New Evidence Suggesting a Dissociated Etiology for *Cribra Orbitalia* and Porotic Hyperostosis

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ABSTRACT

Objectives

Porotic hyperostosis, characterized by porotic lesions on the cranial vault, and *cribra orbitalia*, a localized appearance of porotic lesions on the roof of the orbits, are relatively common osteological conditions. Their etiology has been the focus of several studies, and an association with anemia has long been suggested. Anemia often causes bone marrow hypertrophy or hyperplasia, leading to the expansion in trabecular or cranial diploic bone as a result of increased hematopoiesis. Hypertrophy and/or hyperplasia is often coupled with a disruption of the remodeling process of outer cortical bone, cranially and/or post-cranially, leading to the externally visible porotic lesions reported in osteological remains. In this paper, we investigate whether individuals with *cribra orbitalia* have increased thickness of the diploë, the common morphological direct effect of increased hematopoiesis, and thus test the relationship between the two conditions, as well as explore the type of anemia that underlie it.

Methods

An analysis of medical CT scans of a worldwide sample of 98 complete, young to middle-aged adult dry skulls from the Duckworth Collection was conducted on male and female cribrotic individuals (n= 23) and non-cribrotic individuals (n= 75), all of whom lacked any evidence of porotic lesions on the vault. Measurements of total and partial cranial thickness were obtained by virtual landmark placement, using the Amira 5.4 software; all analyses were conducted in IBM SPSS 21.

Results

Cribriotic individuals have significantly thinner diploic bone and thicker outer and inner tables than non-cribriotic individuals, contrary to the expected diploic expansion that would

result from anemic conditions associated to bone marrow hypertrophy or hyperplasia. Additionally, individuals without *cribra orbitalia* and those with the condition have distinctive cranial thickness at particular locations across the skull and the severity to which *cribra orbitalia* is expressed also differentiates between those with mild and those with a moderate to severe form of the condition.

Conclusions

Our results suggest a complex pattern of causality in relation to the pathologies that may lead to the formation of porotic lesions on the vault and the roof of the orbits. A form of anemia may be behind the osteological changes observed in porotic hyperostosis and *cribra orbitalia,* but it is unlikely to be the same type of anemic condition that underlies both types of osteological lesions. We suggest that *cribra orbitalia* may be associated to anemias that lead to diploic bone hypocellularity and hypoplasia, such as those caused by anemia of chronic disease and, to a lesser extent, of renal failure, aplastic anemia, protein deficiency and anemia of endocrine disorders, and not those that lead to bone marrow hypercellularity and hyperplasia and potential porotic hyperostosis. This leads us to the conclusion that the terms *porotic hyperostosis* and *cribra orbitalia* should be used to reflect different underlying conditions.

Skeletal lesions observed on human remains offer insights onto the general wellbeing of humans in the past, as well as informing on the biological and evolutionary responses to disease today. Porotic hyperostosis, a term used to describe the excessive development of porous lesions in bone tissue, is one of the most common occurring skeletal conditions observed in paleoanthropological studies (Ortner, 2003; Jatautis et al., 2011). There is a general consensus that anemia is the main factor resulting in porotic lesions, whether by acquired anemia due to parasites or nutritional deficiencies, or through genetic conditions, such as thalassemia (major and minor) and sickle cell anemia (Martin and Goodman, 2002; Facchini, 2004; Dabbs, 2011). Anemia leads to irregular hematopoiesis (blood cell production) in the marrow of trabecular bone, which may result in the enlargement of the cancellous structure, either by marrow hypertrophy (enlarged tissue due to increased size of cells) or hyperplasia (enlarged tissue due to increased number of cells) (Martini *et al*., 2011). Although hypertrophy and hyperplasia are different processes, either response may lead to the expansion of the diploë, the trabecular bone of the cranium embedded between the two cortical tables. Exposure of the enlarged diploë may occur when coupled with the irregular remodeling of the outer cranial table, consequently unveiling its structure as observable porotic lesions. In mild cases, these lesions may be simply characterized by the appearance of pores on the outer cranial table, while in severe cases extensive lesion expansion may completely expose the cranial diploë and/or result in bony growths that invade orbital spaces.

The appearance of porotic lesions in different cranial regions has generated a terminological division of this condition, such that lesions occurring on the parietals, and occasionally on the frontal and occipital bones, are referred to as porotic hyperostosis (PH), while lesions on the superior orbital bones are termed *cribra orbitalia* (*CO*). Other terms in the literature include *symmetrical osteoporosis*, *spongy hyperostosis*, and *cribra cranii* (Ales Hrdlička, 1914; Henschen, 1961; Carlson *et al.*, 1974; Ortner, 2003; Facchini, 2004).

Notwithstanding the different terminology, all the terms describe the condition in which bone forms pore-like lesions that may differ in the severity and region of expression, including its occurrence on post-cranial bones (Ortner, 2003). Thus, the terms *porotic hyperostosis* and *cribra orbitalia* are generally used to describe the same osteological condition expressed in different skeletal locations, and are not, by nomenclature, meant to signify different causal afflictions. However, the independent appearance of porotic lesions on the vault or orbital roof, or their co-occurrence in some cases, has led to different etiological interpretations. Some scholars have suggested that orbital lesions may be a preliminary phase to those potentially occurring on the vault (Stuart-Macadam, 1989; Wapler *et al.*, 2004), others that different types of anemia may result in lesions in either area (El-Najjar *et al.*, 1975; Webb, 1982; Sandford *et al.*, 1983; Salvadei *et al.*, 2001; Sullivan, 2005; Dabbs, 2011), while still others hold that each type of lesion reflects separate conditions entirely (Rothschild, 2012). Therefore, despite years of research, whether the same particular etiology may be responsible for these two types of cranial lesion, regardless of their singular or co-occurrence on cranial bones, remains unresolved (Steinbock, 1976; Wapler *et al.*, 2004; Walker et al., 2009; Rothschild, 2012).

Identifying the precise etiology of cranial porotic lesions is challenging because there may be many more illnesses, and co-occurring illnesses, than there are skeletal responses to such (McIlvaine, 2015). In other words, the physiological capacity for bone to respond to pathological stress is essentially limited to irregular bone formation (deformation) and/or remodeling (abnormal bone loss or gain). Thus, there is a very broad range of diseases and a more tightly constrained set of physiological processes that result in a very specific set of skeletal responses. In such cases, it is the investigation of the physiological processes involved that bridges the underlying causal factors with its resulting skeletal condition.

Hemoglobulin disorders, such as the various forms of acquired or genetic anemia, disrupt the physiological processes of hematopoiesis and may (or not) have an effect on bone tissue, particularly in causing cranial diploic expansion associated with boney lesions. Thus, hemoglobulin disorders, specifically anemia, are those most often identified as causative factors in the appearance of PH of the skull (Stuart-MacAdam, 1985; Hershkovitz et al., 1991; Martin and Goodman, 2002). Therefore, when PH is observed on skeletal remains, the expectation is that these individuals likely suffered from some form of anemia, evidenced by a thickened cranial diploë and bone porotic lesions. This study investigates whether, in cases among whom PH appears only in the superior orbital bones, the condition is accompanied by substantial diploic thickness in and across the cranial vault.

MATERIALS AND METHODS

Data on cranial thickness and incidence of *CO* were collected from a worldwide sample of 98 young to middle-aged male and female adult complete dry crania housed in the Duckworth Collection at the Leverhulme Centre for Human Evolutionary Studies, University of Cambridge (Table 1). Of these individuals, 75 represent a cohort with no macroscopic indication of cranial pathology. The remaining 23 individuals were selected on the basis of presence of orbital lesions and absence of vault porotic lesions, as well as of any other macroscopic pathological condition. The reliability of identification of presence of *CO* and its degree of severity was assessed by the independent recording of the condition by the authors. It was recognized that PH and *CO* are conditions often common in children and adolescents; however, immature individuals were excluded to avoid developmental factors in the determination of cranial vault thickness.

(Table 1 Here)

The severity of orbital lesions was graded as five stages of development, similar in scheme to Nathan and Haas (1966), Stuart-Macadam (1985), and Buikstra and Ubelaker (1994), and the presence or absence of healing of some or most of the lesions were noted. No individuals in the sample showed the maximum (grade five) level of severity of the condition as defined in this study. The grades of development of *CO* used here are defined as follows (Fig. 1, Supplementary Figure S1):

1. Porotic (Present): presence of pores on the roof of one or both orbits;
2. Porotic with netting (Present to Moderate): isolated pores with appearance of netting or coalescing;
3. Netting (Moderate): pores cease to be in isolation and merge, forming a net-like or maze structure of coalesced pores;
4. Honeycomb-like netting (Moderate to Severe): enlarged, coalesced pores beginning to thicken or form a honeycomb-like structure (non-protruding);
5. Honeycomb (Severe): protruding trabecular structure or growth beyond outer table.

