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# Investigating histomorphological variations in human cranial bones through ontogeny

## *Enquête sur les variations histomorphologiques des os crâniens humains au cours de l'ontogenèse*

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ABSTRACT

Through ontogeny, human cranial vault bones undergo differentiation in terms of their shape, size and tissue maturation. This differentiation is visible at both the macroscopic and microscopic levels. Preliminary data from a histological and compartmentalisation exploratory analysis of individuals with different ages suggest differences in the modelling and remodelling patterns through ontogeny. Child vault bones are primarily composed of avascular lamellar bone (largely vascularised), late juvenile or adolescent bones present the largest extension of mineralised areas (highly remodelled) and the lowest vascularisation (diploe is highly reduced), and the adult present highly vascularised bone in which the diploe is again largely extended. During childhood, the existence of an avascular lamellar bone promotes the sealing of the cranium bones surfaces whereas adult vault bones seem to become opened ectocranially due to the remodelling. We discuss the possibility that both effects could be related with the head thermoregulation.



*Mots clés :*
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RÉSUMÉ

Au cours de l'ontogenèse, les os de la voûte crânienne humaine subissent une différenciation en termes de forme, de taille et de maturation de tissu. Cette différenciation est visible, non seulement au niveau macroscopique, mais aussi au niveau microscopique. Les données préliminaires d'une analyse exploratoire histologique et de la compartimentation d'individus d'âges différents suggèrent des différences dans les schémas de modelage et de remodelage au cours de l'ontogenèse. Les os de la voûte crânienne de l'individu infantile sont composés principalement d'os lamellaire (largement vascularisé), les os du juvénile

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ou de l'adolescent présentent la plus large extension de zones minéralisées (os très remodelés) et la plus faible extension de vascularisation (le diploé est très réduit), et l'adulte présente un tissu osseux très vascularisé, dans lequel le diploé est aussi très développé. Pendant l'enfance, l'existence d'os lamellaire avasculaire favorise le scellement des surfaces des os du crâne, tandis que les os de la voûte des adultes semblent s'ouvir ectocranialement, en raison du remodelage. Nous discutons de la possibilité que les deux effets soient liés à la thermorégulation de la tête.



## 1. Introduction

As part of the axial skeleton, the skull is considered one of the most important skeletal structures, the growth of which is closely associated with the development of the brain and sense organs. Similar to rest of the skeleton, skull growth is a sequential and ordered process that configures size and shape of bones through ontogeny (Scheuer and Black, 2000). As it is well known, the lower part of the neurocranium (i.e., the basicranium or the cranial base) is derived from mesoderm cells (used as a mould to build different bone morphologies during endochondral ossification, Karaplis, 2008). In contrast, the calvaria or cranial vault (the upper part of the neurocranium) and the major part of the viscerocranium (i.e., bones that constitute the face) course intramembranous ossification (osteoid substance is laid down directly over mesenchyme cells; Lewis, 2007; Scheuer and Black, 2000). Although the intramembranous ossification process of cranial bones has been embryologically well studied, the growth of the vault bones encompasses a more complex process that is not fully understood, and several important aspects, including bone tissues organisation in cranial bones, remain unknown (Bartsiokas, 2002; Hillier and Bell, 2007; Percival and Richtsmeier, 2013; Trammell, 2012). During ontogeny, bone tissues are biomineralised early while bones begin to increase in size and take shape according to their cranial morphological configuration. After the first mineralisation (constituting the trabecular or embryonic-like bone, de Ricqlès et al., 1999), bone tissues are reconfigured within the periosteum (situated in the ectocranial and endocranial surfaces) and the endosteum (the surface of the diploe cavities) (Francillon-Vieillot et al., 1990; Lieberman, 2011) such that the bone becomes thicker and radially enlarged from ossification centres. During this process (i.e., modelling, first named growth remodelling by Enlow, 1963), active periosteal and endosteal membranes replace old structures (younger tissues), depositing new bone via accretion and/or reabsorbing it in the complementary surface (Enlow, 1963, Enlow and Hans, 1996; Lieberman, 2011; McFarlin, 2006). The modelling of cranial bones is accompanied by an increase in the brain size, which provokes their passive movement via primary displacement and relocation (Aguila and Enlow, 1998; Enlow and Hans, 1996; Francillon-Vieillot et al., 1990) until they contact one another at cranial sutures (Enlow and Hans, 1996). The sutures are formed by a fibrous connective tissue derived from the mesenchyme that exhibits the same behaviour as an ossification growth site, and the sutures grow until the brain reaches its final size (Hall, 2005; Lana-Elola et al., 2007; Mishina and Snider, 2014; Morriss-Kay and Wilkie, 2005; Opperman, 2000). Sometimes, sutures present accessory ossification centres that generate isolated bones (sutural or wormian bones, Di Ieva et al., 2013). Remodelling, unlike modelling, replaces old bone by new one (Bayliss et al., 2011; Enlow, 1963; Martínez-Maza et al., 2006; McFarlin, 2006). A specialised type of cells, termed osteoclasts, and osteoblast, which are coordinated to constitute the basic multicellular units (BMUs), multiply after chemical or mechanical stimulations and begin to reabsorb bone, those causing irregular perforations called resorption spaces (RS). Then, finely laminated bone is centripetally deposited, enclosing blood capillary and nerves, constituting a secondary osteon (SO). The number of SOs within a bone section is related to mechanical loading in long bones and is usually used for age estimation (Robling and Stout, 2008). However, due to the absences of such mechanical influences (comparable to long bones) in the vault bones, the true role of SOs in these bones continues to be discussed (Hillier and Bell, 2007; Trammell, 2012).

