forensic sciences

Review

The Estimation of Sex of Human Skeletal Remains in the Portuguese Identified Collections: History and Prospects

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Abstract: The estimation of biological sex is of paramount significance for the identification of anonymous skeletal remains in contemporary medico-legal contexts or bioarcheological studies of past societies. Sex estimation techniques are usually affected by population-specific sexual dimorphism. As such, the need for distinctive standards for each population has long been acknowledged. This paper aims to document and critically address sex estimation methods that have been created, or tested, in Portuguese identified skeletal collections in a historically situated dimension. Moreover, the ever-changing landscape of forensic anthropology calls for a deep reflection about a plethora of issues regarding sex estimation in human remains, including the usage of medical imaging methods and innovative statistical approaches, the biological and social resignification of sex and gender, the problems arising from secular change, and the ethics involving the use of human skeletal collections within the discipline.

Keywords: biological profile; skeletal sex; Portugal; forensic anthropology

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Citation: Curate, F. The Estimation of Sex of Human Skeletal Remains in the Portuguese Identified Collections: History and Prospects. *Forensic Sci.* **2022**, *2*, 272–286. https://doi.org/10.3390/forensicsci2010021

Academic Editors: Francisca Alves Cardoso, Vanessa Campanacho and Claudia Regina Plens

Received: 25 February 2022
Accepted: 18 March 2022
Published: 21 March 2022

1. Introduction

In the beginning there is something: the estimation of biological sex, a parameter of critical importance in the identification of unidentified skeletal remains both in contemporary forensic contexts and bioarcheological studies of past societies. Sex pertains to the biological and/or genetic attributes of an individual, and according to which it is classified as female, male or intersex. The conventional anthropological workflow for the evaluation of a biological profile—i.e., sex, ancestry, age at death and stature—often begins with sex assessment, as the analyses of age at death and stature are sex-contingent [1–3]. The estimation of sex in skeletal remains depends on the identification and evaluation of the phenotypic differences between the skeletons of males and females [4]. Differences in size and shape are unequally expressed throughout the skeleton, and the pelvis is generally considered the most dimorphic skeletal region [5,6].

In humans, pelvic sexual dimorphism is deeply associated with the selective pressures of bipedal locomotion and parturition but adaptative dissimilarities between sexes also stem from sexual selection [7–10]. It has been also suggested that hormonal mediation of the female pelvis is partially dependent on developmental plasticity [11]. Pelvic bones are fragile, particularly the pubic region [12], and when they are absent or extensively fragmented other skeletal elements are used to estimate sex [13]. Within the conventional frameworks of analysis, the cranium is usually the following skeletal region to be considered when estimating sex, but long bones seem to provide a better prediction performance [14].

Molecular methods, namely proteomic and genomic analyses, show high accuracy in the estimation of skeletal sex, but they are costly, technically challenging and reliant on data quality [13,15,16]. Thus, methods for sex assessment classically fall in two comprehensive categories, the morphoscopic (i.e., morphological, or visual) and metric approaches [2,13]. Decision making with morphoscopic methods is inherently more subjective as these approaches are observer-dependent and biased. Metric approaches are more reliable and interpretable, with a less steep learning curve [13,17].

Excepting pelvic-centered methods, both morphological and metric techniques are highly affected by population-specific factors [6,18], including robusticity or body shape and size. Populations also differ regarding the morphological expressions of sexual dimorphism [19] and secular change [19,20]—the regional diversity of the skeletal system obviously constrains the wide-ranging application of sex estimation methods, substantiating the need for geographic-specific methodologies and databases.

Significantly, most of the seminal methods for the assessment of sex are historically rooted in the 20th century North-American academic milieu, within an emerging scientific genealogy that was nurtured by institutional support, interested researchers and the availability of documented skeletal collections [21]. The global development of forensic and bioarcheological research agendas, combined with the establishment of new reference collections around the world, abetted the study of skeletal sexual dimorphism in different populations and a growing number of regional-based methods for the estimation of sex is now available, e.g., [22–32]. Likewise, a plethora of standardized recommendations that can be used to estimate sex from unknown skeletal remains have been developed and/or tested in Portuguese documented collections. These methods hail from a long research tradition within an earlier landscape of typological and descriptive skeletal anatomy, and, of course, from the availability of different skeletal reference collections in the country.

The cardinal purpose of this article consists of a descriptive historical enumeration of the sex estimation methods that have been conceived, or evaluated, in Portuguese documented skeletal collections. Additionally, the ever-changing landscape of forensic anthropology calls for a deep critical reflection about an aggregate of issues regarding sex estimation in human remains, particularly when in Portuguese contexts.

2. The Portuguese Reference Skeletal Collections

There is no resurrection of the dead, as explained by Friedrich Schiller's Phillip the Second, King of Spain (1787), but one is tempted to remember the biblical Lazarus. Osteological laboratories are prone to resurrection myths, as it is known that all skeletons have close ties to the world and its circumstances. This is especially true in the case of documented (also known as «reference» or «identified») skeletal collections, receptacles of human skeletal and dental variation [33], that first emerged in a context where the anthropological Other began to be observed, illustrated, and defined [34]. The standard perspective regarding reference collections portrays them as primary teaching resources and the cornerstone for the creation of fundamental techniques in both forensic anthropology and bioarcheology, including methods for the estimation of sex, age, and stature [35], or the optimization of differential diagnoses in paleopathology. The relevance of these biological and social archives is closely entwined with a series of parameters, such as the number of individuals represented, the completeness and preservation of the skeletons, the sexual and age distribution, and particularly the existence of documentary biographical information about each of the individuals [35–37].

Portugal has been bountiful to those that study human remains, as Francisca Alves-Cardoso [38] aptly described it, and one of the reasons for that stems from the unusually large number of Portuguese documented collections that have been assembled and curated for more than 100 years [38,39]. For comprehensive depictions of the Portuguese documented collections see, e.g., Rocha [40], Alves-Cardoso [38], and Lopes and Fernandes [39]. Ethical and legal considerations primarily focused on these collections have been published in the last few years [35,37,38], outlining a paradigmatic shift towards a more vigilant gaze onto documented skeletal remains.

The physician Francisco Ferraz de Macedo pioneered the first reference collection in Portugal—also one of the earliest in Europe—between 1882 and 1889, that comprised over a thousand skulls and circa 300 skeletons. The Ferraz de Macedo collection was donated in 1907 to the Bocage Museu (now the National Natural History and Science Museum of the University of Lisbon) but, unfortunately, it was almost completely destroyed in 1978 during a fire. During the 1980s and later, Luís Lopes and Hugo Cardoso resumed Macedo's efforts and amassed a new collection—the Identified Collection of the National Natural History and Science Museum of the University of Lisbon—that currently comprises over 1800 individuals, deceased between 1880 and 1975 [41].

By the end of the 19th century, Bernardino Machado began to collect human skulls from the medical schools of Porto and Lisbon, and the anatomical museum of the University of Coimbra, establishing the Medical Schools Collection (632 skulls) in the University of Coimbra. After 1907, Eusébio Tamagnini strived for the creation of two new collections: the Coimbra Identified Skeletal Collection, comprising 505 individuals, and the International Exchanges Collection composed of 1142 skulls. The Coimbra skeletal collections, at present curated at the Department of Life Sciences (DCV) of the University of Coimbra, originate from unclaimed bodies of the largest municipal cemetery, Cemitério da Conchada. The latter collections include individuals that died between 1904 and 1936/37, while the first comprises individuals deceased between 1895 and 1903 [40,42]. Recently, a new identified skeletal collection was accrued at the DCV, University of Coimbra, emerging from a partnership with the Santarém City Council. The 21st Century Identified Skeletal Collection is composed of 302 adult individuals that died between 1982 and 2012 [43,44]. A sub-collection of experimentally burned skeletons is being amassed from the 21st Century Identified Skeletal Collection since 2013 and currently comprises 56 individuals [44].

During the second decade of the 20th century, the anthropologist António Mendes Correia assembled a documented skeletal collection at the University of Porto from unclaimed bodies recovered in cemeteries from the city of Porto. Until recently, the Mendes Correia collection was all but forgotten, obfuscated into the margins of history for unspecified reasons. Even though the original documentation of the collection is lost, basic biographic information about each individual was collected by Hugo Cardoso and Luísa Marinho [33]. The collection is currently curated at the Natural History Museum and the Faculty of Sciences of the University of Porto, and features a total of 99 individuals. The collection includes individuals who died before 1912 and probably much earlier. A partnership between the BoneMedLeg research project and the municipality of Porto is at the basis of another documented skeletal collection, established between 2012 and 2014, which comprises 95 individuals (81 fully identified) [45]. All individuals died between 1969 and 2003. The BoneMedLeg collection is presently being curated at the North Delegation of the National Institute of Legal Medicine and Forensic Sciences, I.P.