(Figure 1 Here)

Nine of the 23 individuals with *CO* showed minor signs of healing, while the remaining fourteen showed no evidence of healing. In the former group, most individuals (n= 6 of 9) had grade 2 *CO*, with two individuals with grade 1 and one individual with grade 3, while most individuals in the latter group (no healing) had either grade 1 *CO* (n= 7 of 9), or grade 4 *CO* (n= 4 of 4), with three individuals with grade 2 *CO*.

Computed tomography (CT) scans of the 98 crania were carried out at Addenbrooke's Hospital, Cambridge, using a Siemens Somatom Definition Flash scanner, and protocol

specifications defined by Dr. Christophe Zollikofer and Dr. Marcia Ponce de León[1] in collaboration with Dr. Marta Mirazón Lahr in 2007. The CT scan specifications included 120 kV tube voltage and a pitch of 0.7 mm. Reconstructions had a slice thickness of 0.6 mm, with an increment of 0.3 mm, and a H60 sharp (FR) kernel with a window width of 4000 Hounsfield Units (HU) centered at 850 HU. These scans have a fixed matrix size of 512 x 512, whereby a 205 x 205 mm field of view (FOV) and pixel size of 0.4 mm were set. At the time of the study, a sufficiently large μCT scanner was not available, so that radiographic resolution was not optimal for further analysis of the trabecular structure of the orbital roof.

Analyses of thickness dimensions were conducted in *Amira 5.4*, where Euclidian distances were calculated from virtual landmark data placed across the cranial vault. To establish clear boundaries for CT landmark placement, a binary image of the scan was set by using the half maximum height method (HMH) as recommended by Spoor *et al.* (1993). A modification of this method, similar to that used by Coleman and Colbert (2007), where the mean voxel (or three dimensional pixel) value was used rather than local voxel values, was implemented. Voxel means were calculated in *Amira* within a region of interest (ROI) for the frontal and occipital bones of every individual, and with one ROI (and mean value) for both parietals in each case. The HMH was then applied according to the voxel mean for each particular bone of each individual (Fig. 2).

(Figure 2 Here)

Four linear measurements were obtained at each cranial landmark – the total thickness of the vault at that point, and the thickness of each of the three cranial layers (outer and inner tables, and diploë). A total of 186 thickness measurements were taken on each individual skull, 56 on the frontal bone measured at 14 landmark points, 96 on the parietal bones measured at 24

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landmark points, and 34 on the occipital bone measured at 10 landmark points, where only 8 offered layer-specific data. Vault thickness was sampled at 48 landmarks locations, and care was taken to avoid landmark placement directly on sutures so that diploic measurements were attained. Landmarks used were as defined by Howells (1973) and Lahr (1992, 1996), and at locations along bisecting lines that form quadrants in the anterior/posterior (frontal bone), medial/lateral (parietal bones), and inferior/superior (occipital bone) directions across each bone (Rivera, 2014). Cranial thickness was also measured in the approximate center of each quadrant devised by bisecting landmark dimensions (Fig. 3).

(Figure 3 Here)

RESULTS

In order to test for differences in cranial thickness between individuals with and without *CO,* independent t-tests, using Benjamini-Hochberg false discovery rate to control for multiple comparisons, were carried out. Table 2 lists the results of total thickness and of each measurement of the cranial vault layers that are significantly different between individuals with *CO* and those without.

(Table 2 Here)

Individuals with and without *CO* have similar total cranial thickness throughout the vault, only differing in thickness at the cranial vertex, which is significantly thinner in individuals with *CO* than in those who do not exhibit the condition (Fig. 4). Individuals with orbital lesions have thicker inner tables than those without, with the exception of a single cranial location – the point of inflection of the occipital bone (lambda subtense fraction, or OCF). At this point, cribrotic individuals show a wide range of values of thickness of the inner table, including several individuals with very thick inner tables, although the overall median inner

table thickness of the sample is lower than in the sample without cribrotic lesions (Fig. 5). No statistically significant differences in the thickness of the outer table across all 48 cranial landmarks were found between the samples. The diploic layer, which is the layer most expected to respond to hematopoietic stress, shows the opposite pattern to that expected. The pathological and non-pathological samples showed a number of significant differences in diploë thickness across the vault (at 14 landmarks), but in every case the individuals with *CO* were found to have thinner diploic bone than individuals without the condition (Fig. 6).

(Figure 4 Here)

(Figure 5 Here)

(Figure 6 Here)

To explore the relationship between *CO* and the different measures of vault thickness multivariately, a series of stepwise discriminant function analyses were conducted to determine whether non-pathological groups may be distinguished from those with *CO* on the basis of different aspects of cranial thickness. The analyses used Wilks' lambda statistic (the proportion of the total variance in the discriminant scores not explained by differences among the groups) to test the discriminant function, with prior probabilities set according to group size (n= 75, no lesions; n= 23, with orbital lesions), and leave-one-out cross-validation to predict group membership. The tests were carried out separately for measures of total vault thickness, thickness of the outer and inner tables, thickness of the diploë, the thickness of each layer as a percentage of total thickness, and for combinations of the three layers. The results are listed in Table 3, organized in increasing discriminatory power.

(Table 3 Here)

The results show that the relationship between incidence of *CO* and vault thickness is complex, and that the total thickness of the vault, or of particular layers, whether compact or diploic, is not singularly associated with the condition. Only when the variation of two or more cranial layers is combined are more than half the cases of *CO* correctly predicted. The strongest statistical association is found when including the variation of the outer and diploic layers (corrected by total vault thickness), and particularly when information on all three cranial layers (corrected by total vault thickness) is considered. The latter analysis achieves the correct discrimination of 93.3% of non-pathological individuals and 82.6% of cases with *CO* (Figure 7). In other words, incidence of *CO* can be predicted in most cases by the relative thickness of the three cranial layers at particular locations in the vault, reflecting a complex interaction between the spatial distribution of thickness and the expression of porotic lesions on the roof of the orbit.

(Figure 7 Here)

This relationship is shaped by 16 variables that correlate positively and negatively with the Discriminant Function (Figure 8a). The Discriminant Group Centroid Scores are positive for cases with *CO* (2.738) and negative for those without lesions (-0.840). Specifically, cases with *CO* tend to have particularly thin diploë along the cranial mid-line (PAFpost, PAF, FRFinf), particularly thick outer tables on the left and right mid-anterior parietals (PQ2r, PTFantl) and inner tables on the left and right mid-posterior parietals (PTFl, PQ4l, PQ4r), and thin outer (PTFsupl) and inner tables (PTFsupr) either side of the mid-sagittal suture (Figure 8b). Individuals without cribrotic lesions show the opposite trend (Figure 8c). Examining the variables that correlate significantly with the discriminant function shows that the most significant univariate differences are found between the relative thickness of the diploë along the cranial mid-line (FRFinf, PAF, PAFpost), the thickness of

the outer table at the mid-anterior parietals and of the inner table at the mid-posterior parietals (Figure 9).

(Figure 8a here)

(Figure 8b here)

(Figure 8c here)

(Figure 9 here)

Individuals with different degrees of severity of *CO* were found to differ in the pattern of cranial vault thickness (Figure 10). Among the 23 individuals in the sample who had *CO*, 9 had small pores on the roof of one or both orbits, and were classified as mild (Grade 1), 9 showed isolated pores with netting, classified as mild to moderate (Grade 2), 1 had clear netting, classified as moderate (Grade 3), and 4 had honeycomb-like netting, classified as moderate to severe (Grade 4). No case of severe *CO* (Grade 5) was observed. For the purpose of analysis, Grades 3 and 4 were merged, so that 3 degrees of severity of *CO* were used (Grades 1 to 3, representing mild, moderate, and pronounced cribrotic lesions).

(Figure 10 here)

Non-parametric analyses Kruskal-Wallis comparisons of means were performed (Table 4). These are indicative only because of the small sample size of individuals with different degrees of development of the condition, particularly of individuals with Grade 3 or pronounced *CO* (n= 5). Nevertheless, individuals with moderate cribrotic lesions (Grade 2; n = 9) are significantly thicker, either in terms of total thickness totality or in one of the cranial layers at certain cranial landmarks than either individuals with either mild (Grade 1) or pronounced (Grade 3) *CO*. This is the case for 11 landmarks - total thickness at frontal mid-midline (frontal fraction), outer table thickness at Bregma (recorded on the left parietal) and

at the mid-posterior left parietal (Quadrant 3), as well as of both the outer and inner tables at 4 landmarks – mid-inferior right frontal bone (Quadrant 2), at opistocranium, and along the occipital midline at the lambda-subtense fraction and inion. No significant difference across the 3 degrees of severity of *CO* was associated with the thickness of the diploë, and at no point measured on the cranial vaults were individuals with mild *CO* significantly thicker than those with more severe expression of the disease.

