FACULDADE DE MEDICINA
UNIVERSIDADE DO PORTO

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Chronology of fusion of the primary and secondary ossification centers in the human sacrum and age estimation in child and adolescent skeletons

Mestrado Integrado em Medicina

Área: Medicina Legal e Ciências Forenses

Trabalho efetuado sob a Orientação de:

Doutor Ricardo Jorge Dinis Oliveira

Trabalho organizado de acordo com as normas da revista:

American Journal of Physical Anthropology

março, 2013

U.PORTO
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Projeto de Opção do 6º ano – DECLARAÇÃO DE REPRODUÇÃO

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Título da Dissertação/~~Monografia~~ (cortar o que não interessa):

Chronology of fusion of the primary and secondary ossification centers in the human sacrum and age estimation in child and adolescent skeletons

Orientador:

Ricardo Jorge Dinis Oliveira

Coorientador (se aplicável):

____

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Ano de conclusão: 2013

Designação da área do projeto:

Medicina Legal e Ciências Forenses

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DEDICATÓRIA

Dedico este trabalho a todos aqueles que se deixaram abraçar por ele:

Ao Doutor Hugo Cardoso, a minha profunda gratidão pela disponibilidade demonstrada no acompanhamento contínuo deste trabalho, contribuindo para o seu rigor e carácter científico e incentivo.

À minha excelente família, por me terem proporcionado todas as condições para atingir os meus objectivos. Ao meu pai, um beijinho especial pelo incansável apoio e toda a ajuda disponibilizada a este trabalho.

Ao meu grande amigo, Alberto, pela ajuda permanente, que foi sem dúvida fundamental e pela grande amizade.

A todos eles, o meu Muito Obrigada!

Chronology of fusion of the primary and secondary ossification centers in the human sacrum and age estimation in child and adolescent skeletons

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Number of text pages: 25

Number of figures: 7

Number of tables: 16

Abbreviated title: Chronology of fusion of ossification centers in the sacrum

KEY WORDS bone maturation, dry bone, Lisbon collection, posterior probabilities of age

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ABSTRACT Little information is known about fusion times of the primary and secondary centers of ossification in the sacrum from dry bone observations. In this study, the timing of union of these centers was studied in a sample of modern Portuguese skeletons (90 females and 101 males) between the ages of 0 and 30, taken from the Lisbon documented skeletal collection. A three stage scheme was used to assess fusion status between ossification centers as unfused, partially fused and completely fused. Posterior probability tables of age, given a certain stage of fusion, were calculated for most anatomical locations studied using both reference and uniform priors. Partial union of primary centers of ossification was observed from 1 to 8 years of age and partial union of secondary centers of ossification was observed from 15 to 21 years of age. The first primary centers of ossification to complete fusion are the neural arch with the centrum of the fifth sacral vertebrae and the last are the costal element with the centrum of the first sacral vertebra. The annular and sacro-iliac epiphyses are the first secondary centers of ossification to complete fusion, after which the lateral margin fuses. Overall, there is no statistically significant sex differences in timing of fusion for either primary or secondary centers of ossification. This study offers information on timing of fusion of diverse locations in the developing sacrum useful for age estimation of complete or fragmented immature human skeletal remains and fills an important gap in the literature, by adding to previously published times of fusion of primary and secondary ossification centers in this sample.

The chronological age of immature human skeletal remains can be estimated from one of the following developmental indicators: appearance and fusion of primary and secondary centers of ossification, the size of certain bones, and tooth eruption and formation. The use of each of the developmental indicators is usually constrained by the overall age group to which the skeleton belongs fetal, neonate, infant, child or adolescent, and by the skeleton's state of preservation which will determine what bones or structures are present (Ubelaker, 1987).

When the skeleton is complete, age estimation is considered most reliable when obtained from patterns of tooth formation, particularly in individuals under 12 years of age or in pre-adolescents (Lewis and Garn, 1960; Ubelaker, 1987; Cardoso, 2007). When teeth are missing or when information from additional developmental indicators wishes to be incorporated, the anthropologist usually turns to diaphyseal measurements of the long bones, when the remains are of pre-adolescent age, or to fusion of secondary centers of ossification or epiphysis, when the remains are of adolescent and young adult age. Tooth eruption is considered less reliable because it is a discontinuous process (Demirjian, 1980) and is heavily influenced by external factors (Ubelaker, 1987).

Published data on the fusion of primary and secondary centers of ossification is readily available. The primary ossification center is the first place where the bone formation begins in the axle of a long bone or in the body of an irregular bone. Conversely, the secondary ossification center is the area of ossification that appears after the primary center of ossification at the epiphysis of edges of bones. For fusion of primary centers there is ample radiographic data. For example, the order, time of appearance and fusion of primary ossification centers has been documented by Todd (1931), Flecker (1932), Pyle and Hoerr (1955) or Greulich and Pyle (1959) but few have actually been obtained from gross inspection of dry specimens. The studies mentioned before, also provide radiographic information for fusion of secondary centers of ossification, but radiographic observations, both from primary and secondary centers will differ from that obtained from dry bone observations due to problems of clarity of the radiographs, training and experience of the observer and the recognition of stages of union (Krogman and Iscan, 1986). On the other hand, and compared to primary centers of ossification, only a few studies have actually provided ages of fusion for secondary centers of ossification in dry bone (Stevenson, 1924; McKern and Stewart, 1957; Veschi and Faschini, 2002; Coqueugniot and Weaver, 2007).

According to Scheuer and Black (2000), there is an alarming paucity of detailed information concerning secondary vertebral centers of ossification in the sacrum. It is also clear from the literature that there is also a lack of dry bone data, particularly, for fusion of the primary centers. The sacrum is developmentally complex bone and ossifies from 21 primary and 14 secondary ossification centers (Scheuer and Black, 2000). The 21 primary ossification centers, which appear in the fetus, include five centers for the vertebral bodies (centra), six centers for the costal elements (S1, S2 and S3), and ten centers for the neural arches, two for each sacral vertebra (Scheuer and Black, 2000). Comparatively, there are 14 constant secondary ossification centers in the sacrum, 10 annular epiphysis for the vertebral bodies – 2 for each vertebrae, 2 auricular epiphysis for the sacro-iliac joint and 2 epiphysis for the lateral margin of the sacrum (Scheuer and Black, 2000). Consequently, the sacrum provides a number of developmental indicators which can be used for estimating the age of child or adolescent skeletons.