Despite the profuse number of investigations regarding the cranium microstructure and histology using noninvasive-nondestructive analytical protocols, such as scanning electron microscope (SEM) (Kranioti et al., 2009; Martínez-Maza, 2007; Martínez-Maza et al., 2006, 2011, 2013; Mowbray, 2005), computed tomography (CT) (Anzelmo et al., 2014), micro-computed tomography (μCT) (Rühli et al., 2007) and synchrotron absorption-based μCT (Sanchez et al., 2012), conventional histological techniques used to assess bone tissue through bone thin sections (e.g., Enlow, 1968), could aid in better understanding human cranium ontogeny and the evolution of the cranium (Bartsiokas, 2002; Martínez-Maza et al., 2006). The present study focused on the histological analysis of cranial vault bones, in which we explored the microstructure of the frontal, parietal and occipital bones of three individuals from the same osteoachaeological collection with different ages (child, adolescent and young adult). Our primary objectives in this preliminary study of vault bones histology were (i) to analyse the tissue typologies and their spatial configurations, (ii) to study the compartmentalisation and spatial distribution of the mineralised and non-mineralised areas of each bone section, and (iii) to discuss possible implications of these variations from an ontogenetical perspective.

## 2. Materials and methods

Following the methodology proposed in Cambra-Moo et al. (2012, 2014), in which we analysed sections of tibia

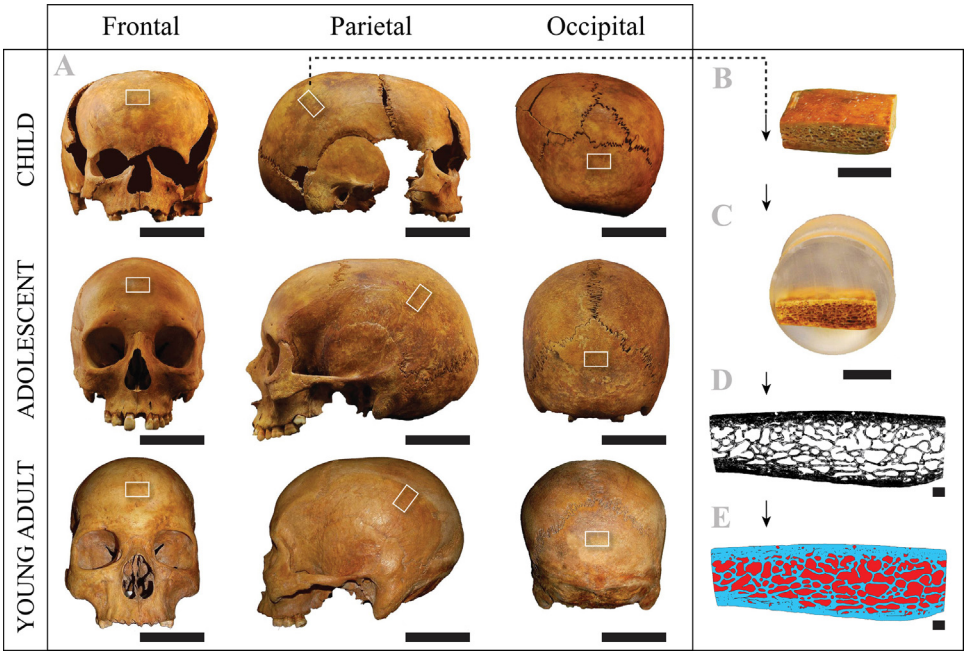


**Fig. 1.** (Color online.) Sampled crania included in the analysis. (A) Anterior, lateral and posterior views of the three crania analysed (child, adolescent and young adult), (B) the block of bone manually extracted from each individual (white squares represent the $2 \times 1.5\,cm^2$ areas sampled in the frontal, parietal and occipital bones of Fig. 1A), (C) the block of bone embedded in the transparent resin before preparing the thin section, (D) photomontage of images obtained using a polarised microscope, and (E) compartments (blue, mineralised area [MA]; and red, vascularisation [VASC]) drawn using geographical information system (GIS) software. Scale bar: 5 cm (A), 1 cm (B, C) and 1 mm (D, E).

