must be known and acceptable (Bonczarowska *et al.* 2021). In the forensic environment, the accuracy of used sex estimation methods is required to be at least 95% (Scheuer 2002). An absolute majority of studies demonstrate the accuracy (percent of correct classification) of currently available sex estimation methods and recommend those methods to be used for sex estimation. However, much more important is the clearly defined risk of errors. This cannot be known, as Galeta and Brůžek (2020) showed, if we use a probability level of 0.5 as the sectioning point or value of the classification technique and ignore the overlapping area of the distribution of the male and female sample. Future research should address this issue.

The second area concerns the methods we use in practice. That is an aspect to bear in mind because biological anthropology is one of the disciplines with a number of methods that must be adapted to many specific circumstances depending on, for example, population, the preservation state, or possible taphonomic alterations (Valsecchi *et al.* 2019). It is still true that there is little emphasis on defining criteria for selecting the most appropriate method. In this context, Buckberry states for age-at-death estimation that anthropologists tend to use methods they have learned during their academic education, or methods they have designed for researchers, rather than using stricter scientific criteria (Buckberry 2015). Historically, qualitative assessments dominated sex estimation in biological anthropology; however, there has been a perceived shift in the past several decades toward the development and greater use of quantitative approaches. The extent to which the standardization of methods for sex estimation has been implemented by the biological anthropology community as a whole is currently

unknown (Klaes 2020a) and "*preferred sex estimation methods will vary according to the anthropologist's personal preferences and experience*" (Garvin 2012). For the future progress of biological anthropology, it will be important to remove the boundaries that divide the world of academics who propose methods and the world of practitioners who, for various reasons, do not use them in practice.

The last area is closely related to the previous area and concerns the facilitation of the use of methods, enabled by software programs and the web interface. "*Statistical programs have revolutionized the way in which forensic anthropologists (as well as bioarchaeologists) conduct casework by allowing practitioners to use computationally complex analytics at the click of a button*" (Berg, Kenyhercz 2017). We did not mention much about the use of geometric morphometry in estimating biological profile parameters. Despite the high probabilities of classification accuracy, the methods of geometric morphometrics are not translatable into a user-friendly method that others can apply practically (e.g. Bertsatos *et al.* 2020, Bytheway, Ross 2010, Čechová *et al.* 2019, Rmoutilová *et al.* 2017, Sorrentino *et al.* 2020). As Garvin and Klaes (2020) point out, increased globalization in communication, awareness and expanded access to collections and resources, are crucial to the development of any type of global standardization in biological profile estimation methods (Garvin, Klaes 2020).

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