(Table 4 here)

The differences in the pattern of cranial thickness in relation to degree of severity of *CO* is sufficiently strong that a discriminant function achieves 100% discrimination (with prior probabilities set at groups sizes and leave-one-out classification) of the three grades of cribrotic lesions used here (Function 1 Eigenvalue 704.173, 71.6% s2; Function 2 Eigenvalue 279.045, 28.4% s2) (Figure 11). Discrimination was achieved on the basis of 15 variables, with cases with moderate (Grade 2) and pronounced (Grade 3) cribrotic lesions separated along Function 1, and cases with mild lesions (Grade 1) separated from those with moderate and pronounced cribrotic lesions (Grades 2 and 3) along Function 2. Function 1 is defined by the combination of (in decreasing order of influence) - increased thickness of the diploë at (a) the left mid-lower frontal bone, of the outer table at (b) the anterior right parieto-temporal fraction, of the diploë (c) at the inferior right parieto-temporal fraction, of the inner table at (d) the right frontal fraction, of the outer table at (e) the cranial vertex, and decreased thickness of the inner table at (f) inion, the outer table at the lambda subtense fraction, and especially, of the diploë at (g) the right mid-superior occipital bone. Function 2 is defined by the combination of increased thickness of the diploë at (a) the right mid-superior occipital bone, of the inner table at (b) the right frontal fraction, of the total thickness at (c) the left mid-inferior frontal bone, and of the outer table at (d) the cranial vertex, and decreased total

thickness at (e) the right supraorbital subtense, of the diploë at (f) the left mid-inferior frontal bone, and especially, of the outer table at (g) lambda subtense fraction.

(Figure 11 here)

However, these differences in cranial thickness among individuals with different degrees of development of *CO* do not describe unique configurations, and a discriminant function that includes individuals without the condition misidentifies a third of the cases of mild *CO* (Grade 1) as non-pathological, as well as approximately 22% of the cases with Grade 2. A bivariate plot of the individual discriminant scores from this analysis including crania with and without *CO* shows how only a portion of the individuals with Grades 2 and 3 of development of cribrotic lesions fall outside the range of variation of the non-pathological sample, while those with a mild version of the disease are largely embedded within the variation of cranial thickness observed among healthy individuals (Figure 12). This suggests that changes in localized cranial vault thickness associated with *CO* take place only once the condition has developed beyond a mild stage.

(Figure 12 here)

Given the differences observed in the combination of cranial vault thickness in the different degrees of *CO*, the possible association between the latter and the healing process was investigated. Among the 23 cribrotic individuals in the sample, 9 show evidence of healing; the majority of individuals with healing cribrotic lesions (6/9) were observed among moderate cases of the condition (Grade 2). A oneway analysis of variance of all individuals (no cribra, healing cribra, no healing) was performed on the discriminant function scores from the analyses above (n=120, no *cribra* n=97, Grade 1 n=9, Grade 2 n=9, Grade 3 n= 5). The differences observed are highly significant for the first two discriminant functions (DF1 $F= 22.895$, $p< 0.001$, df = 119; DF2 $F= 21.808$, $p< 0.001$, df= 119, Table 5). Bonferroni post-

hoc tests show that all three groups (no *cribra*, healing cribrotic lesions, and no healing) are significantly different to each other at $p<0.05$ for the 1st discriminant function, while individuals without *CO* are significantly different from those with both healing and non-healing lesions at $p<0.05$ for the 2nd discriminant function. These results provide further support for the view that particular changes in cranial vault thickness occur during the different stages of development of the disease.

(Table 5 here)

DISSCUSSION

The study of skeletal remains offers the means by which to understand the life, activities, and health of past populations. Porotic hyperostosis is a relatively common osteological pathology that has been linked to the effects of anemia based on bone marrow morphology (Hrdlička, 1910, 1914; Cooley and Lee, 1925; Cooley, Witwer, and Lee, 1927, Angel, 1966; Walker, 2009). This is due to the disruption to hematopoiesis (either through blood loss, impaired or reduced erythropoiesis and/or increased hemolysis), leading to a compensatory reaction that imposes a stress on the hematopoietic tissues (such as the diploë) which, in turn, affects the size and/or number of cells in bone marrow (Halvorsen & Bechensteen, 2002; Stockmann & Fandrey, 2006; Walker, 2009). The most common effect of this process on the cranium is the expansion of the diploë at the expense of the outer table, which becomes resorbed through time, and the appearance of porotic lesions on the cranial vault (Moseley, 1965; Cule and Evans, 1968; El-Najjar *et al.*, 1975; Stuart-Macadam, 1987; Tayles, 1996; Gowland and Western, 2012).This causative link between anemia, one of the most common nutritional disorders in the world (WHO, 2015) and cranial porous lesions, one of the most common palaeopathological conditions observed (El-Najjar *et al.,* 1976; Cohen & Armelagos, 1985; Walker, 1985, 1986; Steckel & Rose, 2002) has been a major focus of

research, with recent studies focusing on the complexity of dietary, hereditary and socio-cultural factors that may affect the incidence of porotic lesions in the past (Walker, 2009). Two major areas of debate are the relationship between porous lesions on the vault (porotic hyperostosis), and on the roof of the orbits (*cribra orbitalia*) as well as the potential causes of both conditions.

A strong relationship between PH and anemia has long been recognized, initially in relation to hemolytic anemias (Caffey, 1937; Angel, 1966; Sebes & Diggs, 1979; Hershkovitz *et al.*, 1997), and later as a result of iron-deficiency anemia, partly to explain the high incidence of porotic lesions in prehistoric Amerindian populations (Dunn, 1965; Agarwal *et al.*, 1970; Moseley, 1974; Walker, 1985; de Zuleta, 1994; Sullivan, 2005). Although the latter causative relationship has been strongly challenged (Walker *et al.*, 2009), the association between PH and anemias that cause expansion of the diploë has found strong support (Moseley & Jarcho, 1966; Moseley, 1974; Schultz, 2001; Ortner, 2003). In the course of the history of studies about porotic cranial lesions and anemias, *CO* came to be considered by some scholars as part of the complex physiological response of the cranium to anemia, and thus with the same causative factors as PH (Hengen, 1971; Stuart-Macadam, 1989; Stodder, 2006). In a detailed review of the possible conditions that may cause PH and *CO*, Walker (2009) challenges current views of both the association between the two conditions and the types of anemia responsible, as well as suggesting alternative etiologies for *CO.*

In this study, we provide further evidence for the independence of the two conditions characterized by cranial porous lesions by testing whether, in the absence of porotic lesions on the vault, the presence of porous lesions on the roof of the orbit results from bone marrow hyperplasia and/or hypertrophy (diploë expansion). Our findings are preliminary because of small sample sizes, but represent the first detailed investigation of the thickness of the different cranial layers across numerous loci on the cranial vault using CT scans. The results

suggest that *CO* in this sample is not associated with an increase in diploic thickness on the cranial vault, and therefore cannot be the result of conditions that lead to bone marrow expansion, but that the relationship with cranial thickness overall is complex. Furthermore, this relationship changes along the stages of development of the condition. These results have implications towards understanding the processes that affect localized changes in cranial thickness, including the relationship between the two most commonly observed porotic conditions (*cribra orbitalia* and porotic hyperostosis), and may throw light on the types of anemia that could be behind the different patterns observed. These issues are discussed below.