The Identified Skeleton Collection of Évora is the southernmost Portuguese reference collection, established through an agreement between the Laboratory of Biological Anthropology of the University of Évora (LABUE) and the Évora City Council. It comprises 208 individuals deceased between 1870 and 1993, and it is housed in the LABUE at the Department of Biology of the University of Évora [39].

These are in brief the Portuguese collections that have been recursively—though unevenly, as we shall see—used to generate and/or test methods for estimating skeletal sex within the procedural landscapes of forensic anthropology and bioarcheology.

3. A Brief History of Sex Estimation in the Portuguese Collections

The development of scientific techniques is a historical process that is open to description and the possibility of commentary about the technical language, protocols, conventions, and research agendas, among others. The methods for the estimation of sex created, or tested, in the Portuguese identified skeletal collections are also embedded in history, occurring (or co-occurring) in a sequence of events dictated by social, political, and scientific injunctions as well as individual objectives and knowledge.

The Portuguese documented collections embody the ideal ground for developing and testing a variety of hypotheses and methods [46] and, from early on, sexual dimorphism as expressed in the human skeleton was studied by different researchers.

In the first decades of the 20th century, António Mendes Correia used the identified collection assembled by himself (the Mendes Correia collection [33]) and produced a number of articles that, among other research objectives, documented sexual differences in the skeleton, including the vertebral column [47], the shoulder girdle [48,49] and the arm [50,51]. Anthropometric reports generated in the Coimbra Identified Skeletal Collection (CISC) that investigate sexual dimorphism in the skeleton also abound, with research efforts surveying the cranial orbit [52], the pelvis [53], the radius [54], the mandible [55], the sternum [56], the scapula [57], and the ulna [58], among others. Eusébio Tamagnini and Daniel Vieira de Campos studied a sample from the (now disappeared) Ferraz de Macedo collection, with a particular focus on the sex characteristics of the femur [59]. The authors propose the distinction of the sexes based on descriptive statistics of the femoral head diameter and the breadth of the distal articular surface. The majority of these research activities evaluated the metric manifestations of sexual dimorphism, but there are some exceptions, namely the morphological depictions of the radius [54] and the ulnar proximal articular area [58] by Maria Augusta Neto, of the sternum by José Antunes Serra [56], and of the scapula by Maria Helena Xavier de Morais [60]. Research focusing on the sexual dimorphism of the human skeleton has endured in Portuguese collections over the years [61,62].

The anatomical description of different skeletal regions with a focus on sex differences has a long tradition in the Portuguese biological anthropology school, and most of these works are still relevant today as they provide early glimpses into the study of skeletal sexual dimorphism in Portugal and a wealth of statistical data (including raw data) that has been employed for newly oriented research on sex estimation [63,64]. Yet, the classical osteometric monographs and reports generally comprise typological, highly descriptive, analyses that cannot formally be considered methods for sex estimation.

In order to identify the relevant literature regarding sex estimation in Portuguese reference collections, a survey was conducted on the Pubmed (https://pubmed.ncbi.nlm.nih.gov/ (accessed on the 31 January 2022)), Web of Science (https://www.webofscience.com/ (accessed on the 31 January 2022)) and Dimensions (https://www.dimensions.ai/ (accessed on the 31 January 2022)) databases. Publication assessment focused on titles, abstracts and keywords with no data constraints, and a multilingual approach was implemented for the sake of search maximization. Keywords used in the survey include "sex estimation", "sex assessment", "sex diagnosis", "sexual dimorphism", "Portugal", "Portuguese collection", "estimativa do sexo", and "estimation du sexe". An experience-based search was also conducted. A potential limitation of the reported results stems from the decision not to include non-peer-reviewed documents, such as PhD theses and MSc dissertations. However, the selected literature embodies certified, reviewed knowledge. All surveyed techniques for the sex estimation of both adult and non-adult skeletons created and/or tested in Portuguese reference skeletal collections are summarized in Tables 1 and 2.

Table 1. Methods for the estimation of sex in adults created and/or evaluated in Portuguese skeletal reference collections.

Study Reference	Skeletal Region	Collection	Sample Size	Statistical Approach
Cogoluenhes (1984) [65]	Humerus	CISC	—	LDA
Cunha and Van Vark (1990) [66]	Skull	CISC	100	LDA
Bruzek (1995) [67]	Tibia	CISC	95	LDA
Carretero et al. (1995) [68]	Humerus	CISC	154	LDA
Houët et al. (1995) [69]	Pelvis	CISC, SC	220	LDA
Silva (1995) [70]	Calcaneus, Talus	CISC	165	LDA
Wasterlain and Cunha (2000) [71]	Humerus, Femur	CISC	200	SP
Bruzek (2002) [72]	Pelvis	CISC, PC	240	Visual
Murail et al. (2005) [73]	Pelvis	CISC, Others	232	LDA
Garcia (2012) [74]	Tibia	LC	160	—
Albanese (2013) [75]	Clavicle, Humerus, Radius, Ulna	CISC, Others	—	LR
Garcia-Parra et al. (2014) [76]	Sternum	GC, CEI21, CISC	50/100	LDA
Santos et al. (2014) [77]	Skull	CISC, Others	107	LDA, LR, SVM
Navega et al. (2015) [78]	Tarsal Bones	CISC, CEI21	300/60	Diverse ML
Gama et al. (2015) [79]	2nd Cervical Vertebra	CISC, CEI21	190/47	LR

Table 1. *Cont.*

Study Reference	Skeletal Region	Collection	Sample Size	Statistical Approach
Curate et al. (2016) [80]	Proximal Femur	LC, CISC	252/196	LR, C4.5
Bruzek et al. (2017) [81]	Pelvis	CISC, Others	232	LDA
Curate et al. (2017) [82]	Proximal Femur (DXA)	CISC, CEI21	224/60	LDA, LR, REPTree, CART
Curate et al. (2017) [83]	Femur	CISC, CEI21	200/58	SVM, LR, LDA, REPTree
Ammer et al. (2019) [84]	Humerus	CEI21	151	LDA
Coelho and Curate (2019) [63]	Pelvis	CISC	256	Diverse ML
Luna (2019) [85]	Teeth (Canines)	CISC	115	LDA, LR
Amores-Ampuero (2020) [86]	Vertebrae	CISC	73	LR
Rozendaal et al. (2020) [87]	Cervical Vertebrae	AC, LC	160	LDA
Franco et al. (2021) [88]	Teeth	CISC	135	LR
Curate et al. (2021) [89]	Calcaneus, Talus	CISC	180	SVM, LR, C4.5
Curate et al. (2021) [64]	Radius	CISC, LC	364/50	SVM, LR, C4.5

CISC, Coimbra Identified Skeletal Collection; SC, Spitalfields Collection; PC, Paris Collection; LC, Lisbon Collection; GC, Granada Collection; CEI21, 21st Century Identified Skeletal Collection; AC, Athens Collection; Sample Size, pertains only to the Portuguese collections; LDA, linear discriminant analysis; SP, sectioning points; LR, logistic regression; SVM, support vector machines; ML, machine learning; C4.5, decision tree algorithm; REPTree, reduce error pruning tree; CART, classification and regression tree.

In 1995, a special number of the journal *Antropologia Portuguesa* dedicated to the Coimbra identified osteological collections featured new methodological proposals for the assessment of sex in the skeleton. The methods employed different bones, including the tibia, the calcaneus and talus, the humerus, and the pelvis, and established novel orientations toward the estimation of sex in Portuguese collections. Nonetheless, the first unequivocal proposal of a statistically complex technique for the estimation of sex created in the Portuguese identified skeletal collections appeared before, in 1990, proposed by Eugénia Cunha and Gerrit Van Vark [66,90]. The authors studied 570 skulls (213 females and 357 males) from the Medical Schools Collection (DCV, University of Coimbra), performing 61 measurements (with 13 angles calculated from these measurements). Linear discriminant analysis was used to generate models for sex prediction with a correct classification of 80.1% (bias of 2.1%). More recently, a cranial sample from the CISC (107 individuals, 54 females and 53 males) was set as a training model with different classical and machine-learning algorithms, and the validity and reliability of the different models were assessed in other samples [77]. Canine teeth metrics were also used to develop models for sex estimation with a sample from the CISC (115 individuals, 53 females and 62 males) [85]. Discriminant and logistic regression formulae produced accuracies between 77.4 and 86.5%, with posterior probabilities ranging from 0.71 to 0.85. Another study evaluated the usefulness of the mandibular first molar and canines for sex estimation in a sample from the CISC (135 individuals, 78 males and 57 females). Logistic regression models attained accuracies ranging from 60.7% to 74.6% [88].