**Fig. 1.** (Couleur en ligne.) Échantillons de crânes inclus dans l'analyse. (A) Vues antérieure, latérale et postérieure des trois crânes analysés (enfant, adolescent et adulte jeune), (B) bloc d'os extrait manuellement de chaque individu (les rectangles blancs représentent les zones de $2 \times 1{,}5\,cm^2$ échantillonnées dans l'os frontal, pariétal gauche et occipital de la Fig. 1A), (C) bloc d'os enrobé de résine transparente avant la préparation de la section mince, (D) photomontage des images obtenues au microscope polarisant, et (E) compartiments (bleu, surface minéralisée [MA] ; et rouge, vascularisation [VASC]) dessinés avec le logiciel *Geographical Information System* (GIS). Échelle : 5 cm (A), 1 cm (B, C) et 1 mm (D, E).

and humeri midshafts at different ontogenetic stages, we selected three complete human crania from subjects of different ages (child, adolescent and young adult; Fig. 1), and we analysed the histology of selected areas of the frontal, parietal and occipital bones. The skeletal remains are part of the ossuary excavated in the *Santa María de la Soledad* mediaeval church that located in Almansa (Castilla-La Mancha, Spain), which was utilised as a cemetery from the 12th to 18th centuries. From each individual, we extracted three different samples from the vault bones, avoiding areas of muscle attachments (Fig. 1A). Before extracting the blocks, we proceeded to photograph and measure the bones of each cranium to preserve the maximum information from each individual. Due to the nature of the deposit (i.e., an ossuary in which the crania were recovered disarticulated), it was not possible to recognize the mandible of the postcranial skeletal elements. Therefore, each individual was aged based on teeth (i.e., the maxilla) development recommendations (AlQahtani et al., 2010; Buikstra and Ubelaker, 1994; Ubelaker, 1978) and Brothwell's methodology (Brothwell, 1981) for surface macrowear of the molars in the case of the young adult individual. Thus, the three crania were categorised as follow: child (5.5 to 6.5 years old), adolescent (16.5 to 17.5 years old) and young adult (25 to 35 years old).

Using a manual trimmer, a $2 \times 1.5\,cm^2$ bone sample was extracted from the middle area of the cranial bones (Fig. 1B). Then, each block was embedded in a solution composed of two components, a transparent resin (EpoFix Resin) and a catalyst (EpoFix hardener) (Fig. 1C). Using a vacuum pump to prevent the formation of bubbles, all bone cavities were completely filled with the mixture. Then, a total of 9 thin sections were obtained from the three individuals, which were cut using a circular diamond ISOMET low-speed saw (Buehler, Lake Bluff. Illinois, USA). After a fine polish with silicon carbide (SiC) sandpaper (4000 grit) using a manual polisher (Metaserv 3000, Buehler, Düsseldorf, Germany), thin sections were fixed on microscope slides with a mounting adhesive (Microtec Epoxy Adhesive, Resin Technology Group, Massachusetts). The resulting slides were later progressively polished using different grits to reach a final thickness of 100 μm. Finally, each thin section was observed and photographed using a linearly polarised light microscope (BX61 microscope equipped with a DP70 camera, both from Olympus, Hamburg, Germany), and a complete high-resolution photomontage was assembled using Photoshop CS5 software (Adobe Systems, San José, USA) (Fig. 1D).

Lastly, we mapped each section using geographical information systems (GIS) software (ArGIS 9.3, Esri, Redlands, USA) (Fig. 1E). The GIS software allowed us to finely draw each structure in the 9 thin sections to automatically calculate its size and spatial distribution and to provide qualitative descriptions regarding its nature (see details in

Cambra-Moo et al., 2012, 2014). In this study, we drew the mineralised area (MA) of the section and the non-mineralised spaces found in each section (diploe cavities, primary osteons-PO, resorption spaces-RS and secondary osteons-SO), which were used to study the vascularisation (VASC) during the compartmentalisation analysis. In addition, we measured the thickness of the thin sections of all the individuals (five measurements were made for each bone section, see the thickness mean and standard deviation values plotted in Fig. 3).

## 3. Results

After mapping the thin sections, histological and compartmentalisation analyses were carried out for the three bones of the three individuals.