Whereas only imagiological data is available for fusion times of primary centers of ossification in the sacrum (Götz, 1993; Bollow et al., 1997; Broome et al., 1998), the available times obtained for secondary centers of ossification from dry bone observations have important limitations. For example, some of the published standards for epiphyseal union provide information only for males (McKern and Stewart, 1957) or collapse information from adjacent epiphyses (Veschi and Faschini, 2002). In addition, some standards provide incomplete data by using truncated samples at either the lower (McKern and Stewart, 1957; Veschi and Faschini, 2002) and upper age limits (Veschi and Faschini, 2002). Finally, some studies actually failed to provide detailed or any information at all for the sacum (Stevenson, 1924; Coqueugniot and Weaver, 2007). Only more recently have some of these issues been addressed by Rios and co-workers (2008) and Passalacqua (2009) who have used developmental indicators of the sacrum for age estimation of young adult human skeletal remains, namely fusion of the first vertebral bodies, among other indicators.

The goal of this study is to document the chronology and fusion sequence of the primary and secondary centers of ossification of the sacrum, between birth and late adolescence, using a sample of immature human skeletons of both sexes and of known age. Results are compared with published age determination standards obtained from skeletal samples and from different imaging techniques, as observations from dry bone specimens are scarcely available.

This study also aims at developing probabilistic means to estimate age-at-death of immature human skeletal remains, based on the established chronology of sacral fusion. Summary tables for ages of fusion can be considered less suitable for age estimation and, therefore, posterior probability tables have been devised for age estimation. Results reported here provide not only further comparative data on the fusion of different ossification centers but also important alternative tools for age estimation in bioarchaeological and forensic studies.

MATERIALS AND METHODS

This study utilized a sample of 191 skeletons from the documented human skeletal collection curated at the National Museum of Natural History in Lisbon, Portugal (Cardoso, 2006). All individuals between the ages of 0 and 30 years were selected, of which 90 are females and the remaining 101 are males. Their births occurred between 1887 and 1973, whereas deaths occurred between 1903 and 1975. The period between 1920 and 1950 is where most births and deaths took place. Reported ages at death were confirmed by comparison with birth and death dates obtained from civil records (Cardoso, 2005), and, are known in calendar days, in most cases. The age and sex distribution of the sample is depicted in Figure 1. The skeletal sample represents the middle-to-low social class of the city of Lisbon, as inferred from the origin of the remains (temporary graves) (Cardoso, 2006). In addition, the occupations of the adult male segment of the collection, as well as the occupations of the fathers of the subadult segment, include a large proportion of menial jobs and, thus, are also suggestive of a lower socioeconomic status for the collection (Cardoso, 2005, 2007).

Sacra were observed in all stages of postnatal skeletal development. The bone is composed by 21 primary centers of ossification (Francis, 1951) and 14 secondary centers of ossification (Fawcett, 1907; Frazer, 1948).

Fusion was recorded between the following primary centers of ossification: neural arch (Ne) with the costal element (Co) in S1 and S2; neural arch (Ne) with the centrum (Ce) in S1 and S2; costal element (Co) with the centrum (Ce) in S1 and S2, fusion of the neural arch (Ne) with the centrum (Ce) in S3, S4 and S5 (Figure 2 and 3). Although the sacrum has been described has having costal elements on S3, and sometimes on S4 (Scheuer and Black, 2000), no separate centers were identified in this study for these sacral vertebrae.

Consequently, fusion of the costal element (Co) with the neural arch (Ne) or with the centrum (Ce) were only recorded on S1 and S2.

Fusion was also recorded in the following three secondary centers of ossification: the superior annular epiphysis of S1 (Figure 4 and 5); the sacro-iliac epiphysis (Figure 4 and 6); and the lateral margin (Figure 4 and 7). Fusion between the vertebral bodies or centra and their respective annular rings, as well as fusion of costal and/neural elements between S1 and S2 through S4 and S5 was not recorded as the information on timing of fusion of these centers in this sample has already been published elsewhere (Rios et al., 2009). In addition, fusion of the posterior synchondrosis in S1 through S5 was also not recorded, due to a high prevalence of spina bifida in the sample. In these circumstances, and in several cases, it was impossible to distinguish an unfused posterior synchondrosis from a case of spina bifida.

Fusion was initially recorded on the right and left side, for paired ossification centers, but only information on the left side was utilized. However, whenever bilateral asymmetry was identified, the asymmetric individual was duplicated in order to include the greatest variation in fusion regardless of side. Due to problems of preservation some anatomical locations could not be observed and thus sample sizes vary accordingly. Pathological skeletons were also excluded from the study.

A three-stage scheme was used for scoring the degree of fusion between these epiphyses: 1) no union, 2) partial union, and 3) completed union (all traces of fusion having disappeared). The presence of the epiphyseal line or scar, which is a persistent gapless line at the junction of ossification centers, was disregarded as it may consistently overestimate the true age of fusion (Stevenson, 1924). Each fusion between ossification centers was scored independently and summary ages of fusion were obtained. For each fusion between primary and secondary ossification centers, the oldest individual at stage 1 (not fused) provides the upper age limit for this stage’s age interval. The youngest individual at stage 3 (completely fused) provides the lower age limit for this stage's age interval. The youngest and oldest individuals at stage 2 (partial union) provide the lower and upper age limits for this stage's age range.

Data was collected by one of the senior authors and intra-observer agreement was estimated by re-assessing stages of union in a random sample of 10 individuals, several weeks after the initial assessment. Inter-observer agreement was estimated by comparing the assessment of the three authors on a random sample of 10 individuals. Percentage of agreement was chosen as the measure of observer error.

Assessment of sex differences in age at which both primary and secondary ossification centers fuse was carried out by dichotomizing observations into "fusion not attained" versus "fusion attained" and then the calculation of an overall logistic regression model with sex and age as the covariates, for each fusion. The significance of sex differences in timing of fusion was obtained by testing whether the coefficient for the variable "sex" is statistically different from zero (no sex differences statistically significant) using the Wald statistic. The analysis was performed on IBM's SPSS v.20.

Posterior probability tables of age for a given stage of fusion, assuming uniform prior probability of age and reference prior probability of age (Lucy et al., 1996; Chamberlain, 2000), were also generated to provide more detailed information about the age variation in fusion of ossification centers which can be used for age estimation of unknown immature skeletal remains. Although the age distribution of the reference sample is not recommended for use as a source of priors in age estimation, posterior probabilities of age based on the reference series provides comparative information that is unique to the study sample. In contrast, the use of uniform priors reflects a more realistic approach where no assumptions are made about the age distribution of the target population.

RESULTS

Intra-observer agreement was 100% and inter-observer agreement was 96% between the two senior authors and 87% between one of the senior and junior authors. In all cases the disagreement refers to partial fusions being mistaken by a complete fusion or vice-versa. This only occurred in fusion between the neural arch and the centra of the smaller sacral vertebrae and, occasionally, in fusion between the neural arch of the centrum when only visible from the vertebral canal (posteriorly). The size of these ossification centers and their frequent poor preservation, in addition to areas of difficult observation are the likely factors behind the intra- and inter-observational variation.