Relationship between *cribra orbitalia* and porotic hyperostosis

The condition known as porotic hyperostosis was first named by Angel (1966) to refer to porous lesions and pitting on the vault that he considered to be the result of hereditary anemias (such as Thalassemia major and Sickle-cell Anemia) that were common among the prehistoric populations of Southeastern Mediterranean Europe, which he was studying. Hematological and radiographic studies further showed that PH was the outcome of anemias, either genetic (such as the conditions mentioned above), or acquired such as through blood loss, leading to lack of iron, or dietary deficiencies of nutrients necessary to maintain red blood cell homeostasis (i.e. the lack of Vitamins A, B12, B6 and B9) (Marin & Ober, 2001; Stockman & Fandrey, 2006). One of the most notable processes by which the body meets the stress from anemia involves an increase in the production of blood cells, which leads to expansion of hematopoietic centers, such as the cranial diploë, with concomitant reduction of the cranial outer table (Stuart-Macadam, 1987; Ortner, 2003; Walker *et al.*, 2009). The link between these effects and PH has been confirmed by clinical and epidemiological studies (Caffey, 1951; Eng, 1958; Moseley, 1974; Stuart-Macadam, 1987; Schultz, 2001; Ortner 2003). In terms of skeletal samples, the state of cranial diploic bone in previously studied

individuals with PH is described as either hypertrophic (Angel, 1966; Tayles, 1996; Wapler *et al.*, 2004; Sullivan, 2005; Walker *et al.*, 2009; Dominguez-Rodrigo *et al.*, 2012; Gowland and Western, 2012) or hyperplastic (Moseley, 1965; Lanzkowsky, 1968; El-Najjar *et al.*, 1975; Williams *et al.*, 1975; Ascenzi, 1979; Von Endt and Ortner, 1982; Stuart-Macadam, 1985; Walker, 1986; Schultz, 1993; Filon *et al.*, 1995; Martin and Goodman, 2002; Yildirim *et al.*, 2005; Lagia *et al.*, 2007; Walker *et al.*, 2009; Jatautis *et al.*, 2011; Santos *et al.*, 2013) due to an expanded diploë.

A number of studies argue for a similar physiological context for *CO* (Hengen, 1971; Cybulisk, 1977; Stuart-Macadam, 1989). However, as argued by Walker (1985, 1986), the association between the two conditions is not strong. Although the two pathologies can be observed in the same individual, and thus reflect a single underlying condition, this is relatively rare. Only 26.9 % (405/1506) of cases with porotic cranial lesions included in the '*History of Health in the Western Hemisphere*' database (of 4419 skeletal remains from the Americas) had both conditions, while the rest had either one or the other form (Steckle *et al.*, 2002). The two conditions also have relatively similar frequencies across the lifespan (Steckel *et al.*, 2002) and, in as far as they reflect responses to anemic conditions, share a particular ontogeny related to the changes in locus of hematopoietic production during development (Halvorsen & Bechensteen, 2002).

None of the individuals in the present study, including those with *CO*, had macroscopic lesions of PH. The absence of an association between the cribrotic cases analyzed here and of expansion of the diploë strongly suggests that, at least in cases in which *CO* is the sole expression of porotic lesions in the cranium, the underlying mechanism is likely to be different from that leading to PH of the cranial vault. This is supported by the fact that, although *CO* has been thought to be an earlier ontogenetic expression of the anemic response that leads to porotic lesions in the cranium, many cases of PH in children shows no

evidence of cribrotic lesions in the roof of the orbits. The causative independence of PH and *CO* in at least a percentage of cases, calls into question the relationship of the latter with the typical physiological response to anemia leading to bone marrow hyperplasia.

Relationship between *cribra orbitalia* and bone marrow hyperplasia

Several histological and radiographic studies have suggested that *CO* may be associated with marrow hypertrophy (Angel, 1966; Walker *et al.*, 2009). However, the evidence for this relationship in many cases is unclear. Wapler *et al.* (2004) found that more than 1 in 4 cases of *CO* among ancient Nubians had no marrow expansion, while other studies have shown that there was minimal expansion of the diploë in the orbital roof in cases of hemolytic anemia (Caffey, 1937, 1951; Hershkovitz *et al.*, 1997). The results of a detailed analysis of distribution of cranial thickness across the vault, as presented here, show that the relationship between *CO* and expansion of the diploë across the skull is not supported. On the contrary, cribrotic individuals have a significantly thinner diploë, particularly along the midsagittal profile of the frontal and parietal bones and adjacent regions on the parietal bones, than individuals without cranial porotic lesions. Furthermore, the analyses show that non-cribrotic and cribrotic individuals differ in cranial layer characterization, showing a complex pattern of association with localized thickening and thinning of the different layers that form the cranial vault. This is further reflected in the distinct combinations of cranial layer thickening and thinning observed in individuals with different degrees of development of cribrotic lesions.

The results presented here are contrary to the expectation that individuals with *CO* show bone marrow expansion as observed in cases of PH. This further suggests that, if anemia is responsible for the appearance of porotic orbital lesions, it would have to be of the type that is characterized by a significant reduction in either erythrocyte size or production,

leading to a decrease in the overall thickness of cranial diploic bone along the superior portion of the vault. Alternatively, *CO* may be, at least in a percentage of cases, unrelated to any form of anemia, as suggested by Ortner (2003) and Walker *et al.* (2009). The possible causative factors behind porotic lesions in the roof of the orbit are discussed below.

The complex etiology of *cribra orbitalia*

Although a case has been made for hereditary anemias, such as Thalassemia major and Sickle-Cell Anemia, as the causative agents of some archaeological cases of porotic hyperostosis and *cribra orbitalia* (Angel, 1964, 1966, 1967, 1984; Tayles, 1996; Hershkovitz *et al.*, 1997), iron deficiency anemia gained prominence as an explanation for the disease due to its more widespread epidemiological incidence, matching the high frequency of cases of cranial porotic lesions particularly in the Americas (Walker, 1985; Sullivan, 2005), and analogies with clinical studies (Jeliffe & Blackman, 1962; Powell *et al.*, 1965; Aksoy *et al.*, 1966; Cule and Evans, 1968; Lanzkowsky, 1968; Moseley, 1974; El Najjar *et al.*, 1975; Webb, 1982; Martin and Goodman, 2002; Oxenham and Cavill, 2010). Indeed, not only is iron deficiency the most common cause of anemia (Ponka, 1997), but, for instance, only 5-20 % of cases of Thalassemia, itself one of the more severe but relatively rare and regional diseases, show development of PH (Tayles, 1996). However, strong arguments against iron deficiency anemia as the cause of bone porotic lesions that result from the expansion of bone marrow have been put forth on the basis that this type of anemia inhibits effective hemoglobin synthesis, particularly in the development of erythrocytes, and thus leads to the reduction in red blood cell production (Rothschild, 2012). While this challenges the long-standing association between iron deficiency anemia and PH found in the literature (Walker *et al.*, 2009), it adds a potential explanation for the cases of *CO* associated with reduced thickness of the diploë. However, several different types of anemia are possible causative

agents for the condition, as well as diseases unrelated to the hemotopoietic process. These are discussed below.

Anemia as a symptom of different disorders

There are various attributes by which the many types of anemia are clinically classified, such as erythrocyte morphology (*i.e.* size, shape, color or content; Erslev, 1983a; Naeim, 1992; Turgeon, 1993), general size (*i.e.* normocytic, microcytic, or macrocytic; Wintrobe, 1934; Fred, 2007), or cell morphology and etiology (Erslev, 1983a, Marcovitch, 2005). Turgeon (1993) suggests that the categorization of anemia should be based primarily on etiology. Thus, she identifies four main forms of anemia, including those due to *blood loss* (both acute and chronic), *impaired production* (*i.e.* aplastic, iron deficiency, sideroblastic, anemia of chronic disease, and megalobalstic), *hemolytic* (inherited and acquired) and *hemolytic-hemoglobin disorders*, as well as sub-categories within these. A more simplified etiological categorization for anemia based on the state of erythropoiesis has also been proposed (Erslev, 1983a; Naeim, 1992). Erslev (1983a) classifies the types of anemia as either due to reduced erythrocyte production or to increased erythrocyte destruction, with sub-categories based on the types of cell (*i.e.* pluripotent or unipotent cells) primarily affected. He recognizes that various forms of anemia may occur due to the co-occurrence of both reduced red-blood cell production and increased destruction, but adds that his categorical division is based on the principal cause that contributes to anemia. This is consistent with the fact that anemias due to increased erythrocyte destruction, and also blood loss, result in bone marrow hyperplasia, while those that result from reduced or impaired erythrocyte production may result in either normal bone marrow cellularity, hypercellularity (and hyperplasia) or hypocellularity (and hypoplasia; Naeim, 1992). Thus, Erslev's (1983a) categorization may best set the basis on which to identify the probable etiology of *CO* - the

state of marrow hematopoiesis is the defining criteria of an anemic physiology, which in turn is largely accepted to influence the development (or not) of porotic lesions in the cranium.