### 3.1. Child: Lamellar bone extraordinarily extended

As shown in Fig. 2, the child (5.5–6.5 years old) was histologically characterised by poorly vascularised lamellar bone (LB) located in external and internal table (indicated by the white-red dashed lines in Fig. 2A1–A4), which represented 27.24% of the total mineralised area (MA) (see the bottom row of bars plotted in Fig. 3). Although the LB reached a similar extension in the external table of all of the bones (frontal, parietal and occipital), it was reduced in the internal table of the frontal bone (Fig. 2A1). Just below the LB, an incipient irregular fibrolamellar bone (FLB) was identified, and several large isolated secondary osteons (SO) and resorption cavities (RS) were visible (see the details marked in red, Fig. 2A4). In the parietal and occipital bones, the secondary structures reached within the proximity of the ectocranial and endocranial surfaces (Fig. 2A2–A4). The medial area of the three thin sections was occupied by the diploe in which primary and secondary structures were also recognized. The largest cavities in the diploe presented surfaces of deposited (fine laminated endosteal bone, EB, similar to that of the inner cortex of the long bone sections) and resorbed bone (irregular borders), contributing to the typically cancellous structure (better defined in the occipital bone, Fig. 2A3). Regarding the distribution of the compartments of each section (compartmentalisation, Fig. 2A5–A7), the child presented large differences between the values of the area occupied by the different compartments (see the upper bars plot, Fig. 3). The MA occupied a large extension in the frontal and parietal bone sections (74.06 and 70.75%, respectively, see Fig. 3), whereas the occipital bone, in which the MA was reduced (55.10%), exhibited the most vascularisation (VASC), which was nearly double (43.90%, see Fig. 3) that in the frontal and parietal bones (25.93 and 29.25% respectively). Remarkably, the child bones varied from 3.2 mm in the frontal bone to 2.90 mm and 5.47 mm in the parietal and occipital bones, respectively (see lines plots in Fig. 3).

### 3.2. Adolescent: Mineralised area reaches the maximum

For the adolescent (16.5–17.5 years old), the area that was occupied by the avascular LB was drastically reduced (frontal: 4.18% and parietal: 3.81%, see Fig. 3). The LB remained visible in a very small fine area in the external table of the frontal bone (Fig. 2B1 and B4) and was nearly completely substituted by a convoluted-like FLB (compact bone produced by the process of cancellous compaction; Enlow and Hans, 1996) in the parietal and occipital external tables (Fig. 2B2 and B3). In the internal table of the three bones, fine laminated LB was still perceivable in highly remodelled areas (note the occipital was poorly preserved, and the details were not well observable). Secondary structures (mainly SO) were more frequent (not quantified data) compared to the child, extending from the area surrounding the diploe to the endo and ectocranial surfaces (see example in Fig. 2B4). Regarding the compartments (Fig. 2B5–B7), the adolescent individual presented the highest MA (mean value of mineralised area in the three bones: 85.39%) and the lowest VASC (mean value: 14.38%, see Fig. 3). Similar to the child, the MA of the juvenile occupied the largest area (88.49%) of the frontal bone section, and the parietal and occipital bones presented higher values of VASC (16.96 and 15.26%, respectively, see Fig. 3). All of the adolescent's bones presented an extraordinarily reduced diploe area in which the largest cavities tended to be concentrated in the upper part (evident in the frontal bone, Fig. 2B5). Compared to the child, the adolescent presented the opposite tendency regarding section thickness, which ranged from 5.76 mm in the frontal bone to 4.61 mm and 4.85 mm in the parietal and occipital bones, respectively (see Fig. 3). Remarkably, the occipital bone presented a large disparity in thickness values that ranged from 4.47 to 6.75 mm.

### 3.3. Young adult: Vascularisation becomes homogeneous and occupies the complete section

In the young adult individual (25–35 years old), avascular LB was very poorly identifiable (see the frontal and parietal bones in Fig. 2C1 and C2, for which the LB represented 6.21 and 3.7%, respectively, as shown in the bottom row bar plot in Fig. 3). The FLB occupied almost all of the compact area of both tables, and in all of the bones, the diploe was largely extended. Secondary structures were identifiable both in the FLB area and in the diploe. Surprisingly, all of the cranial bones from the young adult individual presented thin canals in the outermost part of the external table that permitted contact between the FLB areas and the ectocranial membranes (see the black arrows in Fig. 2C1–C3 and in the detailed image of Fig. 2C4). The compartments were largely differentiated in the young adult, where the MA occupied the lowest values (frontal: 62.81%, parietal: 61.09% and occipital: 58.29%; see Fig. 2C5–C7 and Fig. 3), and the VASC was largely extended, reaching the highest values of all of the individuals (frontal: 37.19%, parietal: 38.19% and occipital: 41.71%, see Fig. 3 for comparison). The young adult presented the highest bone thickness (frontal, 6.92 mm; parietal, 6.25 mm; occipital, 9.94 mm), and similar to the adolescent, the occipital bone exhibited a large disparity in thickness (ranging from 7.82 to 12.59 mm, see Fig. 3).