There were only 11 cases of bilateral asymmetry identified and only 3 actually influenced the construction of summary and posterior probability tables. These asymmetric individuals always refer to cases where the right side showed partial union and the left either unfused or completed fused centers. Consequently, the 3 cases expanded the age range for stage 2 by one year, either inferiorly or superiorly and the anatomical locations involved are fusion between the neural arch (Ne) and the costal element (Co) in S2, the fusion between the neural arch (Ne) and centrum (Ce) in S5, both in males, and the fusion of the lateral margin epiphysis in females.

Table 1 shows the age summary for fusion of the primary ossification centers of the sacrum and Table 2 summarizes the age intervals for fusion of the secondary ossification centers. In these tables, data are broken down by fusion between individual ossification centers and by sex. Ages are established in one year intervals and represent the interval between the value of one age and the next (e.g. 16 years = 16.0-16.9 years). The last three columns of both Table 1 and Table 2 are identified with the headings stage 1, stage 2 and stage 3. The first column (stage 1) indicates the age of the oldest individual at stage 1 (no union), that is, the age after which the ossification center is likely to be either partially or completely fused. The second column (stage 2) shows the age range of individuals at stage 2 (partial union), that is, the youngest and the oldest age at which the ossification center is in the process of becoming fused. Finally, the third column (stage 3) indicates the age of the youngest individual at stage 3 (completed union), that is, the age before which the ossification center is likely to be either unfused or only partially fused. The purpose of these three columns is to provide the researcher summary developmental information and prompt data to assess the age of unidentified skeletal remains.

If the specimen under examination shows partially united (stage 2) epiphysis, tables provide an estimated interval for the specimen's true chronological age. Using the fusion between the neural arch and costal element (Ne-Co) in S1. As an example, if a certain specimen shows a partial union (stage 2), the estimated age interval is likely to be in the 3-7 year range (3-5 years for females and 4-7 years for males). If the specimen shows an unfused or a completely fused epiphysis, Table 1 and Table 2 data will only provide an upper or lower limit for the estimated age interval, respectively. For example, if the union between the neural arch and costal element (Ne-Co) in S1 is not fused, the specimen is likely to be under 4 years of age.

More detailed information about age of union in all ossification centers of the sacrum is shown in Tables 3-16 in the form of posterior probabilities of age given a certain stage of fusion. Tables 3-10 show the probability of age given a certain stage of fusion of primary ossification centers and Tables 11-16 show the probability of age given a certain stage of fusion of secondary ossification centers.

Age can be estimated by using Tables 3-16 where the probability of age given a certain stage of fusion can be obtained by identifying a certain age range for that stage and adding the probabilities for that range. For example, the probability of an individual being between 3 and 5 years of age, given partial fusion of the neural arch and costal element of S1, is 0.78. Similarly, the probability of a female being between 18 and 21 years of age, given partial fusion of the sacro-iliac epiphysis is 0.85. Comparatively, there is a 0.91 probability of an individual being older than 9 years of age given a complete fusion between the costal element and the centrum of S1.

When considering the ages at stage 2 in Table 2 and Tables 3-10, the first ossification centers to fuse are those of the neural arch-centrum in S4 and S5 (Table 9 and 10), followed by the fusion of the neural arch-costal element in S2 (Table 6) after which the neural arch-centrum fusion seems to follow (Table 7). Then fusion seems to occur between the neural arch-centrum in S3 (Table 8), followed by fusion of ossification centers in S1, with the neural arch-centrum fusion (Table 4) occurring first than the neural arch-costal element fusion (Table 3). The last fusion to occur seems to take place between the costal element and centrum (Table 5) in S1.

Overall, primary centers of ossification seem to fuse from bottom to top, following this approximate inferior-superior sequence: S5 Ne-Ce → S4 Ne-Ce → (S2 Ne-Co → S2 Ne-Ce → S3 Ne-Ce) → S1 Ne-Ce → S1 Ne-Co → S1 Co-Ce. The parenthesis denotes approximately simultaneous fusion. This rough sequence is obtained regardless of whether it is based on observations at stage 2 or 3.

As for the chronology of fusion of secondary ossification centers, if only data at stage 2 is considered from Table 2 and Tables 11-16, the first fusion that occurs is that of the annular epiphysis in S1 (Tables 11-12). Fusion of the annular epiphysis is followed by the fusion of the epiphysis for the sacro-iliac epiphysis (Tables 13-14), which seem to occur simultaneously with that of the lateral margin epiphysis (Tables 15-16). When considering stage 3 instead, the annular epiphysis attains complete fusion simultaneously with that of the sacro-iliac epiphysis, both of which are then followed by complete fusion of the epiphysis for the lateral margin.

Visually there are no consistent differences between the sexes in fusion of primary ossification centers, which is confirmed statistically (results not shown). While female show earlier fusion in some ossification centers, in other it is the males who are ahead. Complete fusion is also found to occur first in both females and males, with to consistent pattern. As for the secondary ossification centers, visually, fusion initiates one to two years earlier in females and is complete at the same time as that of males or one year earlier. However, statistically significant sex differences in fusion are only found in the superior annular epiphysis (Wald=7.443, p=0.06)

DISCUSSION

To the best of the authors' knowledge, this is the first study which was able to document in detail the chronology and sequence of fusion of primary and secondary ossification centers in the sacrum from dry bone observations and in both sexes. Data presented in this study provides anatomical information and methodological tools for age estimation of unidentified immature human skeletal remains, which adds to previously published information on the sacrum by Rios and co-workers (2009), but also data from the same collection on fusion of other secondary (Cardoso, 2008b,c; Cardoso and Severino, 2009) and primary centers of ossification (Cardoso et al., 2013). Compared to other previous studies, this provides slightly more complete information as it also includes data for females, it does not collapse information from different centers and the sample is not truncated in the age range.

Fusion of the primary ossification centers analyzed in this study, occurs between 1 and 8 years age, as documented by the age of fusion of the neural arch and centrum of the fifth sacral vertebra, which are the first primary ossification centers to fuse, and by the age of fusion of the costal element and centrum of the first sacral vertebra which are the last, following a caudal-cranial sequence of ossification.

Fusion of the secondary centers of ossification take place between the ages of 15 and 21 years, with the fusion of the superior annular epiphysis occurring first and the fusion of the sacro-iliac epiphysis occurring last. The sequence of fusion occurs in a caudo-cranial direction, as previously documented by Johnston (1961) for the annular epiphysis, sacrum bodies and costal elements.