Erslev (1983a) lists many forms of anemia caused by reduced erythrocyte production (or hypoproliferative disorders; Turgeon, 1993), including those considered by Naeim (1992) as having the greatest effect on bone marrow. These anemias have both acquired and inherited etiologies, such as aplastic anemia (and pancytopenia; Turgeon, 1993), pure red cell aplasia (acute and chronic), B^{12}, folic acid, protein and iron deficiency, β-thalassemia, anemia of chronic and endocrine disorders and of renal failure, among others. However, of these, only five conditions characteristically lead to hypocellular marrow, namely aplastic anemia, protein deficiency anemia, anemia of both chronic and endocrine disorders, and anemia of renal failure (Steinbock, 1976; Erslev, 1983b, d, e; Oski, 1983; Naeim, 1992; Kojima, 1999; Bain *et al.*, 2010). Notably, pure red cell aplasia (acute and chronic) is characterized by marked erythroid hypoplasia; however, hypercellularity and marrow expansion are also a distinctive counter responses to this particular condition. As such, pure red cell aplasia is more likely characterized by both processes, even if hypocellularity and *aplasia* are the initial response. Other potential causes of hypocellular marrow are due to nutritional deficiency of copper, and to toxins (*i.e.* arsenic), or infections. Among the latter, a range of organisms (bacteria, viruses and parasites) can be involved, particularly brucellosis, cytomegalovirus (CMV), anaplasmosis (*i.e. Anaplasma phagocytophilum*), dengue virus (resulting in aplastic anemia), and malaria (*i.e.* caused by *Plasmodium falciparum*). However, with the exception of dengue virus, these other factors lead primarily to hypercellular bone marrow, although hypocellularity is observed in some cases (Naeim, 1992; Bain *et al.*, 2010).

As mentioned above, iron deficiency anemia has been identified as one of the main potential conditions leading to bone marrow hyperplasia and diploic thickening, and eventually, porotic lesions (Cule and Evans, 1968; El Najjar *et al.* 1975; Webb, 1982; Martin

and Goodman, 2002; Oxenham and Cavill, 2010). This view has been questioned many times and on different basis. Fairbanks and Beutler (1972, 1983) argued that, except in cases of chronic iron deficiency, in which extensive marrow hyperplasia is characteristic, severe iron deficiency anemia has less of an impact on bone marrow expansion than other anemias that cause marked hyperplasia, such as thalassemia. Such reduced hyperplastic response to iron deficiency anemia has been observed in other studies (e.g., Naeim, 1992), leading to the suggestion that atrophic or "hypo-regenerative" marrow may be one of the outcomes of this type of anemia (Rothschild, 2012). In fact, Agarwal *et al.* (1970) found that 95 out of 100 individuals with iron deficiency anemia showed atrophy or thinning of the outer cranial table, with only one or two cases[2] of diploic expansion. They attribute this to a delay in diploic widening from the onset of anemia, which would suggest that complication in bone remodeling of the outer cortical table is either a preliminary effect to diploic expansion or that it is simultaneous to even the slightest degree of diploic expansion. Nevertheless, the most common bone marrow response to iron deficiency anemia is toward hyperplasia rather than hypoplasia (and bone marrow atrophy), and can, in some cases, cause an expansion of diploic bone similar to that observed in individuals with thalassemia (Fairbanks and Beutler, 1983). Thus, this form of anemia is unlikely to be the underlying cause of the appearance of *CO* in individuals that not only lack a generalized thickening of the diploë, but are particularly characterized by regions of the vault with absolutely thinner diploë than non cribriotic individuals as observed in this study.

Interestingly, Walker *et al.* (2009) make the case that iron deficiency anemia does not result in PH of the vault because this form of anemia is primarily due to reduced erythrocyte production; thus, they reason that iron deficiency cannot be responsible for marrow expansion as it "inhibits hypertrophy" because of this reduced state of erythropoiesis (p. 112).

[2] In Agarwal *et al.* (1970), it is noted that two individuals have an expanded diploe, but their Table I notes only one such case.

Instead, they propose that megaloblastic and hemolytic anemias, due to increased erythrocyte destruction, are those that markedly lead to diploic hyperplasia. Although the latter is not disputed, it is not the case that all anemias that are primarily due to reduced red blood cell production will have the tendency toward marrow hypocellularity. As noted above, pure red cell aplasia (both acute and chronic), B_{12} and folic acid deficiency, and β thalassemia are primarily caused by reduced or impaired erythropoiesis, and yet they are characterized by hypercellular bone marrow (Erslev, 1983b; Weatherall, 1983; Naeim, 1992). Thus, it remains that iron deficiency anemia does not have a tendency toward bone marrow hypoplasia of the cranial vault despite its etiological categorization of reduced red blood cell production rather than increased destruction. The results in this study suggest an etiology for *CO* that results in a distinctively thin diploë of mid-sagittal portions of the cranial vault and neighboring parts, with significant thickening of the outer table on the mid-anterior aspect of the parietal bones, and of the inner table on the mid-posterior aspects, and no observable indication of PH of the vault. Thus, megaloblastic and hemolytic anemias, which also result in trabecular hyperplasia and thus vault diploic thickening, should be excluded as factors leading to the cases of *CO* studied here.

Hypocellularity, anemias and *cribra orbitalia*

To explain the diploic thinning in individuals with *CO*, the five anemias due to reduced erythropoiesis, resulting in marrow hypocellularity, should be considered - (a) aplastic and (b) protein deficiency anemia, (c) anemia of endocrine disorders, (d) anemia of chronic disorders or disease, and (e) anemias of chronic renal failure.

Aplastic anemia (or pancytopenia if all blood cells are affected), namely the acquired form, is generally attributed to some pharmaceutical drugs (e.g. *nonsteroidal analgesics: phenylbutazone, ibuprofen; anticonvulsants: carbamazepine, hydantoins; antibiotics:*

chloramphenicol and sulfonamides; Shahidi, 1990, pp 26; Brodsky, 2014, pp 966), chemical toxins (e.g. *benzene, trinitrotoluene, chlorophenothane, lindane, chlorodane, hexachlorocyclohexane,* Shahidi, 1990, pp 26) and viral infections (e.g. hepatitis, where 0.1 – 0.2% develop aplastic anemia, Shahidi, 1990), which induce an autoimmune response (Brodsky and Jones, 2005; Brodsky, 2014) whereby the bone marrow and its production of stem cells, particularly those forming erythrocytes, are impaired or destroyed (Shahidi, 1990; Naeim, 1992; Turgeon, 1993; Bain *et al.*, 2010; Brodsky and Jones, 2005; Brodsky, 2014). Given the damage to the marrow and lack of proliferation of these cells, bone marrow hypoplasia is a highly distinctive characteristic of this form of anemia, and although mortality is variable and dependent on the severity of damage to the hematopoietic process, it can often be quite high (Shahidi, 1990; Naeim, 1992; Marín-Fernandez, 1999). Shahidi (1990) reports that most cases of aplastic anemia "range from 5 to 13 per million population per year in the Western hemisphere" (pp 50), with greater prevalence in those over the age of 50, and with more cases documented in Asia. Brodsky (2014) supports this demographic, adding that children as well as young adults are also affected, and notes that calculating the incidence of aplastic anemia is often muddled by the complexity of its diagnosis, with many idiopathic cases of the disease. However, he cites one of the largest longitudinal studies by the International Aplastic Anemia and Agranulocytosis Study (IAAAS) that reports an incidence rate of 2 cases per million. The high mortality of this condition, together with its hypoplastic characteristic, could possibly explain the high numbers of *CO* identified in archaeological collections; however, aplastic anemia is quite rare (Shahidi, 1990; Brodsky, 2014), an autoimmune response poorly understood, with most cases idiopathic or largely triggered by pharmaceutical drugs (e.g. some antibiotics and anti-inflammatory painkillers) or chemical industrial products (e.g. gasoline and pesticides), and, to a lesser degree, viral infections,

making it an unlikely explanation for the large numbers of prehistoric skeletal remains with *CO*.

Protein deficiency anemia interrupts the production of erythropoietin, and is reported to result in a decrease in bone mass and possibly result in osteoporosis (Oski, 1983; Holick and Dawson-Hughes, 2004). This pattern of generalized outcomes is inconsistent with the localized thick outer and inner tables of cribrotic individuals found in this study. Furthermore, Oski (1983) notes that, while hypocellularity is possible, most individuals with this form of nutritional deficiency have normal bone marrow cellularity. In cases of Kwashiorkor, the form of protein deficiency most commonly observed in malnourished infants and children but also in adults, phases of bone marrow hypoplasia may occur (Davies, 1948; Adams, 1969). However, protein deficiency anemia may not be the most likely condition to result in *CO*, given that individuals in this study have localized thicker compact tables and are not osteoporotic, despite the diploic tendency in individuals with this deficiency to be hypocellular or have hypoplastic marrow.