The pelvic girdle is consensually considered the most reliable skeletal element for the ascription of sex in human remains and has been thoroughly investigated in the Portuguese reference collections since the seminal descriptive work by J.A. Serra in 1938 [53]. Almost 60 years later, Houët et al. [69] presented a series of discriminant functions based on measurements taken from 163 individuals (89 females and 74 males) of the CISC and 57 individuals of the Spitalfields Collection (30 females and 27 males). The correct prediction of sex ranged from 91.4 to 98.6%. A sample of hip bones from the CISC (232 individuals, 102 males and 132 females) was also included in the worldwide study base (2040 adults of known sex from 12 reference populations) of the Diagnose Sexuelle Probabiliste (DSP) [73], a highly accurate free-access metrical tool for the estimation of sex, and its developments [81]. The original raw data provided by Serra [53] were used to create osteometric models for sex assessment executed through an online decision support system, CADOES (https://osteomics.com/CADOES/ (accessed on the 7 February 2022)) [63]. This method, centered around a machine-learning approach, offers user-customized models that can be applied with high accuracy and low bias in Portuguese populations. Finally, a visual method for the determination of sex using the os coxae was proposed by Jaroslav Bruzek [72], providing a correct sex estimation in 95% of cases. The method employed two reference samples, of which one was from the CISC (240 individuals, 106 males and 134 females).

Table 2. Methods for the estimation of sex in non-adults created and/or evaluated in Portuguese skeletal reference collections.

Study Reference	Skeletal Region	Collection	Sample Size	Statistical Approach
Vlak et al. (2008) [91]	Ilium	LC	56	—
Cardoso and Saunders (2008) [92]	Ilium	LC	97	—
Cardoso (2008) [93]	Teeth (adults and non-adults)	LC	107/49	SP, LR
Luna et al. (2017) [94]	Ilium	CISC	34	LDA, LR
Calleja et al. (2020) [95]	Ilium	LC	61	—
Lamer et al. (2021) [96]	Long bones	LC	102	—
Luna et al. (2021) [97]	Ilium	LC, GC	40	—

LC, Lisbon Collection; CISC, Coimbra Identified Skeletal Collection; GC, Granada Collection; Sample Size, pertains only to the Portuguese collections; LDA, linear discriminant analysis; SP, sectioning points; LR, logistic regression.

Contrary to long-held beliefs, postcranial elements, and particularly long bones, present better performance metrics in the assessment of sex when compared with the skull [14]. Population-specific methods based on different long bones have also been created in the Portuguese reference collections.

A multivariate analysis of the humerus in a sample of the CISC (154 individuals, 76 females and 78 males) showed the importance of this bone for sex estimation, and a discriminant analysis classification function with three metric variables correctly classified 94.2% of the individuals [68]. In the same collection, Wasterlain and Cunha [71] studied 200 individuals (100 females and 100 males) and proposed sectioning points for three metric variables of the humerus, with a reported accuracy of 90.0–90.5%. Sex estimation through visual analysis (geometric morphometrics) of the distal humerus was also undertaken in a sample from the 21st Century Identified Skeletal Collection (CEI21, 151 individuals, 80 females and 71 males). The trochlear constriction performed inadequately, properly classifying 63.6% of the individuals, while the olecranon fossa presented accuracies ranging from 88.1 to 94.0%. This technique was implemented through a web-based application (Ammer-Coelho, https://osteomics.com/Ammer-Coelho/ (accessed on the 7 February 2022)) that does not require training or knowledge in geometric morphometrics. In addition to these methods developed for the humerus, a diminutive—and methodologically insufficient—paper published in 1984 tested a discriminant function created for a prehistoric site in a humeral sample from the CISC with an accuracy of 84.0% [65].

The radius has been seldom employed in the creation of methods for sex estimation, with only one technique developed in Portuguese identified collections. Ten radius measurements amassed in a sample of 364 individuals (166 females and 198 males) from the CISC were employed to generate models for sex estimation that were evaluated in the Identified Collection of the National Natural History and Science Museum of the University of Lisbon (Lisbon Collection, 50 individuals, 25 females and 25 males) [64]. The models were created with a classical (logistic regression) and two machine-learning (support vector machines and decision trees) algorithms. Accuracy was generally high both under cross-validation (77.0–93.4%) and in the test sample (70.0–90.0%).

The femur is the strongest bone in the human skeleton, and is sexually dimorphic, with a long-recognized relevance for sexing skeletal remains. As previously mentioned, Tamagnini and Vieira de Campos provided an ingenuous technique for the estimation of sex with the femur [59]. Subsequently, two sectioning points for the femoral head diameters were proposed by Wasterlain and Cunha, with a reported accuracy of 85.5% [71]. Logistic regression and the C4.5 algorithm were employed by Curate et al. [80] to develop a technique based on measurements of the proximal femur. The models were created with a sample from the Lisbon Collection (252 individuals, 114 females and 138 males) and tested with a sample from the CISC (196 individuals, 98 males and 98 females). All models were contrived in a web application (SEUPF, https://osteomics.com/SeuPF/ (accessed on the 7 February 2022)) and reported cross-validated accuracies varying from 82.5 to 85.7%, while in the holdout sample they fluctuated between 80.1 and 86.2%. Another method used dual X-ray absorptiometry, a medical imaging technique, to calculate the total area of the proximal femur and generate models with classical and machine-learning classifiers [82]. This method was created with a sample from the CISC (224 individuals, 112 females and 112 males), and evaluated with a sample from the 21st Century Identified Skeletal Collection (60 individuals, 30 females and 30 males), yielding accuracies between 90.2 and 92.0% under cross-validation, and between 90.0 and 91.7% in the independent test sample. Finally, a set of 15 femoral measurements was acquired in a sample (200 individuals, 100 females; 100 males) from the CISC and models for sex classification were generated with classical and machine-learning algorithms [83]. Under cross-validation, the models correctly estimated sex in 60.0–92.5% of cases. All models were evaluated in a holdout sample (58 individuals, 24 females and 34 males) from the CEI21, with an allocation accuracy ranging from 56.9 to 89.7%.

A technique based on measurements of the tibia [67] utilized a sample of 95 individuals (46 males, 49 females) from the CISC to create models for sex estimation through discriminant analysis, with reported accuracies ranging from 70.5% to 88.4%. More recently, the value of the tibial shaft circumference for sex determination was assessed in the Lisbon Collection (sample of 160 individuals, 80 males and 80 females), with a stated accuracy of 77.0% [74].

A study by Albanese et al. [75] combined standard and unorthodox measurements of the clavicle, humerus, radius, and ulna, proposing several equations that are not population-specific. The method was developed using samples from the Terry Collection and the CISC, and all of the models were tested in samples from the Grant Collection and the Lisbon Collection. Prediction accuracies were consistently good (87.4–97.5%).

In several recovery circumstances, the most dimorphic bones—the pelvis, long bones, and skull—are not available for analysis; hence, other postcranial bones have been used to predict the sex of unknown skeletal remains. The relevance of the calcaneus and talus for the assessment of sex in the Portuguese population, for example, was first established by Ana Maria Silva in 1995 [70]. Silva's method was tailored with a sample from the CISC (165 individuals, 85 females and 80 males) using discriminant analysis. The models—appropriate for incomplete or fragmented calcaneus and/or talus—presented an accuracy ranging from 80.1 to 92.9%. Another sample from the same collection, with 180 individuals (93 females and 87 males), was used to generate models for sex estimation with the calcaneus and talus implementing different statistical approaches, viz., logistic regression (LR), support vector machines (SVM), and a decision-tree algorithm [89]. The univariable and multivariable models yielded an accuracy under cross-validation from 78.3 to 91.3%. The models were operationalized in an open access, user-friendly, responsive website application named CalcTalus (http://osteomics.com/CalcTalus/ (accessed on the 7 February 2022)), that enables a probabilistic estimation of sex.

Two samples of tarsal bones from identified skeletal individuals of Portuguese origin were used to establish another technique for sex estimation. A total of 300 individuals from the CISC (150 males and 150 females) were used to develop sex prediction models based on classical and machine learning algorithms, and the models were evaluated in a test sample from the Identified Skeletal Collection of the 21st Century (60 individuals, 30 males and 30 females) [78]. Overall accuracy under cross-validation varied between 86.3 and 89.7%, ranging from 80.0% to 88.3% in the holdout sample.

The second cervical vertebra was employed to create a predictive model based on logistic regression analysis [79]. Model fitting was attained with 190 individuals (99 males and 91 females) from the CISC, and the resulting model was tested in a sample from the CEI21 (47 individuals, 24 males and 23 females). The model accurately projected sex in 86.7 to 89.7 % of cases. Another study employed the seven cervical vertebrae to develop discriminant functions for the estimation of sex [87] with samples from the Athens

Collection and the Lisbon Collection (160 individuals, 87 males and 73 females). Seven discriminant functions presented accuracy rates between 80.3% and 84.5%. Finally, sexual dimorphism of the first, second and seventh cervical and twelfth thoracic vertebrae was investigated in the CISC (73 individuals, 38 males and 35 females). Logistic regression models yielded accuracy rates between 80.0% and 92.5%.