There are no significant sexual differences in fusion of primary ossification centers, with both males and females showing earlier and later completion times alternatively. Since fusion of these centers occurs before puberty, sex differences are likely to have not been fully expressed. Comparatively, the three secondary ossification centers start to fuse earlier in females, the superior annular and auricular epiphysis completes fusion at the same time in both sexes and the lateral margin achieves complete fusion one year earlier in females.

Although results seem to suggest an earlier maturation in females, only the annular epiphysis showed statistically significant differences. This is consistent with timing of fusion in secondary ossification centers of the vertebral column (Cardoso and Rios, 2011), where there is also a trend toward early maturation in females, but that cannot be systematically confirmed statistically.

Lack of significant sex differences in fusion of secondary centers of ossification may be an expression of little variation being sampled. In fact, although samples of subadult skeletons of known age are rare, their size can be effectively small and, for that reason, can limit the amount of variation that is being sampled. In addition to less variation being sampled, ages of fusion for some centers could not be effectively documented, as it is the case of partial fusion between the costal element and the centrum (Co-Ce) of the second sacral vertebra fusion in both sexes because it was not observed in any of the individuals in the sample. This results in zero probabilities of age in certain age groups for fusion of ossification centers.

Despite some of its obvious limitations, results in this study can be particularly useful for the estimation of age in immature human skeletal remains, mainly in the absence of teeth. Facing unidentified skeletal remains, and being in presence of the sacrum, it is possible to limit the probable age to an interval, as long as the ossification centers are only partially fused. Whenever the union has not commenced or is complete, only a maximum or a minimum age can be obtained, respectively. The age of unidentified skeletal remains can be established to within 3-4 years using the timing of fusion of primary ossification centers of S5, S4 and S3, and within 5-6 years using the timing of primary ossification centers of S2 and S1. A similar range can be obtained when using the timing of fusion of secondary ossification centers. These wide ranges can be of limited practical use when ageing very young individuals, particularly in a forensic context. Since females are almost always in advance of males in skeletal maturation in adolescence, it is desirable to determine the sex of the remains prior to the estimation of age from fusion of secondary centers of ossification. This may not be required for when age is estimated from fusion of primary ossification centers.

Due to differences between imagiological (x-ray, CT-scan, MRI) and gross inspection of fusion, it is also preferable to estimate age from data presented here when estimating the age of dry bone remains.

Comparing the results of this study with that of other published materials may be useful to assess potential population variation in bone maturity, but this is actually constrained by the source material for fusion of the sacrum, which is largely imagiological in nature. Although differences between imagiological and gross (dry bone) inspections would require comparisons to be made with other similar skeletal samples, this cannot be accomplish entirely due to scarcity of dry bone data.

With respect to fusion of primary ossification centers, only radiographic data has been compiled by Scheuer and Black (2000) and made available by Broome and co-workers (1998) from radiographs, CT scans and MRI's. According to Scheuer and Black (2000) the union between the neural arch and the costal element (Ne-Co) occurs between 2-5 years age and the fusion between the neural arch and the center of the vertebra (Ne-Ce) and the costal element and the center of the vertebra (Co-Ce), occur between 2-6 years age. This information is consistent with that of this study, but only if S1 and S2 and considered.

Broome and co-workers (1998) report similar age ranges for fusion between most primary centers of ossification, but show 2-4 years earlier fusion between the centers of S1, between the neural arch and the costal element in S2 and the neural arch and centrum in S3.

With respect to fusion of secondary centers of ossification, data published by Rogers and Cleaves (1935) and by Bollow and co-workers (1997) are only indicative of fusion times as the samples in both studies comprise children with pathological disorders and were obtained from imaging techniques. In these cases, the sacro-iliac epiphysis shows complete fusion by 17-18 years of age (Rogers and Cleaves, 1935), which is somewhat earlier than that found in this study, and the lateral epiphysis is reported as showing progressive ossification between 9 and 17 years of age (Bollow and co-workers, 1997), also earlier than that observed in this study. In both studies, fusion of these epiphyses was observed earlier in females compared to males.

Broome and co-workers (1998) also report on ages of fusion for the secondary centers of ossification. In their study, commencing fusion of the lateral margin and of the sacro-iliac epiphysis take place between 18-21 and 18-19 years of age, respectively. Both age ranges are well within the ranges provided by the study sample. Additionally and according to McKern and Stewart (1957), the superior annular epiphysis, the lateral epiphysis and the sacro-iliac epiphysis all fuse between 17-21 years of the age. Only the lateral margin seems to fuse slightly later in McKern and Stewart's (1957) sample compared to the study sample. First ages of fusion cannot be compared directly given that McKern and Stewart's (1957) sample is inferiorly truncated at the age of 17 years.

Veschi and Fachini (2002) also provide timings of fusion in a skeletal sample, but here secondary centers in the vertebra are shown combined with that of the fusion of sacral bodies, so it is uncertain whether these secondary centers also refer to the sacrum. In any case, overall fusion seems to occur between 16 and 24 years in these locations. The very wide age range can include fusion of all secondary centers and is not particularly useful for meaningful comparisons.

Stevenson (1924) and Coqueugniot and Weaver (2007), who report timings of secondary center fusion in several anatomical locations from dry bone observations in two different skeletal samples, do not provide comparative data for the sacrum.

Fusion of the secondary ossification centers in the cervical, thoracic and lumbar vertebrae (Cardoso and Rios, 2011) occurs generally sooner than that in the sacrum. The only secondary ossification center of the sacrum shared with the pre-sacral vertebrae is the annular epiphysis, which fuses one year earlier in the fifth lumbar vertebra compared to that of the sacrum. This chronology is, perhaps, expected as the fifth lumbar vertebra precedes the first sacral vertebra and fusion of epiphysis seems to follow the caudo-cranial gradient discussed above and by Cardoso and Rios (2011).

Overall, and despite the differences in scoring methodologies between imaging techniques used in several studies and dry bone observations adopted in this study, the age ranges obtained are similar to those reported in other studies, with some notable discrepancies. Unfortunately, there is very little data with which meaningful comparisons can be carried out. In fact, if comparisons can be done across dry bone or radiographic studies, as to eliminate methodological differences, there is data which suggests that socioeconomic status will probably explain most of the variation in skeletal maturation between samples.

This is true whether these socioeconomic differences span time periods (secular trends) or geographical locations (developed versus developing countries). For example, secular acceleration in skeletal maturation has been clearly demonstrated for British (Himes, 1984) and South African children (Hawley et al., 2009) from hand-wrist radiographs, with a mean advancement of about 1 year. Comparatively, in a group of malnourished populations from Central America, Frisancho and co-workers (1970a,b) found that skeletal maturation in poor adolescents was 5-9% delayed relative to US standards, compared with a delay of 36-38% in early childhood. Similar results have also been reported by Dreizen and co-workers (1967) and Pickett and co-workers (1995) for US and Guatemalan malnourished childen, respectively. These findings suggest that secular trend effects within the same population and different levels of social and economic development between populations have a smaller effect on timing of fusion of secondary centers of ossification than on fusion of primary centers. However, none of the effects, particularly in younger children, can be considered negligible.