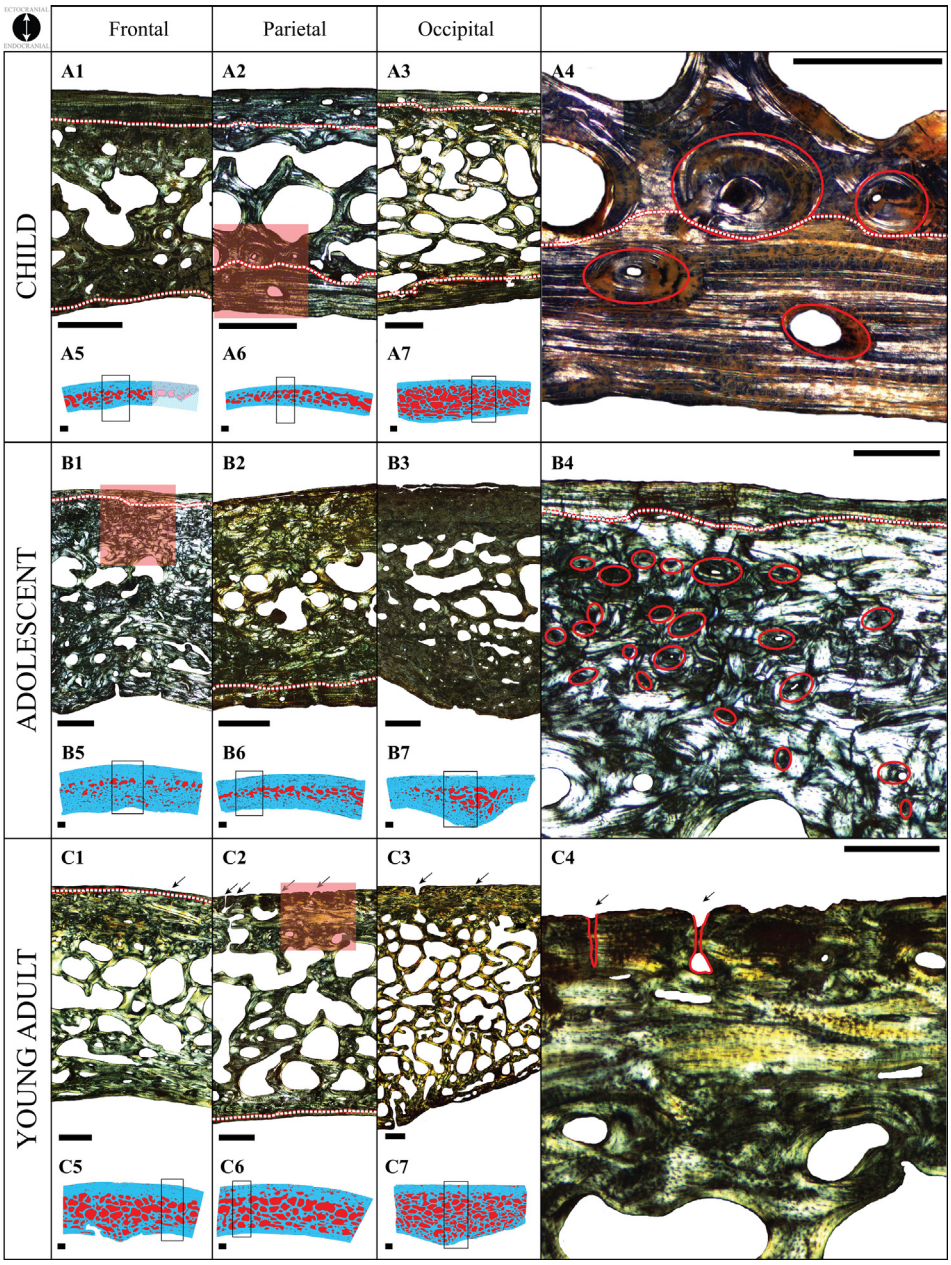


**Fig. 2.** (Color online.) Histomorphological data for the different vault bones (frontal, parietal and occipital). (A) Child, (B) adolescent, and (C) young adult. Images 1 to 3 represent areas of the thin sections viewed under the linearly polarised microscope extracted from the black line square remarked in images 5 to 7, which refer the different compartments analysis drawn using GIS software (mineralised area [MA] and vascularisation [VASC]). Red-white dotted lines identify the lamellar bone (LB) extensions in the different individuals. Detailed images (obtained from the reddish squared areas, A2, B1 and C2) in the three thin sections are shown in A4, B4 and C4 (red lines identified secondary osteons-SO and resorption cavities-RS in A4 and B4 and thin canals in the outermost part of the external table in C4). The black arrows indicate thin ectocranial canals disposition in the three bones. The black scale bars represent 1 mm with the exception of A4, B4 and C4, which represents 0.5 mm.