The most helpful skeletal sex indicators are modestly expressed before puberty and adolescence; consequently, sexing non-adult remains is fraught with difficulties [2]. In the past few years, the investigation of several dimorphic non-adult skeletal traits has suggested that estimating the sex of non-adults is feasible and reasonably accurate [95,98]. The study of non-adults' sexual dimorphism in the Portuguese reference collections has provided interesting results. Vlak et al. [91] evaluated Schutkowski's method for juvenile sex determination in the Lisbon collection (56 individuals, 23 females and 33 males), suggesting that neither sciatic notch morphology nor metrics are good indicators of sex in non-adult individuals (<15 years old). Another study in the Lisbon Collection (97 individuals, 57 males and 40 females) assessed the accuracy of the arch criterion and the composite arch of the ilia in the estimation of sex in non-adults [92]. Overall reported accuracy was low (between 26.7 and 52.6%), and the recording of both traits showed a poor agreement between observations and observers. Sample-specific sex estimation techniques of non-adults based on permanent tooth dimensions were created through sectioning points and logistic regression [93]. Both adults (107 individuals, 52 females and 55 males) and non-adults (49 individuals, 22 females and 27 males) of the Lisbon Collection were included. The canines presented the highest sexual dimorphism and sex assessment based on canine dimensions provided correct prediction accuracies between 58.8 and 100%.

In 2017, Luna et al. [94] proposed a methodology that evaluates the auricular surface of non-adult ilia. Morphological and metric variables were collected in 34 individuals (21 females and 13 males) from the CISC; the prediction models presented classification accuracies that ranged between 82.4 and 88.2%. A test of this method was conducted at the Lisbon Collection (61 individuals, 31 females and 30 males) showing a good prediction performance, between 73.4 and 86.7%. Accuracy rates were especially good in individuals younger than two years old [95]. This technique was also evaluated in fetuses and children under five years of age [97] from the Granada and the Lisbon (24 individuals, 13 females and 11 males) collections. The method showed good reliability in both sexes and in all age categories.

Finally, a test of the Stull et al. [99] method in the Lisbon Collection (102 individuals, 45 females and 57 males) suggests that the proposed models for diaphyseal dimensions of the long bones yield poor allocation accuracies in independent samples [96].

4. The Perplexing Bones of Sex—Achievements and Opportunities

The previous account of sex estimation techniques created in the Portuguese identified skeletal collections intends to be more than a sequence of events, experiences, and results: it provides a constellational depiction of the most relevant projects committed to the protocols and conventions of sexing human remains. A cumulative inventory such as this reveals an underlying structure in the scientific gaze towards the body, and the pervasive motifs that define and restrict the mapping of the skeletal differences between sexes. The Portuguese reference collections represent, to an extent, the empirical tradition within biological anthropology that boosted a methodological concern with the development of more accurate, sample-specific methods for sexing human remains. Notwithstanding, the historical consciousness of the technical apparatuses intertwined in these collections reveals how they are an imprecise form of knowledge.

The Coimbra Identified Skeletal Collection is omnipresent in studies concerning adult sex estimation in Portugal. Samples garnered from this collection appear in 88.9% of the methods created and/or tested in Portuguese reference collections. The collection is on par—or almost—with other famous identified skeletal assemblages, such as the Hamman–Todd or Terry collections, sustaining several research works in different areas within anthropology [40,42]. The CISC is composed of individuals that were born between

1822 and 1921, and died between 1904 and 1936; thus, it is important to consider the short-term effects of environment on skeletal anatomy and the potential consequences of secular change (i.e., biological modifications that occur over decades or generations, apparently ascribable to environmental factors) in the accuracy of sexing methods when employed in medico-legal contexts. Secular changes affect adult skeletal morphology, including height, cranial size and shape, and long bone length and proportions [20]. As metric variation in the skeleton presents diachronic modifications, there are implications for the assessment of sex from skeletal dimensions [20,100], namely an increased bias in sex-specific accuracy. Some studies have suggested that methods fitted in chronologically older samples—such as the CISC—perform worse in more recent collections, with a decrease in accuracy rates and increased bias [83,100]. This is frequently related to a higher misclassification of females [80]: in general, the linear dimensions of the long bones are larger for both sexes in more recent samples, but the increase is relatively larger in females [83]. Incorrect evaluations imply the collapse of evidence and expert testimonies, particularly affecting forensic cases involving recent deceased individuals. As the overwhelming majority of adult sex estimation methods in Portugal derive from the CISC, this poses the challenge to test and update long-standing methodologies with appropriate reference samples, particularly those that originate in chronologically more recent collections such as the BoneMedLeg collection [33] or the 21st Century Identified Skeletal Collection [43,44].

In the last few years, the CEI21 has been increasingly employed to create and test sexing methods, but the collection shows demographic characteristics that can affect the creation of new methodologies, namely the fact that it is mostly composed of elderly individuals [44]. This issue can be envisioned as a caveat or as an opportunity. Research has suggested that some skeletal changes occurring after adulthood tend to obfuscate sexual dimorphism [2]; hence, this collection provides an excellent opportunity to map dimorphism in aging individuals, and also to evaluate and develop methods that are more reliable in older age classes.

The «Portuguese» techniques for assessing sex in non-adults mostly rely in the Lisbon Collection, with a somewhat limited number of skeletons available for study, and mostly composed of individuals that died before 1960 [41,96]. The limitations of existing reference collections, including the dearth of non-adults and of contemporary (21st century) individuals, can be partially surmounted with the aid of medical imaging techniques [101]. Conversely, medical imaging approaches, such as computer tomography, conventional radiography, or bone densitometry, have been widely used to address the prediction of sex in cranial and postcranial bones [82], but with a restricted usage in Portugal, at least partially related to funding constraints. Alongside this chronological and demographic benefit, the use of medical imaging data to develop new methods would also have an effect on the curation of identified skeletal collections, by decelerating the bone damage caused by frequent handling. Basic ethical tenets, such as dignity and respect, will only be upheld if the unjustified physical disturbance of human skeletal remains ceases [35].

The described methods for the assessment of sex are at times insular, with a narrow geographical scope—they were created in Portuguese collections to be employed in the Portuguese medico-legal or archeological contexts. Thus, the evaluation of these methods in samples of different geographical origins would certainly feature an interesting research path in the study of region-specific sexual dimorphism.

The spatialization and patterning of biological sex throughout the skeleton requires an anthropological gaze that is also (although not only) a statistical perception of reality. Statistical analyses are of particular significance for forensic anthropology, and efforts to implement both groundbreaking and trustworthy analytical statistics in forensic research are becoming ubiquitous [77,102]. The recent explosion of statistical plurality is noticeable in the diachronic narrative of sexing methods created in Portuguese collections: linear discriminant analysis (LDA) reigned unchallenged for years, but during the second decade of the 21st century the use of different statistical approaches became routine. LDA, the oldest classifier still in use, identifies a linear combination of predictor variables that optimally separates mutually exclusive groups [82]. The resulting discriminant function provides a cutoff point that induces a strict dichotomic estimation of sex [103]. The utilization of sectioning points is, in this regard, similar to LDA.

Sexual dimorphism in the skeleton, however, is expressed along a continuum of phenotypic variation with a substantial overlap in dimorphic trait manifestations [103,104]. In fact, skeletal morphologies have a range of variation and the dimorphic traits based on shape and size show both variation between and within the sex groups; hence, human skeletal variation is the norm, and the conflation between the morphologies of different a priori categories (such as sex) will often occur [104]. Visual standards [105] for sexing human remains recognize the overlap and the uncertainty associated with it by using a scale of five categories, «hyperfeminine», «feminine», «sex indeterminate», «masculine», and «hypermasculine». Another classical statistical algorithm, logistic regression, allows a probabilistic assessment of sex that is both consentaneous with the clinal expression of sexual dimorphism and the standards enacted in the wake of the Daubert v Merrell Dow ruling [103,106]. Thus, the non-binary spectrum of sex variation should always be acknowledged by presenting the uncertain or unclear sex attributions that can simply reverberate biological features [107].

The more sophisticated machine-learning classifiers can lower the error rate but should be used cautiously, particularly artificial neural networks, which present less than desirable interpretability [102]. Moreover, the results of some studies focusing on sex estimation in Portuguese collections suggest that the type of statistical classifier—classical or machine-learning—does not impact accuracy and bias in a substantial way [63,64,83]. Even when the performance of the machine-learning algorithms is better, the difference is not overwhelming [82] and further practical research indicates that complex machine-learning models do not necessarily attain better performance metrics [108]. Again, the flexibility, elegance and simplicity of logistic regression accommodates much of the most advantageous characteristics in a statistical technique that aims to estimate the sex of unidentified skeletal remains [103].