Although age intervals for maximum error in estimating age are about 3-4 years in the primary centers and 5-6 years in secondary centers, errors of assessment due to socioeconomic status or secular effects should not be considered irrelevant, as they are likely to shift the starting and ending points of the probable age ranges, during which centers are fusing. This will be particularly notorious as the greater delay in skeletal maturation can be expected for fusion of primary centers of ossification (Frisancho et al., 1970a,b). A 5-9% development delay can be translated into a bias of about 0.75 to 2 years in estimated age from fusion of primary ossification centers, whereas a 35-38% delay results in a bias of about 0.35 to 3 years in estimated age from fusion of secondary ossification centers.

Other studies have confirmed this motion that socioeconomic status can affect the timing of bone maturation and have shown that earlier reference standards for epiphyseal union are not appropriate for ageing modern human skeletons in a forensic context, by identifying a secular acceleration in skeletal maturation (Meijerman et al., 2007; Langley-Shirley and Jantz, 2010). For example, Meijerman and co-workers (2007) have shown that socioeconomic status has a negative impact on epiphyseal union of the medial clavicle, by decreasing the predicted probability of individuals having mature clavicles at each age. The decrease in probability suggests a delay of about one year in low socioeconomic status individuals. One year is approximately the amount of absolute skeletal delay that one would expect from the studies carried out by Frisancho et al. (1970a,b), who report a 5-9% delay.

Being that most births and deaths in the study sample occurred between 1920 and 1949, and taking into consideration improvements in social economic conditions of the population from which the study individuals were drawn (Cardoso, 2008a), the sample may not be representative of the current population. In fact, the study sample has been described as representing many populations experiencing lower levels of social and economic development (Cardoso, 2005; Cardoso, 2007), which means that its individuals may show, on average, the typical delay in bone maturation in infancy and adolescence that results from malnutrition and which has been documented in developing countries (Dreizen et al., 1967; Frisancho et al., 1970a,b; Pickett et al., 1995). The study sample individuals would also show a maturational delay which is typical of a country which has not gone through a secular trend similar to that which most developed nations have experienced in the last century.

Although the impact of socioeconomic status on bone maturation can be relatively small, compared to the wide age intervals with which age can be established using epiphyseal union, it is not necessarily irrelevant (Cardoso, 2008b). Therefore, for a correct use of the age-based reference standards for fusion of primary or secondary centers of ossification, the forensic anthropologist and the bioarchaeologist should pay special attention to the different levels of social modernization and/or economic development of the population from which the standard was derived and from which the skeletal remains that are being aged originate.

Although the children and adolescents in the study sample do not represent either the well off or the extremely disadvantaged segments of the early 20^{th} Portuguese society, they may serve as a reference sample for many populations experiencing lower levels of social and economic development. This may include prehistoric or historic populations studied by bioarchaeologists or people from developing countries investigated by forensic anthropologists.

CONCLUSIONS

This study documents ages of fusion of primary and secondary centers of ossification in the sacrum and provides means for age estimation of unidentified human skeletal remains from fusion of those ossification centers. It is possibly the first occasion where such detailed information is systematically available for dry bone observations in a known age skeletal sample, particularly for fusion of primary ossification centers and in both sexes.

With the exception of fusion of sacral vertebrae (centra), fusion between ossification centers of the sacrum occurs between the age of 1 year and the age of 21 years, with primary centers initiating fusion by 1 year of age and being completely fused by 8 years of age; and secondary centers initiating fusion by 15 years of age and being completely fused by 21 years of age. The sequence of fusion follows a caudal-cranial and anterior-posterior gradient in the primary centers of ossification. There are no sex differences in the primary centers and sexes differ only in fusion of secondary centers. Tables of posterior probabilities of age provide a means to estimate age of individuals in early childhood and adolescence/early adulthood.

The age variation in fusion of secondary centers of ossification in the vertebra described here can provide important information for aiding the estimation of age of adolescent and young adult skeletons, increasing the available information from previously published works. The data provide additional information which can be useful in a variety of contexts. Although age ranges obtained do not differ significantly from those provided by radiographic studies, differences in methodology might prove them unsuitable for use in skeletal samples. Data presented here, however, scarce is useful for age estimation of unidentified skeletal remains, but may not be considered representative of modern living populations. Nonetheless, data can still be used to evaluate archaeological samples and possibly forensic cases from developing nations.

ACKNOWLEDGMENTS

We thank the National Museum of Natural History (University of Lisbon) for providing access to the collections in their care.

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Table 1 - Summary of age (in years) of fusion of primary ossification centers of the sacrum.

Vertebra	Centers fused	Sex	Stage 1	Stage 2	Stage 3
S1	Ne-Co	F	≤4	3-5 (n = 5)	≥5
	Ne-Co	M	≤4	4-7 (n = 4)	≥5
	Ne-Co	F+M	≤4	3-7 (n = 9)	≥5
	Ne-Ce	F	≤5	3-6 (n = 6)	≥5
	Ne-Ce	M	≤4	2-7 (n = 4)	≥3
	Ne-Ce	F+M	≤5	2-7 (n = 10)	≥3
	Co-Ce	F	≤5	3-8 (n = 7)	≥5
	Co-Ce	M	≤4	3-8 (n = 4)	≥5
	Co-Ce	F+M	≤5	3-8 (n = 11)	≥5
S2	Ne-Co	F	≤4	2-3 (n = 2)	≥5
	Ne-Co	M	≤3	1-4 (n = 4)	≥3
	Ne-Co	F+M	≤4	1-4 (n = 6)	≥3
	Ne-Ce	F	≤4	2 (n = 1)	≥5
	Ne-Ce	M	≤4	3 (n = 1)	≥3
	Ne-Ce	F+M	≤4	2-3 (n= 2)	≥3
	Co-Ce	F	≤4	-	≥5
	Co-Ce	M	≤4	-	≥3
	Co-Ce	F+M	≤4	-	≥3
S3	Ne-Ce	F	≤2	2-5 (n = 4)	≥4
	Ne-Ce	M	≤4	2-3 (n = 2)	≥2
	Ne-Ce	F+M	≤4	2-5 (n = 6)	≥2

S4	Ne-Ce	F	≤2	1-3 (n = 3)	≥2
	Ne-Ce	M	≤2	2-3 (n = 2)	≥2
	Ne-Ce	F+M	≤2	1-3 (n = 5)	≥2
S5	Ne-Ce	F	-	2-3 (n = 2)	≥2
	Ne-Ce	M	≤2	1 (n = 1)	≥1
	Ne-Ce	F+M	≤2	1-3 (n = 3)	≥1

Ne – neural arch, Co – costal element, Ce - centrum

Table 2 - Summary of age (in years) of fusion of secondary ossification centers of the sacrum.