**Fig. 2.** (Couleur en ligne.) Données histomorphologiques pour les différents os de la voûte crânienne (frontal, pariétal gauche et occipital). (A) Enfant, (B) adolescent, et (C) adulte jeune. Les images de 1 à 3 représentent les zones de la section mince observées au microscope polarisant linéaire, extraites du carré noir marqué sur les images 5 à 7 et qui concerne l'analyse des différents compartiments dessinés au moyen du logiciel GIS (zone minéralisée [MA] et vascularisation [VASC]). Les lignes pointillées rouges et blanches indiquent l'extension de l'os lamellaire (LB) chez les différents individus. Les images de détail (obtenues à partir des zones carrées rougeâtres, A2, B1 et C2) des trois sections minces sont montrées en A4, B4 et C4 (les lignes rouges identifient des ostéones secondaires-SO et des cavités de réabsorption-RS dans A4 et B4, et les canaux minces dans la partie la plus externe de la planche en C4). Les flèches noires indiquent la disposition de fins canaux ectocraniaux dans les trois os. Les barres d'échelle noires représentent 1 mm, à l'exception de celles en A4, B4 et C4, qui représentent 0,5 mm.

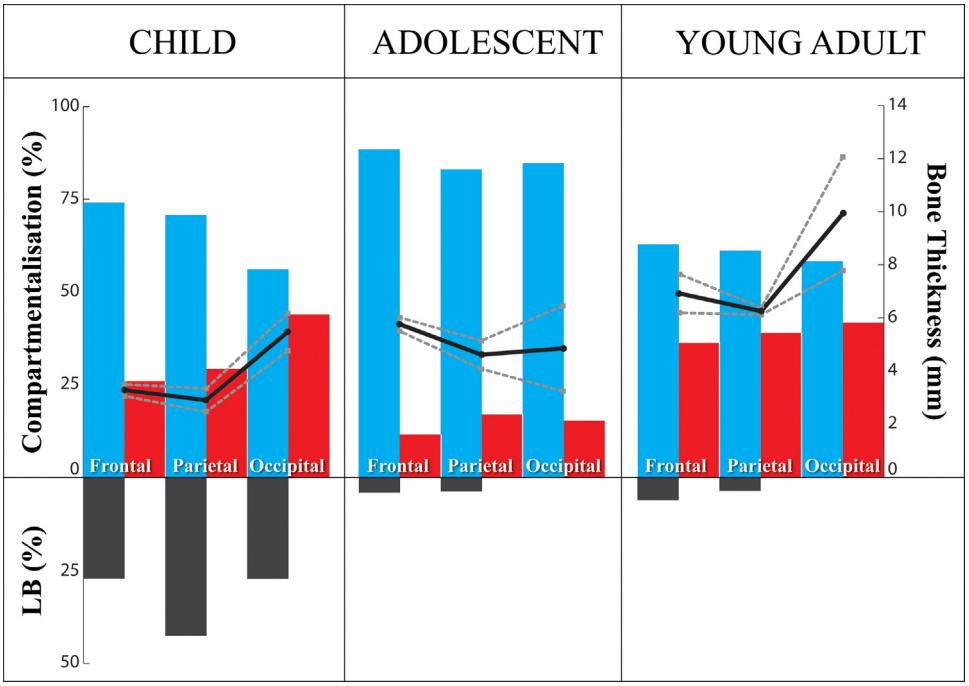


**Fig. 3.** (Color online.) Compartments proportions in the different cranium bones. The upper bar plots shows the percentage extension of the mineralised area (MA, blue color) and vascularisation (VASC, red color). The bottom bars plot illustrates the extension of lamellar bone (LB, dark grey color) in the three bones. The embedded line plots show the mean (black line) and standard deviations (grey dotted line) for the thin section thickness of all of the bones.
**Fig. 3.** (Couleur en ligne.) Proportions des compartiments dans les différents os crâniens. Les barres supérieures montrent le pourcentage de l'extension de la surface minéralisée (MA, couleur bleue) et de la vascularisation (VASC, couleur rouge). Les barres inférieures illustrent l'extension d'os lamellaire (LB, couleur gris foncé) dans les trois os. Les lignes incluses montrent la moyenne (ligne noire) et les déviations standard (ligne pointillée grise) de l'épaisseur de la section mince de tous les os.

## 4. Discussion

Enlow (1963) was one of the first to investigate the histology of cranial bones. His work not only described the most important aspects of skull histology, but he also postulated a complete model for the growth of the skull bones (Enlow, 1968; de Ricqlès, 2007). Despite this revolutionary contribution and many other notable histological studies that have been carried out on the cranial bones (mostly focused in the facial growth; Martínez-Maza, 2007), there is limited standardised data regarding the histology of cranium cross-sections that include tissue types descriptions (Cho and Hwang, 2012; Trammell, 2012), and many histological variations that occur during cranial ontogeny and evolution remain poorly understood (Bartsiokas, 2002).