Fittingly, in recent years, the prediction of sex has mostly become a probability statement. However, beyond the technicalities that inform much of the contemporary statistical research, the estimation of skeletal sex is still somewhat typological, and well within the confines of sex as a binary dichotomy. Constructionist arguments contend that, as sex can be fluid, the binary partition of sex into males and females is a product of discourse [104]. During the long 18th century (1688–1815), a fundamental change in the scientific discourse solidified the notion of masculine and feminine as diametrically opposed, rather than contiguous and hierarchical [109]. Anthropologists must be conscious about the possibility that this binary rift was peripheral in many societies who recognized human variation in ways that did not fall into a precise two-sex dichotomy [110,111]. Even chromosomal sex is multifarious and does not map clearly onto two bodily morphologies [110].

The anthropological responsibility to dismantle the rigid binary sex categorization stems, among other factors, from the fact that transgender and gender non-conforming people experience higher rates of violence [112,113]. Moreover, a distinction between sex and gender is almost never presented (regrettably, this is generalized), and the conflation of the two concepts is thus acritically acknowledged. Although gender—the expected social roles grounded in the sex of the person (gender role) or personal identification of one's own gender based on a personal awareness (gender identity)—cannot be directly observed in the skeleton, there are skeletal expressions of gender delimited by skeletal plasticity [104,110]. This thorny legacy inherited by contemporary anthropologists needs to be recognized, pondered, and contested. A first step to address the embodiment of gender in the skeleton pertains, for example, the evidence of gender-based division of labor: patterns of musculoskeletal stress markers or of degenerative joint disease have been used in bioarcheology to convey the potential disparity of sex and gender. Activity reconstruction, mortuary analysis (including the study of material culture associated with the dead body), intentional body modification, among others, are being referenced and mapped in gender-based bioarcheological analyses [111,114].

An interesting trend observed in the methods created in Portuguese identified collections pertains to the expanding relevance of proper goodness-of-fit metrics (measures of goodness-of-fit classically synopsize the disagreement between observed values and the values anticipated under the proposed model), including accuracy under cross-validation and in independent test samples. The majority of the early published methods merely displayed resubstitution classification errors, recognized for being optimistically biased. Cross-validation is probably the most common method to evaluate prediction error, but often appropriately assesses only the expected prediction error. Thus, the generalization error of any classification model should be evaluated with an independent test sample [115]—and that is increasingly the case in sex estimation techniques created in the Portuguese identified collections.

Web-based decision support systems and data sharing are contingent to the ongoing use of imaging methods and cutting-edge statistical approaches in forensic research. Online applications of interest for skeletal sex estimation are usually straightforward responsive interfaces that offer comprehensive documentation about methods, and include data and graphical analyses as well as classification outputs. Additionally, most of the available web-based applications—particularly those that feature models generated in Portuguese collections—bestow an educational diorama focused on human skeletal variation and statistical analyses [102]. Data sharing encourages networked collaboration models, global learning opportunities and diversity of research. The sharing of raw data also allows the iteration of research, enabling the validation of the original project or the development of new avenues of study [116,117]. Interestingly, some early studies concerning sexual dimorphism in Portuguese reference collections provided a wealth of raw data, e.g., [53,54], a valuable practice that has not been pursued in more recent research. Even though there are clear advantages in data sharing and online archiving, some ethical plights need to be addressed, including, but not limited to, the protection of identities by fully anonymizing the data, the consultation with living or descendant communities, and the clarification of data ownership rights by academic institutions or museums [116,117].

5. Concluding Remarks

The large number of Portuguese reference skeletal collections that have been accrued and curated for more than one century have supported—and continue to support—a myriad of research projects in the scientific axes of forensic anthropology and bioarcheology. Among these, the systematic recording of sexual dimorphism, and the creation and/or evaluation of models for skeletal sex estimation have been paramount. The historical engagement with the different technical projects developed in the Portuguese collections shows that these have been essential to the understanding of sexual dimorphism as expressed in the human skeleton, and to the improvement of sex estimation methods. It also provided an opportunity to consider the limitations of the estimators. There are numerous technical matters for enhancement regarding sexing methods, including the increase of accuracy rates and the reduction of bias, the development of methods that can be used on fragmentary or incomplete remains, the routine usage of medical imaging techniques, and robust and probabilistic statistical approaches. The introduction of probabilistic thought in the estimation of skeletal sex recognizes how uncertainty is a mark of complexity. Sex—and, of course, gender—is not fixed or transhistorical, and the skeletal estimation of such an intricate feature will not be solved just with technological optimism; there is also a dire necessity for conceptual and discourse innovation.

Funding: This research was funded by the Foundation for Science and Technology through CIAS (UIDB/00283/2020).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: I am grateful to the Special Issue Editor, Francisca Alves-Cardoso, and two anonymous reviewers which helped to improve the manuscript.

Conflicts of Interest: The author declares no conflict of interest.

References

1. Garvin, H.M. Adult Sex Determination: Methods and Application. In *A Companion to Forensic Anthropology*; Dirkmaat, D.C., Ed.; Blackwell Publishing Ltd.: Chichester, UK, 2012; pp. 239–247.
2. İscan, M.Y.; Steyn, M. *The Human Skeleton in Forensic Medicine*; Charles C Thomas: Springfield, IL, USA, 2013.
3. Bethard, J.D.; VanSickle, C. Applications of Sex Estimation in Paleoanthropology, Bioarchaeology, and Forensic Anthropology. In *Sex Estimation of the Human Skeleton*; Klales, A., Ed.; Academic Press: London, UK, 2020; pp. 25–34, ISBN 9780128157671.
4. Christensen, A.M.; Passalacqua, N.V.; Bartelink, E.J. *Forensic Anthropology: Current Methods and Practice*; Elsevier: Amsterdam, The Netherlands, 2014; ISBN 9780124172906.
5. Chapman, T.; Lefevre, P.; Semal, P.; Moiseev, F.; Sholukha, V.; Louryan, S.; Rooze, M.; Van Sint Jan, S. Sex Determination Using the Probabilistic Sex Diagnosis (DSP: Diagnose Sexuelle Probabiliste) Tool in a Virtual Environment. *Forensic Sci. Int.* **2014**, *234*, 189.e1–189.e8. [CrossRef] [PubMed]
6. Steyn, M.; Patriquin, M.L. Osteometric Sex Determination from the Pelvis-Does Population Specificity Matter? *Forensic Sci. Int.* **2009**, *191*, 113.e1–113.e5. [CrossRef] [PubMed]
7. Ridley, M. Pelvic Sexual Dimorphism and Relative Neonatal Brain Size Really Are Related. *Am. J. Phys. Anthropol.* **1995**, *97*, 197–200. [CrossRef] [PubMed]
8. Hager, L.D. Sex and Gender in Paleoanthropology. In *Women in Human Evolution*; Hager, L.D., Ed.; Routledge: New York, NY, USA, 1997; pp. 1–27.
9. Garvin, H.M.; Passalacqua, N.V. Current Practices by Forensic Anthropologists in Adult Skeletal Age Estimation. *J. Forensic Sci.* **2012**, *57*, 427–433. [CrossRef]
10. Betti, L. Sexual Dimorphism in the Size and Shape of the Os Coxae and the Effects of Microevolutionary Processes. *Am. J. Phys. Anthropol.* **2014**, *153*, 167–177. [CrossRef]
11. Huseynov, A.; Zollikofer, C.P.E.; Coudyzer, W.; Gascho, D.; Kellenberger, C.; Hinzpeter, R.; Ponce de León, M.S. Developmental Evidence for Obstetric Adaptation of the Human Female Pelvis. *Proc. Natl. Acad. Sci. USA* **2016**, *113*, 5227–5232. [CrossRef]
12. Rissech, C.; Estabrook, G.F.; Cunha, E.; Malgosa, A. Using the Acetabulum to Estimate Age at Death of Adult Males. *J. Forensic Sci.* **2006**, *51*, 213–229. [CrossRef]
13. Krishan, K.; Chatterjee, P.M.; Kanchan, T.; Kaur, S.; Baryah, N.; Singh, R.K. A Review of Sex Estimation Techniques during Examination of Skeletal Remains in Forensic Anthropology Casework. *Forensic Sci. Int.* **2016**, *261*, 165.e1–165.e8. [CrossRef]
14. Spradley, M.K.; Jantz, R.L. Sex Estimation in Forensic Anthropology: Skull Versus Postcranial Elements. *J. Forensic Sci.* **2011**, *56*, 289–296. [CrossRef]
15. Buonasera, T.; Eerkens, J.; de Flamingh, A.; Engbring, L.; Yip, J.; Li, H.; Haas, R.; DiGiuseppe, D.; Grant, D.; Salemi, M.; et al. A Comparison of Proteomic, Genomic, and Osteological Methods of Archaeological Sex Estimation. *Sci. Rep.* **2020**, *10*, 11897. [CrossRef]
16. Thomas, R.M. Sex Determination Using DNA and Its Impact on Biological Anthropology. In *Sex Estimation of the Human Skeleton*; Klales, A.R., Ed.; Academic Press: London, UK, 2020; pp. 343–350.
17. Baumgarten, S.E.; Kenyon-Flatt, B. Metric Methods for Estimating Sex Utilizing the Pelvis. In *Sex Estimation of the Human Skeleton*; Klales, A.R., Ed.; Academic Press: London, UK, 2020; pp. 171–184.
18. Albanese, J.; Eklics, G.; Tuck, A. A Metric Method for Sex Determination Using the Proximal Femur and Fragmentary Hipbone. *J. Forensic Sci.* **2008**, *53*, 1283–1288. [CrossRef] [PubMed]
19. Ubelaker, D.H.; DeGaglia, C.M. Population Variation in Skeletal Sexual Dimorphism. *Forensic Sci. Int.* **2017**, *278*, 407.e1–407.e7. [CrossRef] [PubMed]
20. Langley, N.R.; Jantz, R.L. Secular Change. In *Sex Estimation of the Human Skeleton*; Klales, A.R., Ed.; Academic Press: London, UK, 2020; pp. 295–306, ISBN 9780128157671.
21. Ubelaker, D.H.; DeGaglia, C.M. Factors of Population Variation in Sex Estimation Methodology. In *Sex Estimation of the Human Skeleton*; Klales, A.R., Ed.; Academic Press: London, UK, 2020; pp. 281–293.
22. Charisi, D.; Eliopoulos, C.; Vanna, V.; Koilias, C.G.; Manolis, S.K. Sexual Dimorphism of the Arm Bones in a Modern Greek Population. *J. Forensic Sci.* **2011**, *56*, 10–18. [CrossRef] [PubMed]
23. Alunni-Perret, V.; Staccini, P.; Quatrehomme, G. Sex Determination from the Distal Part of the Femur in a French Contemporary Population. *Forensic Sci. Int.* **2008**, *175*, 113–117. [CrossRef]
24. Kranioti, E.F. Radiometry versus Osteometry in Sex Assessment: A Study of the Cretan Radius. *Aust. J. Forensic Sci.* **2019**, *51*, 135–148. [CrossRef]
25. Patriquin, M.L.; Steyn, M.; Loth, S.R. Metric Analysis of Sex Differences in South African Black and White Pelves. *Forensic Sci. Int.* **2005**, *147*, 119–127. [CrossRef]
26. Peckmann, T.R.; Scott, S.; Meek, S.; Mahakkanukrauh, P. Sex Estimation from the Scapula in a Contemporary Thai Population: Applications for Forensic Anthropology. *Sci. Justice* **2017**, *57*, 270–275. [CrossRef]
27. Cuzzullin, M.C.; Curate, F.; Freire, A.R.; Costa, S.T.; Prado, F.B.; Daruge Junior, E.; Cunha, E.; Rossi, A.C. Validation of Anthropological Measures of the Human Femur for Sex Estimation in Brazilians. *Aust. J. Forensic Sci.* **2020**, *54*, 61–74. [CrossRef]