Epiphysis	Sex	Stage 1	Stage 2	Stage 3
Annular	F	≤16	15-21 (n = 18)	≥18
	M	≤18	17-21 (n = 11)	≥16
	F+M	≤18	15-21 (n = 29)	≥16
Sacro-iliac	F	≤19	16-21 (n = 9)	≥18
	M	≤18	17-21 (n = 9)	≥16
	F+M	≤19	16-21 (n = 18)	≥16
Lateral margin	F	≤19	16-19 (n = 3)	≥18
	M	≤21	18-20 (n = 3)	≥18
	F+M	≤21	16-20 (n = 6)	≥18

Table 3 - Posterior probabilities of age (in years) given a certain stage of union between the neural (Ne) and costal (Co) centers of ossification of the first sacral vertebra (sexes combined).

Age	Reference priors			Uniform priors		
	Stage 1	Stage 2	Stage 3	Stage 1	Stage 2	Stage 3
<2	0.72	0.00	0.00	0.66	0.00	0.00
3	0.23	0.11	0.00	0.20	0.07	0.00
4	0.05	0.11	0.00	0.15	0.22	0.00
5	0.00	0.67	0.01	0.00	0.49	0.01
6	0.00	0.00	0.04	0.00	0.00	0.04
7	0.00	0.11	0.01	0.00	0.22	0.03
>8	0.00	0.00	0.93	0.00	0.00	0.92
	1.00	1.00	1.00	1.00	1.00	1.00

Table 4 - Posterior probabilities of age (in years) given a certain stage of union between the neural (Ne) and centrum (Ce) centers of ossification of the first sacral vertebra (sexes combined).

Age	Reference priors			Uniform priors		
	Stage 1	Stage 2	Stage 3	Stage 1	Stage 2	Stage 3
<1	0.41	0.00	0.00	0.42	0.00	0.00
2	0.30	0.10	0.00	0.19	0.06	0.00
3	0.19	0.20	0.01	0.15	0.14	0.00
4	0.08	0.00	0.00	0.21	0.00	0.00
5	0.03	0.50	0.01	0.03	0.44	0.01
6	0.00	0.10	0.04	0.00	0.12	0.03
7	0.00	0.10	0.01	0.00	0.24	0.03
>8	0.00	0.00	0.93	0.00	0.00	0.93
	1.00	1.00	1.00	1.00	1.00	1.00

Table 5 - Posterior probabilities of age (in years) given a certain stage of union between the costal (Co) and centrum (Ce) centers of ossification of the first sacral vertebra (sexes combined).

Age	Reference priors			Uniform priors		
	Stage 1	Stage 2	Stage 3	Stage 1	Stage 2	Stage 3
<2	0.69	0.00	0.00	0.61	0.00	0.00
3	0.21	0.18	0.00	0.16	0.11	0.00
4	0.08	0.00	0.00	0.20	0.00	0.00
5	0.03	0.45	0.01	0.03	0.34	0.01
6	0.00	0.09	0.04	0.00	0.09	0.03
7	0.00	0.09	0.01	0.00	0.18	0.03
8	0.00	0.18	0.01	0.00	0.27	0.02
>9	0.00	0.00	0.92	0.00	0.00	0.91
	1.00	1.00	1.00	1.00	1.00	1.00

Table 6 - Posterior probabilities of age (in years) given a certain stage of union between the neural (Ne) and costal (Co) centers of ossification of the second sacral vertebra (sexes combined).

Age	Reference priors			Uniform priors		
	Stage 1	Stage 2	Stage 3	Stage 1	Stage 2	Stage 3
0	0.07	0.00	0.00	0.27	0.00	0.00
1	0.37	0.17	0.00	0.25	0.11	0.00
2	0.33	0.50	0.00	0.20	0.31	0.00
3	0.19	0.17	0.01	0.19	0.17	0.01
4	0.04	0.17	0.01	0.09	0.41	0.01
>5	0.00	0.00	0.98	0.00	0.00	0.98
	1.00	1.00	1.00	1.00	1.00	1.00

Table 7 - Posterior probabilities of age (in years) given a certain stage of union between the neural (Ne) and centrum (Ce) centers of ossification of the second sacral vertebra (sexes combined).

Age	Reference priors			Uniform priors		
	Stage 1	Stage 2	Stage 3	Stage 1	Stage 2	Stage 3
<1	0.50	0.00	0.00	0.46	0.00	0.00
2	0.36	0.50	0.00	0.21	0.31	0.00
3	0.07	0.50	0.01	0.09	0.69	0.02
4	0.07	0.00	0.00	0.23	0.00	0.00
>5	0.00	0.00	0.99	0.00	0.00	0.98
	1.00	1.00	1.00	1.00	1.00	1.00

Table 8 - Posterior probabilities of age (in years) given a certain stage of union between the neural (Ne) and centrum (Ce) centers of ossification of the third sacral vertebra (sexes combined).

Age	Reference priors			Uniform priors		
	Stage 1	Stage 2	Stage 3	Stage 1	Stage 2	Stage 3
<1	0.55	0.00	0.00	0.66	0.00	0.00
2	0.41	0.50	0.01	0.23	0.26	0.00
3	0.00	0.33	0.01	0.00	0.56	0.02
4	0.05	0.00	0.01	0.11	0.00	0.02
5	0.00	0.17	0.04	0.00	0.19	0.03
>6	0.00	0.00	0.93	0.00	0.00	0.92
	1.00	1.00	1.00	1.00	1.00	1.00

Table 9 - Posterior probabilities of age (in years) given a certain stage of union between the neural (Ne) and centrum (Ce) centers of ossification of the fourth sacral vertebra (sexes combined).

Age	Reference priors			Uniform priors		
	Stage 1	Stage 2	Stage 3	Stage 1	Stage 2	Stage 3
0	0.08	0.00	0.00	0.43	0.00	0.00
1	0.54	0.20	0.00	0.38	0.13	0.00
2	0.38	0.40	0.03	0.20	0.19	0.01
3	0.00	0.40	0.01	0.00	0.68	0.01
>4	0.00	0.00	0.96	0.00	0.00	0.98
	1.00	1.00	1.00	1.00	1.00	1.00

Table 10 - Posterior probabilities of age (in years) given a certain stage of union between the neural (Ne) and centrum (Ce) centers of ossification of the fifth sacral vertebra (sexes combined).