Anemia of endocrine disorders occurs when either the production of erythropoietin, the hormone that induces erythropoiesis in the thyroid or pituitary glands is disrupted (Naeim, 1992; Bain *et al.*, 2010). Of the two, a hypopituitary gland has a greater tendency to cause bone marrow hypoplasia, while thyroid dysfunction may not lead to a particularly discernable reduction in bone marrow cellularity (Erslev, 1983b). In a study considered to be the only population assessment of the incidence and prevalence of hypopituitarism (Schneider *et al.*, 2007), Regal et al. (2001) determined that this condition occurs in 4.21 persons per 100,000 per year, with a prevalence of 45.5 per 100,000, with a similar incidence in both men and women, and where pituitary and/or peripituitary tumors were the main cause, although they cite hormone deficiency also as a contributor. Thus, these rates suffice to determine that the

hypocellularity associated with anemia originating from hypopituitarism is unlikely to explain the ubiquity of *CO* observed in anthropological studies.

Anemia of chronic disorders or disease refers to a condition associated with both infectious and non-infectious illness (e.g. typhoid and tuberculosis, and vasculitis, sarcoidosis and rheumatoid arthritis, respectively; Erslev, 1983e; Weiss and Goodnough, 2005). It tends to cause the premature destruction of erythrocytes, disruption to regular iron uptake (Weiss and Goodnough, 2005; Weiss, 2009), and may lead to bone marrow hypocellularity. Naeim (1992) specifically notes that anemia of chronic disorders have no tendency toward hyperplasia. Interestingly, it is the second most prevalent form of anemia after iron deficiency anemia (Weiss and Goodnough, 2005), with which it also shares certain physiological characteristics (Erslev, 1983e; Bridges and Pearson, 2008), such as the reduction in serum concentration of iron and transferrin saturation (Weiss and Goodnough, 2005). However, while the two conditions share a hypoferremia phenotype (lack of iron in circulation), the underlying processes are different. In the case of anemia of chronic disease, reduced circulating iron is due to the uptake of iron by the reticuloendothelial system, given the reduced uptake by transferrin (iron transporter) (Weiss and Goodnough, 2005). The authors further state that in iron deficiency anemia, the low level of transferrin saturation could be caused by an increase in transferrin itself, creating this disproportion, while in anemia of chronic disease this is either not the case or transferrin levels are relatively less differential (Weiss and Goodnough, 2005). Furthermore, while ferritin (a protein binder for iron) levels in iron deficiency anemia may be low in absolute terms, normal levels are either maintained or higher in anemia of chronic disease since iron is not depleted but rather stored by the macrophage system. Although these physiological processes require clinical analyses to identify, there is a critical difference between both forms of anemia that contributes to anatomical differentiation - anemia of chronic disorders or disease tends toward hypoplastic

bone marrow, while iron deficiency anemia tends toward bone marrow hyperplasia. This characteristic, together with the fact that anemia of chronic disorder or disease has a sufficiently high prevalence to account for the high frequency of archaeological cases, makes it a better candidate as the causative agent of *CO* associated with thinning of the diploë as observed in the present study than iron deficiency anemia.

Anemia of chronic renal failure is an outcome of failing kidney function (Boulton-Jones, 1981) that may be caused by multiple factors (e.g. hypertension, congenital kidney abnormalities, obstructions due to tumors or stones, diabetes, gout, tuberculosis, malaria). Since the kidneys are responsible for the production of erythropoietin (the hormone that initiates erythropoiesis in the marrow), reduced erythropoiesis is one of the main factors contributing to anemia in individuals with chronic renal failure, and can result in severe bone marrow hypoplasia (Naeim, 1992). Thus, of the anemias leading to bone marrow hypocellularity, anemia of chronic renal failure may have one of the most dramatic effects on the reduction of cellularity and, therefore, a greater potential response of hypoplastic or atrophic marrow. However, chronic renal failure (stage 5 of chronic kidney disease, and when accompanying anemia becomes more prevalent) is relatively rare, affecting 120 individuals per million of the population per year, or less (Boulton-Jones, 1981; Stel *et al.*, 2017). Furthermore, the fact that the individuals more likely to express hypoplastic bone marrow are those at the end-stage of kidney disease, or renal failure, with relatively high mortality rates when untreated, makes it inconsistent with the degree of healing of cribrotic lesions widely observed in archaeological human remains as well as considering the high incidence of *CO* among infants and children.

Thus among the various anemic conditions that lead to marrow hypocellularity and hypoplasia, only anemias caused by chronic disorders or diseases meet the physiological pathway, and incidence and prevalence criteria to make it a possible explanation for cases of

CO associated with thinning of the cranial diploë. Notably, non-anemic conditions have also been suggested as the underlying mechanism behind the condition.

Non-anemic potential causes of *cribra orbitalia*

Two main potential causes of *CO*, unrelated to any type of anemia, have been proposed. Both hypotheses focus on physical properties of the cranial vault, and particularly the roof of the orbits. The first of these suggests that diploic expansion can cause "pressure atrophy" on the outer cortical table, allowing the diploic bone to extend into the orbit, expressing *CO*, in severe cases (Steinbock, 1976). Following this logic, thickened outer and inner cranial tables, as observed in this study, may similarly produce an atrophic pressure on the diploë and its resulting counter-pressure would reduce its thickness, as observed by McElhaney (1970) and Peterson and Dechow (2002). However, the regions of the vault where diploic thinning is observed in individuals with *CO* in this study show no particular thickening of the cortical layers, and the only compensatory co-variation between thickening and thinning observed in this study involved the two cortical layers, whereby individuals showed thickening of the outer table at the mid-anterior aspect of the parietal bone with concomitant thinning of the inner table, and thinning of the inner table of the mid-posterior aspects of the parietal bones with concomitant thickening of the outer table – and vice-versa. Therefore, physiological pressure does not offer a consistent explanation for the patterns of vault thickness observed in this study.

The second hypothesis was first suggested by Ortner and colleagues (Ortner and Ericksen, 1997; Ortner *et al.*, 1999, 2001), and developed by subsequent scholars to account for the absence of bone marrow hypertrophy among some individuals with *CO* (Walker *et al.*, 2009). Other researchers had observed that diseases such as scurvy, rickets, and hemangiomas, as well as trauma can cause subperiosteal hematomas that lead to porotic

lesions in the roof of the orbits (Wolter, 1979; Griffeth *et al.*, 1997; Ortner, 2003). These conditions lead to localized hematomas, which lift the periosteum away from bone and form blood clots in the process of healing, which in turn are transformed into plaques of highly vascular, subperiosteal new bone (Woo & Kin, 1997; Sabet *et al.*, 2001; Schultz, 2001; Ma'luf *et al.*, 2002; Brickley & Ives, 2006). Although skeletal lesions associated with scurvy are most commonly reported for the long bones of the lower limbs (Ortner and Putschar, 1981), lesions in the cranial vault are well-known, from the distinctive cranial porous bossing known as Parrot's swellings (Barlow, 1883) to lesions that may be macroscopically undistinguishable from cases of *CO* (Fraenkel, 1929). Such subperiosteal hematomas are most common in children, among whom the periosteum is not as tightly attached as in adults and who have greater density of blood vessels connecting the periosteum to the underlying bone (Tonna, 1974; Ortner and Eriksen, 1997; Ma'luf *et al.*, 2002; Augustin *et al.*, 2007). This would account for the higher prevalence of *CO* observed in infants and young children in archaeological skeletal remains (Steckel *et al.*, 2002).

In particular, scurvy, a disease caused by deficiency or absence of Vitamin C (ascorbic acid) in the diet has been implicated in the formation of cribrotic lesions (Fraenkel, 1929; Ortner and Eriksen, 1997). The subperiosteal hematoma in scurvy occurs on the inferior surface of the orbital plate of the frontal bone (Jaffe, 1972), causing an inflammatory condition that may lead to *CO.* Accordingly, cribrotic lesions in the orbital roof were observed in 12% of autopsy cases of scurvy in infants (Fraenkel, 1929). Vitamin C is critical for the formation of connective tissues, including collagen and cement material that binds the endothelial layer in blood vessels, which if defective, leads to increased susceptibility to haemorrhage (Ortner and Putschar, 1981). Particularly in relation to *CO,* Vitamin C deficiency is known to weaken the Sharpey's fibers that attach the connective tissue covering the orbital roof, thus leading to minor traumatic lesions and bleeding, and potentially the

formation of cribrotic lesions (Walker *et al.*, 2009). Thus, cases of *CO* with absence of diploic vault expansion as observed in this study could be associated with scurvy. A recent study using CT scans to measure the cranial vault thickness in crania of children with scurvy and anemia found that individuals with scurvy had greater vault thickness at most of the landmarks studied than crania from a non-pathological control group, particularly among children below the age of 8 years, as well as a strong relationship between degree of severity of *CO* and PH and patterns of cranial thickness at different landmarks across the vault (Zuckerman *et al*., 2014). The latter results are consistent with the observations on severity of cribrotic lesions in adults and cranial thickness made in this study, although the former differ from those of the present study in which total cranial thickness was more often greater among non-pathological than cribrotic individuals, although the different age groups being considered and the comparatively small sample of cases of scurvy (n= 11) in the study by Zuckerman *et al.* (2014) may account for some of the differences observed.