28. Moore, M.K.; DiGangi, E.A.; Niño Ruíz, F.P.; Hidalgo Davila, O.J.; Sanabria Medina, C. Metric Sex Estimation from the Postcranial Skeleton for the Colombian Population. *Forensic Sci. Int.* **2016**, *262*, 286.e1–286.e8. [CrossRef]
29. Attia, M.H.; Aboulnoor, B.A.E.-S. Tailored Logistic Regression Models for Sex Estimation of Unknown Individuals Using the Published Population Data of the Humeral Epiphyses. *Leg. Med.* **2020**, *45*, 101708. [CrossRef]
30. Ríos Frutos, L. Brief Communication: Sex Determination Accuracy of the Minimum Supero-Inferior Femoral Neck Diameter in a Contemporary Rural Guatemalan Population. *Am. J. Phys. Anthropol.* **2003**, *122*, 123–126. [CrossRef] [PubMed]
31. Gualdi-Russo, E. Sex Determination from the Talus and Calcaneus Measurements. *Forensic Sci. Int.* **2007**, *171*, 151–156. [CrossRef] [PubMed]
32. Ogawa, Y.; Imaizumi, K.; Miyasaka, S.; Yoshino, M. Discriminant Functions for Sex Estimation of Modern Japanese Skulls. *J. Forensic Leg. Med.* **2013**, *20*, 234–238. [CrossRef] [PubMed]
33. Cardoso, H.F.V.; Marinho, L. Lost and Then Found: The Mendes Correia Collection of Identified Human Skeletons Curated at the University of Porto, Portugal. *Antropol. Port.* **2016**, *32–33*, 29–46. [CrossRef]
34. Tobias, P.V. On the Scientific, Medical, Dental and Educational Value of Collections of Human Skeletons. *Int. J. Anthropol.* **1991**, *6*, 277–280. [CrossRef]
35. Cardoso, H. An Ethical, Cultural and Historical Background for Cemetery-Based Human Skeletal Reference Collections. *J. Contemp. Archaeol.* **2021**, *8*, 21–52. [CrossRef]
36. Alves-Cardoso, F.; Campanacho, V. The Scientific Profiles of Documented Collections via Publication Data: Past, Present, and Future Directions in Forensic Anthropology. *Forensic Sci.* **2022**, *2*, 37–56. [CrossRef]
37. Alves-Cardoso, F. Lives Not Written in Bones: Discussing Biographical Data Associated with Identified Skeletal Collections. In *Identified Skeletal Collections: The Testing Ground of Anthropology?* Henderson, C., Alves-Cardoso, F., Eds.; Archaeopress Publishing: Oxford, UK, 2018; pp. 151–167, ISBN 9781784918064.
38. Alves-Cardoso, F. "Not of One's Body": The Creation of Identified Skeletal Collections with Portuguese Human Remains. In *Ethical Approaches to Human Remains*; Squires, K., Errickson, D., Márquez-Grant, N., Eds.; Springer: Cham, Switzerland, 2019; pp. 503–518.
39. Lopes, C.; Fernandes, T. The Identified Skeleton Collection of Évora: Importance for Forensic Science and Bioarchaeology in the Southern Inland of Portugal. *Int. J. Leg. Med.* **2021**, 1–8. [CrossRef]
40. Rocha, M.A. Les Collections Ostéologiques Humaines Identifiées Du Musée Anthropologique de l'Université de Coimbra. *Antropol. Port.* **1995**, *13*, 7–38.
41. Cardoso, H.F.V. Brief Communication: The Collection of Identified Human Skeletons Housed at the Bocage Museum (National Museum of Natural History), Lisbon, Portugal. *Am. J. Phys. Anthropol.* **2006**, *129*, 173–176. [CrossRef]
42. Cunha, E.; Wasterlain, S. The Coimbra Identified Osteological Collections. In *Skeletal Series in Their Socioeconomic Context*; Grupe, G., Peters, J., Eds.; M. Leidorf: Rahden, Germany, 2007; Volume 5, pp. 23–33.
43. Ferreira, M.T.; Vicente, R.; Navega, D.; Gonçalves, D.; Curate, F.; Cunha, E. A New Forensic Collection Housed at the University of Coimbra, Portugal: The 21st Century Identified Skeletal Collection. *Forensic Sci. Int.* **2014**, *245*, e1–e5. [CrossRef]
44. Ferreira, M.T.; Coelho, C.; Makhoul, C.; Navega, D.; Gonçalves, D.; Cunha, E.; Curate, F. New Data about the 21st Century Identified Skeletal Collection (University of Coimbra, Portugal). *Int. J. Leg. Med.* **2021**, *135*, 1087–1094. [CrossRef] [PubMed]
45. Cardoso, H.F.V.; Marinho, L.; Caldas, I.M.; Puentes, K.; Andrade, M.; Toso, A.; Assis, S.; Magalhães, T. Historical, Demographic, Curatorial and Legal Aspects of the Bonemedleg Human Skeletal Reference Collection (Porto, Portugal). *Anthropol. Anz.* **2020**, *77*, 57–73. [CrossRef] [PubMed]
46. Henderson, C. Introduction. In *Identified Skeletal Collections: The Testing Ground of Anthropology?* Henderson, C., Alves-Cardoso, F., Eds.; Archaeopress Publishing: Oxford, UK, 2018; pp. 1–10.
47. Mendes Correia, A. Osteometria Portuguesa: I Coluna Vertebral. *Ann. Sci. Da Acad. Polytech. Do Porto* **1917**, *XII*, 227–254.
48. Mendes Correia, A. Osteometria Portuguesa: II Cintura Escapular. *Ann. Sci. Da Acad. Polytech. Do Porto* **1918**, *XIII*, 102–123.
49. Mendes Correia, A. Osteometria Portuguesa: II Cintura Escapular (Continuação). *Ann. Sci. Da Acad. Polytech. Do Porto* **1918**, *XIII*, 172–195.
50. Mendes Correia, A. Osteometria Portuguesa: IV Esqueleto Do Braço e Do Antebraço. *Ann. Sci. Da Acad. Polytech. Do Porto* **1920**, *XIV*, 243–253.
51. Mendes Correia, A. Osteometria Portuguesa: IV Esqueleto Do Braço e Do Antebraço (Continuação). *Ann. Sci. Da Acad. Polytech. Do Porto* **1927**, *XV*, 25–56.
52. Themido, A.A. O Índice Orbitário Nos Portugueses. *Contrib. Para O Estud. Da Antropol. Port.* **1950**, *2*, 177–200.
53. Serra, J.A. A Pelve Nos Portugueses: Morfologia Da Pelve No Homem. *Contrib. Para O Estud. Da Antropol. Port.* **1938**, *3*, 1–174.
54. Neto, M.A.M. Estudo Osteométrico Do Antebraço Nos Portugueses: I—Rádio. *Contrib. Para O Estud. Da Antropol. Port.* **1957**, *VI*, 143–217.
55. Albuquerque, R.M. Estudo Antropológico Da Mandíbula Nos Portugueses. *Contrib. Para O Estud. Da Antropol. Port.* **1952**, *5*, 1–196.
56. Serra, J.A. O Esterno Nos Portugueses: Caracteres Métricos e Morfológicos Do Esterno No Homem. *Contrib. Para O Estud. Da Antropol. Port.* **1941**, *4*, 5–131.
57. Xavier de Morais, M.H. Estudo Antropológico Da Omoplata Nos Portugueses: I. Caracteres Métricos. *Contrib. Para O Estud. Da Antropol. Port.* **1966**, *VIII*, 21–97.