Age	Reference priors			Uniform priors		
	Stage 1	Stage 2	Stage 3	Stage 1	Stage 2	Stage 3
0	0.25	0.00	0.00	0.59	0.00	0.00
1	0.50	0.33	0.01	0.29	0.26	0.01
2	0.25	0.33	0.03	0.12	0.21	0.02
3	0.00	0.33	0.01	0.00	0.53	0.02
>4	0.00	0.00	0.96	0.00	0.00	0.95
	1.00	1.00	1.00	1.00	1.00	1.00

Table 11 - Posterior probabilities of age (in years) given a certain stage of the annular epiphysis (reference priors).

Age	Females			Males			Sexes combined		
	Stage 1	Stage 2	Stage 3	Stage 1	Stage 2	Stage 3	Stage 1	Stage 2	Stage 3
<14	0.97	0.00	0.00	0.86	0.00	0.00	0.91	0.00	0.00
15	0.00	0.06	0.00	0.02	0.00	0.00	0.01	0.03	0.00
16	0.03	0.33	0.00	0.07	0.00	0.04	0.05	0.21	0.02
17	0.00	0.06	0.00	0.04	0.09	0.00	0.02	0.07	0.00
18	0.00	0.06	0.07	0.02	0.27	0.00	0.01	0.14	0.04
19	0.00	0.33	0.04	0.00	0.27	0.00	0.00	0.31	0.02
20	0.00	0.11	0.07	0.00	0.18	0.08	0.00	0.14	0.08
21	0.00	0.06	0.18	0.00	0.18	0.25	0.00	0.10	0.21
>22	0.00	0.00	0.64	0.00	0.00	0.63	0.00	0.00	0.63
	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

Table 12 - Posterior probabilities of age (in years) given a certain stage of the annular epiphysis (uniform priors).

Age	Females			Males			Sexes combined		
	Stage 1	Stage 2	Stage 3	Stage 1	Stage 2	Stage 3	Stage 1	Stage 2	Stage 3
<14	0.99	0.00	0.00	0.85	0.00	0.00	0.91	0.00	0.00
15	0.00	0.21	0.00	0.06	0.00	0.00	0.03	0.14	0.00
16	0.01	0.18	0.00	0.05	0.00	0.02	0.03	0.14	0.01
17	0.00	0.21	0.00	0.04	0.12	0.00	0.03	0.14	0.00
18	0.00	0.07	0.07	0.01	0.26	0.00	0.01	0.16	0.03
19	0.00	0.18	0.01	0.00	0.35	0.00	0.00	0.24	0.01
20	0.00	0.11	0.05	0.00	0.18	0.06	0.00	0.14	0.05
21	0.00	0.04	0.08	0.00	0.09	0.09	0.00	0.06	0.07
>22	0.00	0.00	0.79	0.00	0.00	0.83	0.00	0.00	0.84
	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

Table 13 - Posterior probabilities of age (in years) given a certain stage of the sacro-iliac epiphysis (reference priors).

Age	Females			Males			Sexes combined		
	Stage 1	Stage 2	Stage 3	Stage 1	Stage 2	Stage 3	Stage 1	Stage 2	Stage 3
<15	0.84	0.00	0.00	0.90	0.00	0.00	0.87	0.00	0.00
16	0.10	0.22	0.00	0.07	0.00	0.04	0.08	0.11	0.02
17	0.02	0.00	0.00	0.02	0.11	0.00	0.02	0.06	0.00
18	0.02	0.11	0.04	0.02	0.33	0.00	0.02	0.22	0.02
19	0.02	0.33	0.07	0.00	0.33	0.00	0.01	0.33	0.04
20	0.00	0.11	0.07	0.00	0.11	0.15	0.00	0.11	0.11
21	0.00	0.22	0.15	0.00	0.11	0.26	0.00	0.17	0.20
>22	0.00	0.00	0.67	0.00	0.00	0.56	0.00	0.00	0.61
	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

Table 14 - Posterior probabilities of age (in years) given a certain stage of the sacro-iliac epiphysis (uniform priors).

Age	Females			Males			Sexes combined		
	Stage 1	Stage 2	Stage 3	Stage 1	Stage 2	Stage 3	Stage 1	Stage 2	Stage 3
<15	0.88	0.00	0.00	0.91	0.00	0.00	0.90	0.00	0.00
16	0.04	0.16	0.00	0.05	0.00	0.02	0.04	0.08	0.01
17	0.05	0.00	0.00	0.03	0.19	0.00	0.04	0.15	0.00
18	0.02	0.19	0.03	0.01	0.29	0.00	0.02	0.26	0.01
19	0.01	0.28	0.03	0.00	0.39	0.00	0.01	0.30	0.02
20	0.00	0.19	0.07	0.00	0.08	0.09	0.00	0.11	0.07
21	0.00	0.19	0.07	0.00	0.05	0.10	0.00	0.10	0.07
>22	0.00	0.00	0.80	0.00	0.00	0.79	0.00	0.00	0.82
	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

Table 15 - Posterior probabilities of age (in years) given a certain stage of the epiphysis for the lateral margin (reference priors).

Age	Females			Males			Sexes combined		
	Stage 1	Stage 2	Stage 3	Stage 1	Stage 2	Stage 3	Stage 1	Stage 2	Stage 3
<15	0.83	0.00	0.00	0.86	0.00	0.00	0.85	0.00	0.00
16	0.10	0.67	0.00	0.06	0.00	0.00	0.08	0.33	0.00
17	0.02	0.00	0.00	0.02	0.00	0.00	0.02	0.00	0.00
18	0.02	0.00	0.07	0.02	0.67	0.04	0.02	0.33	0.05
19	0.02	0.33	0.07	0.03	0.00	0.04	0.03	0.17	0.05
20	0.00	0.00	0.10	0.00	0.33	0.15	0.00	0.17	0.13
21	0.00	0.00	0.76	0.02	0.00	0.78	0.01	0.00	0.77
	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

Table 16 - Posterior probabilities of age (in years) given a certain stage of the epiphysis for the lateral margin (uniform priors).

Age	Females			Males			Sexes combined		
	Stage 1	Stage 2	Stage 3	Stage 1	Stage 2	Stage 3	Stage 1	Stage 2	Stage 3
<15	0.87	0.00	0.00	0.84	0.00	0.00	0.86	0.00	0.00
16	0.04	0.53	0.00	0.05	0.00	0.00	0.04	0.25	0.00
17	0.05	0.00	0.00	0.05	0.00	0.00	0.05	0.00	0.00
18	0.02	0.00	0.06	0.01	0.71	0.03	0.02	0.39	0.04
19	0.01	0.47	0.04	0.03	0.00	0.04	0.02	0.19	0.04
20	0.00	0.00	0.09	0.00	0.29	0.09	0.00	0.17	0.08
21	0.00	0.00	0.81	0.01	0.00	0.85	0.00	0.00	0.85
	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

Fig. 1. Sex and age distribution of the sample.