This study, at this phase, represents an introductory approximation of the histomorphological variations of vault bones, and despite the study explores a small number of individuals (which are not representative of the different age classes), from a late mediaeval ossuary (skeletal remains appeared disarticulated) in which intra- or inter-population differences were not under control (sex and/or dietary differences of the individuals were not possible to establish), the histological descriptions and the analysis of the tissue compartmentalisation of the three individuals viewed, made on an ontogenetic perspective, has revealed interesting preliminary results. First, it is important to highlight that, as has been largely described before (Scheuer and Black, 2000), bone section thickness increases with age (see the mean thickness variations in the lines plots of Fig. 3), and the occipital bone exhibits the large disparity in thickness. Second, the enlargement of the vault bones is critical until the end of the childhood (vault bones reach their final extension). Afterwards (during youth), the increasing bones thickness becomes more relevant (see the proposal to describe the main changes in cranium growth, Fig. 4). Third, these changes that describe the size increase are accompanied by important modifications in the histology and compartmentalisation of vault bone tissues (Fig. 3).

Regarding the child, the presence of an important mineralised area occupied by avascular lamellar bone (LB, mean extension: 32.37% of the total mineralised area [MA]; Fig. 3) is *a priori* unexpected. In most mammals, this bone tissue type is usually related to low rates of bone deposition (in adult individuals), which does not match the rapid growth rates described during the human infancy and childhood (Currey, 2002; Scheuer and Black, 2000). However, the presence of an avascular LB in an individual approximately 6 years old could be easily assumable because, at this age, the complete cranium size is almost reached (90% of the brain size is reached by the 6th year, Aguila and Enlow, 1998; Scheuer and Black, 2000). In addition, the vault bones have almost reached their final extension and may begin to increase in thickness (to reach adult values), which could imply a change in the growth rate while the growth rate on other bones of the cranium

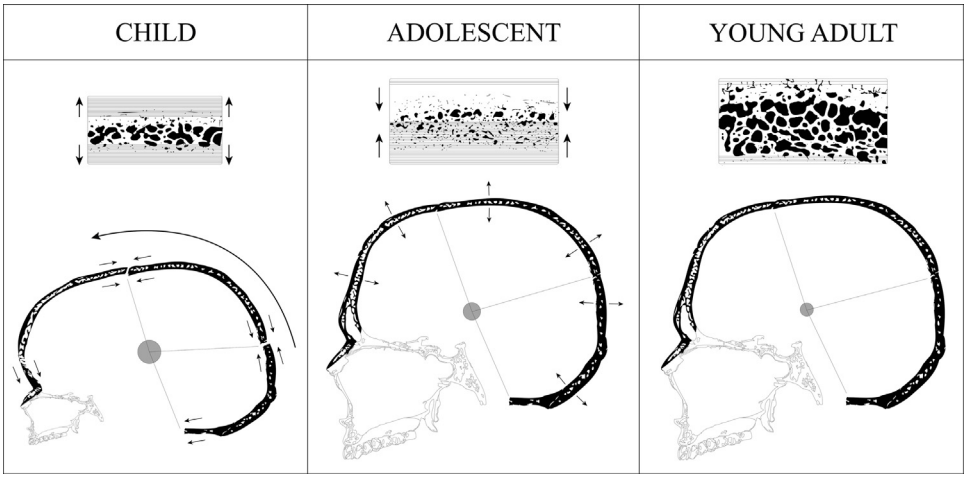


**Fig. 4.** Proposed for main growth patterns in the vault bones during ontogeny. The upper row shows a schematic of the spatial relationship (histological and GIS data) between the thin section compartments (mineralised area [MA] and vascularisation [VASC]) and the tissue types extension (lamellar bone [LB]) for the three individuals (child, adolescent and young adult). The bottom row exemplifies the main changes proposed for the cranium growth through ontogeny (the increase in bone extension during childhood and the thickness increase during youth). The black curved arrow indicates the preferred sense of growth in vault bone.

**Fig. 4.** Principaux modèles de croissance proposés des os de la voûte au cours de l'ontogenèse. La partie supérieure de la figure montre un schéma de la relation spatiale (données histologiques et du GIS) entre les compartiments de la section mince (zone minéralisée [MA] et vascularisation [VASC]) et l'extension des types de tissu (os lamellaire [LB]) chez les trois individus (enfant, adolescent et adulte jeune). La partie inférieure met en évidence les principaux changements proposés pour la croissance du crâne au cours de l'ontogenèse (augmentation de l'extension d'os pendant l'enfance et augmentation de l'épaisseur durant la jeunesse). La flèche courbe noire incurvée indique le sens préférentiel de la croissance dans l'os de la voûte.