58. Neto, M.A.M. Acerca Do Valor Da Grande Cavidade Sigmóide Do Cúbito Como Caráter Sexual. *Contrib. Para O Estud. Da Antropol. Port.* **1959**, *7*, 1–12.
59. Tamagnini, E.; Vieira de Campos, D.S. O Fémur Português. *Contrib. Para O Estud. Da Antropol. Port.* **1949**, *2*, 1–69.
60. Xavier de Morais, M.H. Estudo Antropológico Da Omoplata Nos Portugueses: II Caracteres Morfológicos. *Contrib. Para O Estud. Da Antropol. Port.* **1968**, *8*, 103–151.
61. Cardoso, H.F.V.; Cunha, E. Sexual Dimorphism in Upper Limb Skeletal Proportions. *Biom. Hum. Anthropol.* **2000**, *18*, 55–61.
62. Arsuaga, J.L.; Lorenzo, C. Sexual Dimorphism of the Hip Bone in the Coimbra Population (Portugal). *Antropol. Port.* **1995**, *13*, 171–191.
63. d'Oliveira Coelho, J.; Curate, F. CADOES: An Interactive Machine-Learning Approach for Sex Estimation with the Pelvis. *Forensic Sci. Int.* **2019**, *302*, 109873. [CrossRef]
64. Curate, F.; Mestre, F.; Garcia, S.J. Sex Assessment with the Radius in Portuguese Skeletal Populations (Late 19th–Early to Mid 20th Centuries). *Leg. Med.* **2021**, *48*, 101790. [CrossRef]
65. Cogoluenhes, A. Travail Sur Les Humérus de La Collection Anthropologique de l'Université de Coimbra. *Antropol. Port.* **1984**, *2*, 5–8.
66. Cunha, E.; Van Vark, G. Calculo de Funçoes Discriminantes Para a Diagnose Sexual Do Crânio. *Antropol. Port.* **1990**, *8*, 17–37.
67. Bruzek, J. Diagnose Sexuelle à l'aide de l'analyse Discriminante Appliquée Au Tibia. *Antropol. Port.* **1995**, *13*, 93–106.
68. Carretero, J.; Lorenzo, C.; Arsuaga, J. Análisis Multivariante Del Húmero En La Colección de Restos Identificados de La Universidad de Coimbra (Portugal). *Antropol. Port.* **1995**, *13*, 139–156.
69. Houët, F.; Bruzek, J.; Murail, P. Etablissement Des Nouvelles Fonctions Discriminantes à Partir de l'os Coxal Applicables Dans d'autres Populations. *Antropol. Port.* **1995**, *13*, 157–170.
70. Silva, A.M. Sex Assessment Using the Calcaneus and Talus. *Antropol. Port.* **1995**, *13*, 107–119.
71. Wasterlain, R.S.N.; Cunha, E. Comparative Performance of Femur and Humerus Epiphysis for Sex Diagnosis. *Biom. Hum. Anthropol.* **2000**, *18*, 9–13.
72. Bruzek, J. A Method for Visual Determination of Sex, Using the Human Hip Bone. *Am. J. Phys. Anthropol.* **2002**, *117*, 157–168. [CrossRef]
73. Murail, P.; Bruzek, J.; Houët, F.; Cunha, E. DSP: A Tool for Probabilistic Sex Diagnosis Using Worldwide Variability in Hip-Bone Measurements. *Bull. Mem. Soc. Anthropol. Paris* **2005**, *17*, 167–176. [CrossRef]
74. Garcia, S. Is the Circumference at the Nutrient Foramen of the Tibia of Value to Sex Determination on Human Osteological Collections? Testing a New Method. *Int. J. Osteoarchaeol.* **2012**, *22*, 361–365. [CrossRef]
75. Albanese, J. A Method for Estimating Sex Using the Clavicle, Humerus, Radius, and Ulna. *J. Forensic Sci.* **2013**, *58*, 1413–1419. [CrossRef]
76. García-Parra, P.; Pérez Fernández, Á.; Djorojevic, M.; Botella, M.; Alemán, I. Sexual Dimorphism of Human Sternum in a Contemporary Spanish Population. *Forensic Sci. Int.* **2014**, *244*, 313.e1–313.e9. [CrossRef]
77. Santos, F.; Guyomarc'h, P.; Bruzek, J. Statistical Sex Determination from Craniometrics: Comparison of Linear Discriminant Analysis, Logistic Regression, and Support Vector Machines. *Forensic Sci. Int.* **2014**, *245*, 204.e1–204.e8. [CrossRef] [PubMed]
78. Navega, D.; Vicente, R.; Vieira, D.N.; Ross, A.H.; Cunha, E. Sex Estimation from the Tarsal Bones in a Portuguese Sample: A Machine Learning Approach. *Int. J. Leg. Med.* **2015**, *129*, 651–659. [CrossRef]
79. Gama, I.; Navega, D.; Cunha, E. Sex Estimation Using the Second Cervical Vertebra: A Morphometric Analysis in a Documented Portuguese Skeletal Sample. *Int. J. Leg. Med.* **2015**, *129*, 365–372. [CrossRef] [PubMed]
80. Curate, F.; d'Oliveira Coelho, J.; Gonçalves, D.; Coelho, C.; Ferreira, M.T.; Navega, D.; Cunha, E. A Method for Sex Estimation Using the Proximal Femur. *Forensic Sci. Int.* **2016**, *266*, 579.e1–579.e7. [CrossRef]
81. Brůžek, J.; Santos, F.; Dutailly, B.; Murail, P.; Cunha, E. Validation and Reliability of the Sex Estimation of the Human Os Coxae Using Freely Available DSP2 Software for Bioarchaeology and Forensic Anthropology. *Am. J. Phys. Anthropol.* **2017**, *164*, 440–449. [CrossRef]
82. Curate, F.; Albuquerque, A.; Ferreira, I.; Cunha, E. Sex Estimation with the Total Area of the Proximal Femur: A Densitometric Approach. *Forensic Sci. Int.* **2017**, *275*, 110–116. [CrossRef]
83. Curate, F.; Umbelino, C.; Perinha, A.; Nogueira, C.; Silva, A.M.; Cunha, E. Sex Determination from the Femur in Portuguese Populations with Classical and Machine-Learning Classifiers. *J. Forensic Leg. Med.* **2017**, *52*, 75–81. [CrossRef]
84. Ammer, S.; d'Oliveira Coelho, J.; Cunha, E.M. Outline Shape Analysis on the Trochlear Constriction and Olecranon Fossa of the Humerus: Insights for Sex Estimation and a New Computational Tool. *J. Forensic Sci.* **2019**, *64*, 1788–1795. [CrossRef]
85. Luna, L.H. Canine Sex Estimation and Sexual Dimorphism in the Collection of Identified Skeletons of the University of Coimbra, with an Application in a Roman Cemetery from Faro, Portugal. *Int. J. Osteoarchaeol.* **2019**, *29*, 260–272. [CrossRef]
86. Amores-Ampuero, A.; Viciano, J. Sexual Dimorphism from Vertebrae: Its Potential Use for Sex Estimation in an Identified Osteological Sample. *Aust. J. Forensic Sci.* **2020**, *00*, 1–13. [CrossRef]
87. Rozendaal, A.S.; Scott, S.; Peckmann, T.R.; Meek, S. Estimating Sex from the Seven Cervical Vertebrae: An Analysis of Two European Skeletal Populations. *Forensic Sci. Int.* **2020**, *306*, 110072. [CrossRef] [PubMed]
88. Franco, S.F.; Azevedo, Á.; Matos, V.M.J.; Mongiovi, D.; Franco, S.F. Odontometric Patterns in Human Mandibular Molars for Sex Estimation in a Forensic Context. *Dent. Anthropol.* **2021**, *34*, 36–43.