Reports of scurvy in archaeological remains have increased in recent years as its possible skeletal manifestations become better known (e.g, Maat, 2004; Brickley and Ives, 2006; Mays, 2008; Brown and Ortner, 2011; Geber and Murphy, 2012) and researchers become aware of the potential over-diagnosis of anemia as the explanation for the common porotic lesions observed in past populations. However, while scurvy is a possible explanation for those cases of *CO* that show no expansion of the bone marrow such as those included in the present study, the skeletal lesions of scurvy overlap substantially with those caused by other diseases, making diagnosis in adults uncertain (Brickley and Ives, 2006; Armelagos *et al.*, 2014). Nevertheless, of all the possible conditions that may lead to the form of *CO* dissociated with porotic lesions on the vault and diploë expansion observed in the sample studied here, scurvy is the most likely of causes if anemia, particularly due to chronic disease, can be excluded.

CONCLUSION

Many studies attribute PH and *cribra orbitalia* to some form of anemia due to its hypertrophic and hyperplastic effect on cranial diploic bone (*e.g.* Moseley, 1965; Angel, 1966; Lanzkowsky, 1968; El-Najjar, 1975; Williams *et al.*, 1975; Ascenzi, 1979; Von Endt and Ortner, 1982; Stuart-Macadam, 1985; Walker, 1986; Filon *et al.*, 1995; Tayles, 1996; Schultz, 1993; Martin and Goodman, 2002; Wapler *et al.*, 2004; Gowland and Western, 2012). Most of the studies focused specifically on *CO* also cite other potential etiologies, such as inflammation (*i.e.* lacrimal gland, craniofacial sinuses), and infection (*i.e.* trachoma), which do not have an effect on the diploic bone of the cranium (Steinbock, 1976; Schultz, 2001). However, not all significant detriment to cranial bone marrow is expressed by expansion. To the contrary, the individuals with *CO* included in this study have significantly thinner diploic bone along the cranial midline of the vault and neighboring parietal regions, and no evidence of PH. Such cases of *CO* with no associated expansion of the diploë are reported in the literature, and contribute to hypotheses that argue against all anemias associated with marrow hypertrophy as a causative factor behind cribrotic lesions. Our results add to this body of evidence, and provide a detailed description of the complex pattern of changes in localized cranial vault thickness in individuals with different degrees of severity and healing of *CO*.

While a form of anemia remains the most likely underlying condition for *CO*, the macroscopic lesions observed in skeletal remains can be caused by different forms. *Cribra orbitalia* associated with bone marrow hyperplasia and/or porotic hyperostosis, is likely to be related to one of the major anemias that compensate an impairment or deficiency in blood cells by stimulating increased hematopoiesis, such as with iron-deficiency, hereditary (e.g., Thalassemia, Sickle-Cell) and some acquired anemias. *Cribra orbitalia* in cases with the absence of bone marrow expansion, however, can only be related to those anemias that result

in localized diploic atrophy or hypoplasia. Of these, anemia of chronic renal failure may have the most dramatic effect on diploic bone, although its rarity and demographic makes it an unlikely cause of the widespread archaeological prevalence of *CO*, while anemia of endocrine disorders (particularly of the pituitary) may also contribute. However, anemia of chronic disease is highly prevalent (only second in occurrence to iron deficiency anemia), and it is characteristically hypoplastic, which is consistent with the lack of diploic expansion in cribrotic individuals in this study. However, scurvy can also cause cribrotic lesions in the roof of the orbits that is dissociated from cranial vault marrow expansion, and should be considered as a likely cause of CO if anemia, particularly those of chronic disease, can be excluded. The overlapping expression of these conditions on the skeletal system makes differential diagnosis difficult, and highlights the importance of considering the spatial and structural pattern of cranial vault thickness when investigating palaeopathological cases of *CO* and PH.

Lastly, given that porotic hyperostosis of the vault is firmly associated with diploic hyperplasia, and that at least some individuals with *cribra orbitalia* are significantly characterized by localized hypoplasia, it is suggested that the terms should be connoted to reflect their general, but clearly separate etiologies.

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Table 1 List of crania included in the analyses

Regional Distribution of Non-cribrotic Individuals	Sample Size (n)	Sex	
		Male	Female
Asia (Burma, India & Sri Lanka, Andaman & Nicobar Islands)	8	3	5
Africa (West, Southern, Central, East & North Africa)	6	2	4
Africa (Kenya, East Africa)	10	5	5
Africa (Somalia, East Africa)	7	6	1
Africa (Tanzania, East Africa)	8	8	0
Africa (Sudan, East Africa)	5	5	0
Europe (France, Austria, Hungry, Switzerland, Sweden, Germany, Serbia, Russia, Czechoslovakia, Italy, Minorca, Sardinia, England, Malta, Greece)	9	3	6
America (South & Central)	6	3	3
America (North)	9	6	3
Australia	7	3	4
TOTAL	**75**	**44**	**31**
Regional Distribution of Cribrotic Individuals			
Africa (South Africa, Kenya, Somalia, Tanzania, Sudan)	16	8	8
Asia (Mainland India & Andaman Islands)	5	2	3
Europe (Germany)	1	1	0
South America (Peru)	1	1	0
TOTAL	**23**	**12**	**11**

Table 2 Cranial vault thickness measurements that differ between cribrotic and non-cribrotic individuals

Measurement in ranking order	**Benjamini-Hochberg p value (0.25 false discovery rate)**
TOTAL THICKNESS (n=48)	
Vertex	p = 0.110
OUTER TABLE THICKNESS (n-46)	
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DIPLOIC THICKNESS (n=46)	
Bregma subtense fraction posterior (PAFpost)	p = 0.015
Nasion subtense fraction inferior (FRFinf)	p = 0.015
Bregma subtense fraction anterior (PAFant)	p = 0.015
Bregma subtense fraction (PAF)	p = 0.018
Ophryon (O)	p = 0.041
Frontal quadrant 4 (FQ4)	p = 0.045
Parietal quadrant 4 left (PQ4l)	p = 0.051
Parietal quadrant 2 right (PQ2r)	p = 0.051
Frontal quadrant 2 (FQ2)	p = 0.057
Bregma (B)	p = 0.060
Vertex (V)	p = 0.112
Parietal quadrant 2 left (PQ2l)	p = 0.167
Glabella (G)	p = 0.167
Nasion subtense fraction superior (FRFsup)	p = 0.196

INNER TABLE THICKNESS (n= 46)	
Opistocranium (Op)	p = 0.040
Parietal quadrant 4 left (PQ4l)	p = 0.040
Parietal temporal fraction superior left (PTFsupl)	p = 0.058
Inion (In)	p = 0.109
Parietal quadrant 3 left (PQ3l)	p = 0.149
Frontal quadrant 1 (FQ1)	p = 0.149
Nasion subtense fraction (FRF)	p = 0.149
Lambda subtense fraction (OCF)	p = 0.149
Parietal temporal fraction posterior right (PTFpostr)	p = 0.149
Parietal quadrant 3 right (PQ3r)	p = 0.149
Nasion subtense fraction left (FRFl)	p = 0.239
Nasion subtense fraction right (FRFr)	p = 0.242
Lambda subtense fraction left (OCFl)	p = 0.242
Parietal temporal fraction posterior left (PTFpostl)	p = 0.242

Table 3: Canonical discriminant function results for cranial measurements in increasing order