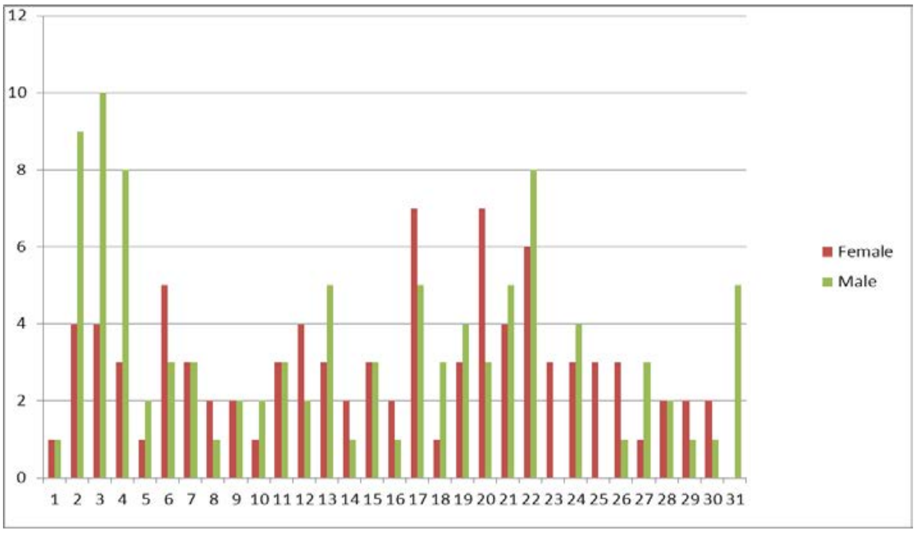

Fig. 2. Diagram illustrating the primary ossification centers of the sacrum scored in this study. Ne – neural arch, Co – costal element, Ce – centrum.

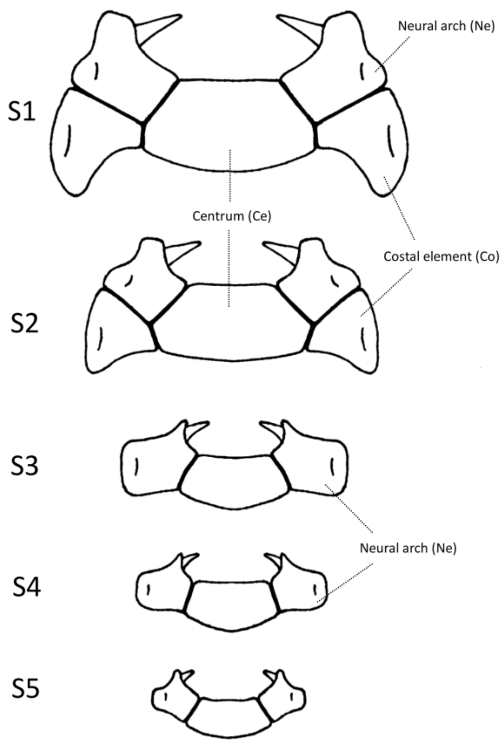

Fig. 3. – A two year-old female showing primary ossification centers in an unfused state, with the exception of S2 Ne-Co, Ne-Ce and Co-Ce, S3, S4 and S5 Ne-Ce which are partially fused.

Fig. 4. Diagram illustration the secondary ossification centers of the sacrum scored in this study.

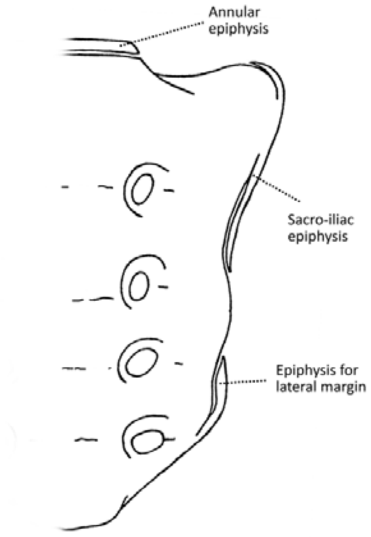

Fig. 5. A fifteen year-old female (same as in Fig. 7) showing partial union of the annular epiphysis.

Fig. 6. A twenty year-old male showing partial union of the sacro-iliac epiphysis.

Fig.7. A fifteen year-old female (same as in Fig. 5) showing partial union of the lateral margin.

American Journal of Physical Anthropology Guide for Authors

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Research Articles

General instructions. The manuscript should have a uniform style and be submitted exactly as it is to appear in print. It should consist of the following subdivisions in order, each prepared as a unit on separate pages or in separate files, as indicated in the “Online Submission” section:

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. . . analysis by Ward (2001) reveal . . .
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. . . an earlier report (Hutchinson, 1999) . . .
. . . earlier reports (Arriaza et al., 2000; Paoli et al., 2002; Ross, 2003) . . .

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Benefit BR, McCrossin ML. 1993b. On the lacrimal fossa of Cercopithecinae, with special reference to cladistic analysis of Old World monkey relationships. Folia Primatol 60:133–145.

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Book
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Book chapter
Gruner O. 1993. Identification of skulls: A historical review and practical applications. In: Iscan MY, Helmer RP, editors. Forensic analysis of the skull. New York: Wiley-Liss. p 29–45.

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	Abbreviations
AchE	Acetylcholinesterase
CP	Cortical plate
SmI	Primary somatosensory cortex
V	Ventral

Please reference relevant text pages in order to reduce the length of legends and avoid redundancy.

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Abbreviations. Use the following abbreviations for most common measurements of length, area, volume, and weight:

LENGTH

Km kilometer
m meter
cm centimeter
mm millimeter
µm micrometer (micron)
nm nanometer
pm picometer
Å Ångstrom unit (10 Å = 1 nm)

AREA

km^2 square kilometer
m^2 square meter
cm^2 square centimeter
mm^2 square millimeter
μm^2 square micrometer
nm^2 square nanometer
km^3 cubic kilometer
m^3 cubic meter
cm^3 cubic centimeter
mm^3 cubic millimeter
μm^3 cubic micrometer
nm^3 cubic nanometer

VOLUME

Kl kiloliter
liter spell out
ml milliliter
µl microliter
nl nanoliter
pl picoliter

WEIGHT

kg kilogram
gm gram
mg milligram
µg microgram
ng nanogram
pg picogram

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