89. Curate, F.; d'Oliveira Coelho, J.; Silva, A.M. CalcTalus: An Online Decision Support System for the Estimation of Sex with the Calcaneus and Talus. *Archaeol. Anthropol. Sci.* **2021**, *13*, 74. [CrossRef]
90. Cunha, E.; van Vark, G.N. The Construction of Sex Discriminant Functions from a Large Collection of Skulls of Known Sex. *Int. J. Anthropol.* **1991**, *6*, 53–66. [CrossRef]
91. Vlak, D.; Roksandic, M.; Schillaci, M.A. Greater Sciatic Notch as a Sex Indicator in Juveniles. *Am. J. Phys. Anthropol.* **2008**, *137*, 309–315. [CrossRef] [PubMed]
92. Cardoso, H.F.V.; Saunders, S.R. Two Arch Criteria of the Ilium for Sex Determination of Immature Skeletal Remains: A Test of Their Accuracy and an Assessment of Intra- and Inter-Observer Error. *Forensic Sci. Int.* **2008**, *178*, 24–29. [CrossRef]
93. Cardoso, H.F.V. Sample-Specific (Universal) Metric Approaches for Determining the Sex of Immature Human Skeletal Remains Using Permanent Tooth Dimensions. *J. Archaeol. Sci.* **2008**, *35*, 158–168. [CrossRef]
94. Luna, L.H.; Aranda, C.M.; Santos, A.L. New Method for Sex Prediction Using the Human Non-Adult Auricular Surface of the Ilium in the Collection of Identified Skeletons of the University of Coimbra. *Int. J. Osteoarchaeol.* **2017**, *27*, 898–911. [CrossRef]
95. Monge Calleja, Á.M.; Aranda, C.M.; Santos, A.L.; Luna, L.H. Evaluation of the Auricular Surface Method for Non-Adult Sex Estimation on the Lisbon Documented Collection. *Am. J. Phys. Anthropol.* **2020**, *172*, 500–510. [CrossRef]
96. Lamer, M.; Spake, L.; Cardoso, H.F.V. Testing the Cross-Applicability of Juvenile Sex Estimation from Diaphyseal Dimensions. *Forensic Sci. Int.* **2021**, *321*, 110739. [CrossRef] [PubMed]
97. Luna, L.H.; Aranda, C.M.; Monge Calleja, Á.M.; Santos, A.L. 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. Leg. Med.* **2021**, *135*, 993–1003. [CrossRef]
98. Sutter, R.C. Nonmetric Subadult Skeletal Sexing Traits: I. A Blind Test of the Accuracy of Eight Previously Proposed Methods Using Prehistoric Known-Sex Mummies from Northern Chile. *J. Forensic Sci.* **2003**, *48*, 2002302. [CrossRef]
99. Stull, K.E.; L'Abbé, E.N.; Ousley, S.D. Subadult Sex Estimation from Diaphyseal Dimensions. *Am. J. Phys. Anthropol.* **2017**, *163*, 64–74. [CrossRef] [PubMed]
100. Gonçalves, D. Evaluation of the Effect of Secular Changes in the Reliability of Osteometric Methods for the Sex Estimation of Portuguese Individuals. *Cad. Do GEEvH* **2014**, *3*, 53–65.
101. Dirkmaat, D.C.; Cabo, L. Forensic Anthropology: Embracing the New Paradigm. In *Companion to Forensic Anthropology*; Dirkmaat, D.C., Ed.; Wiley-Blackwell: West Sussex, UK, 2012.
102. d'Oliveira Coelho, J.; Curate, F.; Navega, D. Osteomics: Decision Support Systems for Forensic Anthropologists. In *Statistics and Probability in Forensic Anthropology*; Obertová, Z., Stewart, A., Cattaneo, C., Eds.; Academic Press: London, UK, 2020; pp. 259–273.
103. Bartholdy, B.P.; Sandoval, E.; Hoogland, M.L.P.; Schrader, S.A. Getting Rid of Dichotomous Sex Estimations: Why Logistic Regression Should Be Preferred Over Discriminant Function Analysis. *J. Forensic Sci.* **2020**, *65*, 1685–1691. [CrossRef]
104. Sofaer, J.R. *The Body as Material Culture: A Theoretical Osteoarchaeology*; Cambridge University Press: Cambridge, UK, 2006.
105. Buikstra, J.; Ubelaker, D. *Standards for Data Collection from Human Skeletal Remains*; Arkansas Archaeological Survey: Fayetteville, AR, USA, 1994.
106. Klales, A.R.; Ousley, S.D.; Passalacqua, N.V. Statistical Approaches to Sex Estimation. In *Sex Estimation of the Human Skeleton*; Klales, A.R., Ed.; Academic Press: London, UK, 2020; pp. 203–217.
107. Marino, R.; Tanganelli, V.; Pietrobelli, A.; Belcastro, M.G. Evaluation of the Auricular Surface Method for Subadult Sex Estimation on Italian Modern (19th to 20th Century) Identified Skeletal Collections. *Am. J. Phys. Anthropol.* **2021**, *174*, 792–803. [CrossRef]
108. Hassan Attia, M.; Attia, M.; Tarek Farghaly, Y.; Ahmed El-Sayed Abulnoor, B.; Curate, F. Performance of the Supervised Learning Algorithms in Sex Estimation of the Proximal Femur: A Comparative Study in Contemporary Egyptian and Turkish Samples. *Sci. Justice* **2022**, *62*, 288–309. [CrossRef]
109. Laqueur, T. *Making Sex: Body and Gender from the Greeks to Freud*; Harvard University Press: Cambridge, MA, USA, 1990.
110. Joyce, R. Sex, Gender, and Anthropology: Moving Bioarchaeology Outside the Subdiscipline. In *Exploring Sex and Gender in Bioarchaeology*; Agarwal, S.C., Wesp, J.K., Eds.; University of New Mexico: Albuquerque, NM, USA, 2017; pp. 1–12.
111. Hollimon, S.E. Bioarchaeological Approaches to Nonbinary Genders: Case Studies from Native North America. In *Exploring Sex and Gender in Bioarchaeology*; Agarwal, S.C., Wesp, J.K., Eds.; University of New Mexico: Albuquerque, NM, USA, 2017; pp. 51–69.
112. Tallman, S.; Kincer, C.; Plemons, E. Centering Transgender Individuals in Forensic Anthropology and Expanding Binary Sex Estimation in Casework and Research. *Forensic Anthropol.* **2021**. [CrossRef]
113. Schall, J.L.; Rogers, T.L.; Deschamps-Braly, J.C. Breaking the Binary: The Identification of Trans-Women in Forensic Anthropology. *Forensic Sci. Int.* **2020**, *314*, 110356. [CrossRef] [PubMed]
114. Hollimon, S.E. Sex and Gender in Bioarchaeological Research: Theory, Method, and Interpretation. In *Social Bioarchaeology*; Agarwal, S.C., Glencross, B.A., Eds.; Blackwell Publishing Ltd.: Hoboken, NJ, USA, 2011; pp. 147–182, ISBN 9781405191876.
115. Hastie, T.; Tibshirani, R.; Friedman, J. *The Elements of Statistical Learning: Data Mining, Inference and Prediction*; Springer: New York, NY, USA, 2009; ISBN 978-0-387-84857-0.
116. Smith, S.E.; Hirst, C.S. 3D Data in Human Remains Disciplines: The Ethical Challenges. In *Ethical Approaches to Human Remains*; Squires, K., Errickson, D., Márquez-grant, N., Eds.; Springer: Cham, Switzerland, 2019; pp. 315–346.
117. Turner, T.R.; Mulligan, C.J. Data Sharing in Biological Anthropology: Guiding Principles and Best Practices. *Am. J. Phys. Anthropol.* **2019**, *170*, 3–4. [CrossRef] [PubMed]