remains high (Lieberman, 2011). From the foetus phase to early infancy, when the cranium bones are not yet fused, the vault bones are highly porous in their macroscopic appearance (Scheuer and Black, 2000), and it is possible that they are configured by cancellous trabecular tissue in which the diploe cavities contact the ectocranial and endocranial membranes (highly vascularised bone that is considered embryonic-like, de Ricqlès et al., 1999). The presence of the avascular LB in the child could indicate that vault bones become "sealed", minimizing their porosity, while simultaneously increasing their size. The data of the histological and GIS analysis are summarised in the upper row in Fig. 4, and a schematic of our proposal for the main growth changes in the cranium development is shown in the bottom row of Fig. 4. We speculate if this "sealing" process could be implicated in improving the fine control of the brain-head thermoregulation until it reaches its final maturation, which could be associated with decreasing the "temperature shielding length" from childhood to adulthood, as described by Zhu et al. (2006). However, we observed a large area of LB in the child frontal bone, and we also observed that the same LB began to remodel in the parietal and occipital bones (we identified SO and RS, Fig. 2A2–A4). Moreover, all of the individuals presented a similar pattern in which the VASC increased from the frontal to occipital bones (see Fig. 2A5–A7, B5–B7, C5–C7 and Fig. 3). Both characteristics are in accordance with the idea that the bone microstructure reproduces the preferential sense of growth in the vault bones, as described in the literature (occipital comes from the Latin *occipio* that means to begin; Scheuer and Black, 2000), which seems to be initiated from the posterior side of the head (see the black curved arrow in the bottom row of Fig. 4).

In contrast, the adolescent and young adult presented a different pattern. In the adolescent, the MA occupied a larger area of the bone section (mean value: 85.39%) and the diploe appeared markedly reduced (the VASC mean value was under 20%, Fig. 3). The MA was occupied by a highly remodelled fibrolamellar bone (FLB), which presented a convoluted-like structure in the external table (Fig. 2B4), and is partially constituted by LB (also highly remodelled) in the internal tables. The high values of the MA in the adolescent are in accordance with the maximum of mineralisation ascribed to cortical bone during youth (the largest bone robustness in the long bones) when the final cortex size is reached (Cambra-Moo et al., 2014; Ruff and Jones, 1981; Ruff et al., 1994) (Fig. 3). In addition, as has been previously described in other studies, the number of secondary osteons (SO) increased during youth compared to infancy and childhood, and the SOs were small in size (Curtis and Nawrocki, 2010). Finally, the young adult presented the largest VASC of the three crania (mean value: 38.87%), which was highest in the occipital bone (similar to the child). The LB appeared extremely reduced and was only clearly visible in the external table of the frontal bone and in the internal table of the parietal bone (Fig. 2C1 and C2). The most striking result regarding the young adult was the existence of several fine canals by which capillaries could contact the ectocranial membrane (black arrows in Fig. 2C1–C4 and the upper row in Fig. 4). As previously proposed in the literature, cerebral blood flow increases during ontogeny, and therefore, the irregular appearance of LB and the presence of these fine canals in several areas of the vault bones could be related to local changes in heat exchange through the scalp (Zhu et al., 2006, 2009). Future investigations should analyse the possible relationships between

these changes in the adult cortex remodelling process and the local variations in the endocranial and ectocranial surface remodelling areas that have been described by many authors (e.g., Kranioti et al., 2009; Martínez-Maza et al., 2006; Mowbray, 2005). Our proposal establishes that during maturity, the microstructure of vault bones continues to change locally throughout the remodelling process. It is of interest to assess if these changes are related with heat dissipation through the vault bones during head thermoregulation (Bruner et al., 2011; Hershkovitz et al., 1999; Jivraj et al., 2009).

The tissue type characterisation via the histological analysis using thin sections could be crucial in achieving not only a better understanding of the morphological variations of cranial bones but also in understanding the variations in the physiological regime during human ontogeny and human evolution. Through the histological analyses, we can describe both the changes in the size and shape of bones structures during ontogeny and the maturation of the different tissues. Additional individuals must be analysed, and the data of the biological characteristics of each individual must be improved to infer the biological aspects involved in ontogenetic variations. Notwithstanding, this study represents the first attempt to approximate the histomorphological variations in the cranium vault based on an integrative perspective. Further studies are necessary to explore more sections from each bone and additional bones from other locations in the cranium.

## 5. Conclusions

Analyses of the morphological variations in the cranial bones are important in anthropological studies. Histological analyses of cranium bones could contribute to a better understanding of these variations, not only from a developmental perspective but also regarding cranium evolution. The present preliminary approach analysed the histology of the frontal, parietal and occipital bones of three individuals with different ages (child, adolescent and young adult). The presence of different types of bone and the differential spatial distribution of the bone tissues during growth revealed that cranium development is a complex process. We found that child vault bones become larger during childhood and that at the same time they are sealed by an avascular lamellar bone (decreasing their porosity); adolescent bones increase in thickness (reaching almost adult size) and largely reduce in their vascularity; and adult bones become completely remodelled and interconnected with the ectocranial surface via fine vascularisation.

A deep understanding of these transformations during human ontogeny will allow us to analyse the complex processes of bone diseases and better understand the histological variations among different human groups or variations due to sex-age differentiation. Further studies are necessary to unveil important aspects of the histology of cranial vault bones that remains unresolved.

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