Analysis	Eigenvalue	Canonical Correlation	Wilk's λ	X^2	Cross-validated classification			
					Not observed or predicted *CO*	Not observed but predicted *CO*	Observed but not predicted *CO*	Observed and predicted *CO*
DT (uncorr)	0.133	0.342	0.883	11.888	**75 (100.0%)**	0 (0.0 %)	23 (100.0%)	**0 (0.0%)**
IT (corr)	0.259	0.453	0.795	21.850	**71 (94.7%)**	4 (5.3%)	16 (69.6%)	**7 (30.4%)**
TT (uncorr)	0.269	0.460	0.788	22.395	**72 (96.0%)**	3 (4.0%)	16 (69.6%)	**7 (30.4%)**
IT (uncorr)	0.298	0.479	0.770	24.549	**71 (94.7%)**	4 (5.3%)	15 (65.2%)	**8 (34.8%)**
OT (uncorr)	0.378	0.524	0.725	30.009	**71 (94.7%)**	4 (5.4%)	13 (56.5%)	**10 (43.5%)**
DT (corr)	0.486	0.572	0.673	37.004	**68 (90.7%)**	7 (9.3%)	10 (43.5%)	**13 (56.5%)**
OT (corr)	0.661	0.631	0.602	46.915	**69 (92.0%)**	6 (8.0%)	13 (56.5%)	**10 (43.5%)**
OT + IT (corr)	0.683	0.637	0.594	48.175	**67 (89.3%)**	8 (10.7%)	10 (43.5%)	**13 (56.5%)**
IT + DT (corr)	0.725	0.648	0.580	50.723	**68 (90.7%)**	7 (9.3%)	7 (30.4%)	**16 (69.6%)**
OT + DT + IT (uncorr)	0.886	0.685	0.530	58.387	**71 (94.7%)**	4 (5.3%)	8 (34.8%)	**15 (65.2%)**
OT + DT (corr)	0.849	0.678	0.541	56.570	**71 (94.7%)**	4 (5.3%)	6 (26.1%)	**17 (73.9%)**
OT + DT + IT (corr)	2.347	0.837	0.299	105.706	**70 (93.3%)**	5 (6.7%)	4 (17.4%)	**19 (82.6%)**

Table 4 Results of the Kruskal-Wallis test of differences across average cranial thickness among levels of severity of cribrotic lesions (Grades 1 to 3)

Landmark	X2	Df	P	Av thickness Grade 1	Av thickness Grade 2	Av thickness Grade 3
Frontal fraction total thickness	8.043	2	0.018	6.3367	8.1578	6.3376
Parietal Bregma-left outer table thickness	6.285	2	0.043	1.8124	2.4629	1.6590
Parietal quadrant 3 left outer table thickness	9.665	2	0.008	1.7829	2.5212	1.7855
Frontal quadrant 2 outer table thickness	7.752	2	0.021	1.4891	2.1668	1.7467
Frontal quadrant 2 inner table thickness	7.531	2	0.023	0.9258	1.5477	1.0745
Opistocranium outer table thickness	6.747	2	0.034	1.8257	2.3726	1.7983
Opistocranium inner table thickness	6.708	2	0.035	1.2925	2.2144	1.2560
Lambda subtense fraction outer table thickness	10.374	2	0.006	2.1208	3.1491	2.0901
Lambda subtense fraction inner table thickness	6.100	2	0.047	1.2658	2.1187	1.2017
Inion outer table thickness	7.553	2	0.023	2.2159	3.2817	2.0026
Inion inner table thickness	7.350	2	0.025	1.6648	2.7493	1.6749

Table 5 Discriminant function scores differentiating individuals without cribra, and with and without cribrotic healing

	No *cribra* (n=97)	Healing *cribra* (n= 9)	No healing of cribrotic lesions (n= 14)
DF1	-0.35439 ± 0.92	2.702847 ± 2.76	0.717893 ± 2.42
DF2	0.328181 ± 1.05	-0.99299 ± 1.58	-1.63548 ± 1.45

Figure 1: *Cribra orbitalia* and grades of severity

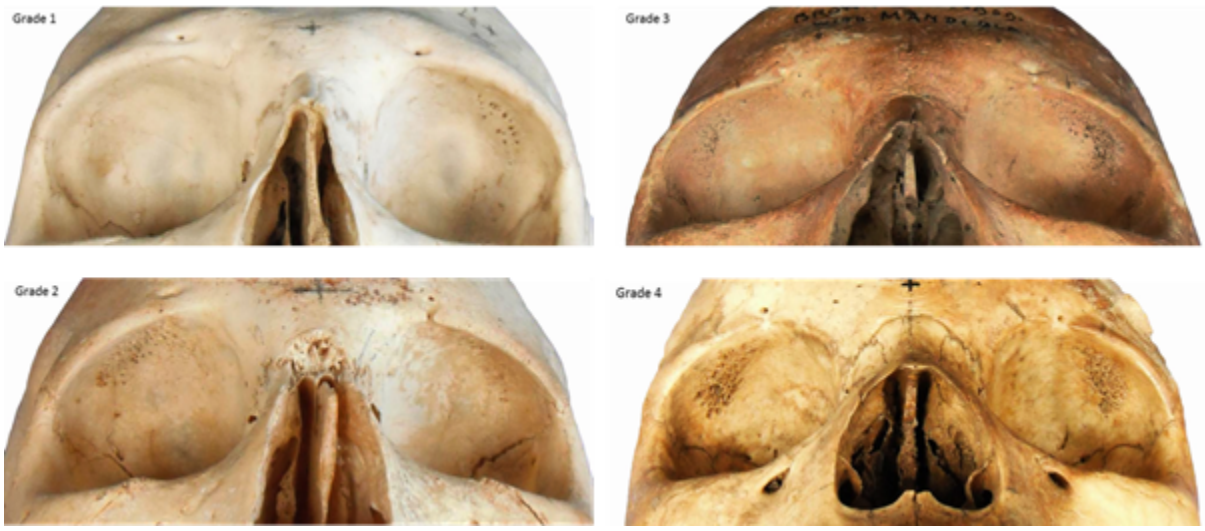


Figure 2: CT boundaries: grey scale and binary images using Half Maximum Height (HMH)

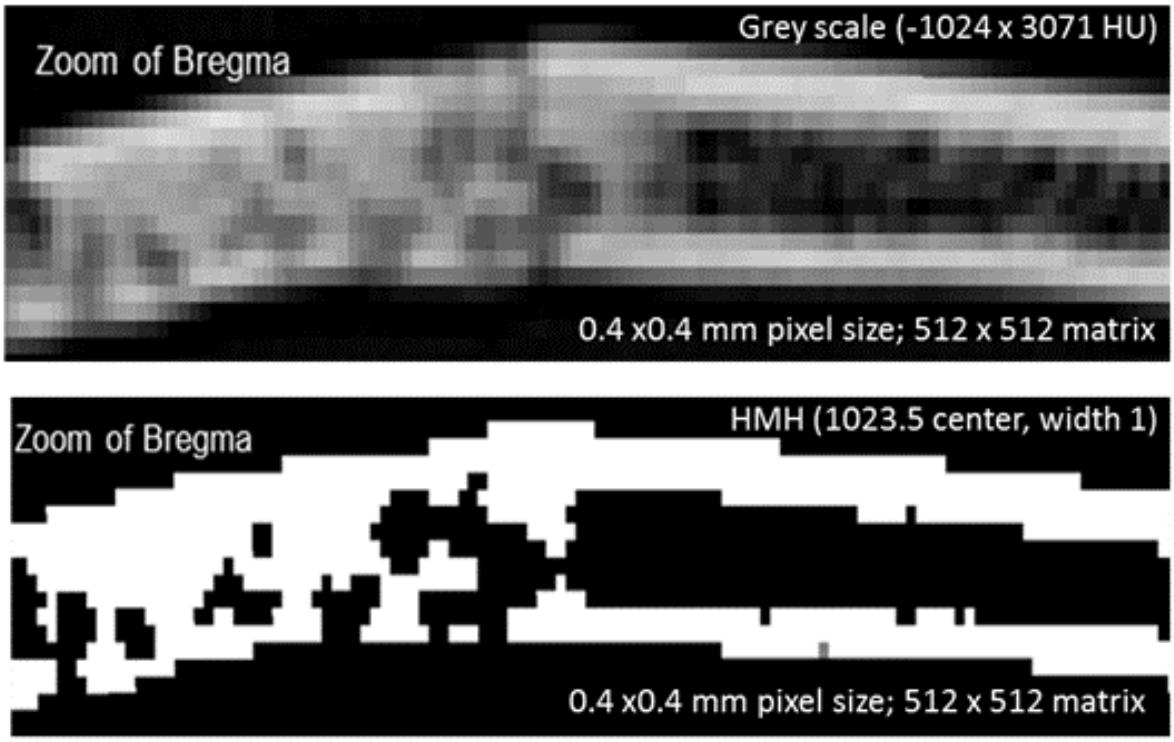
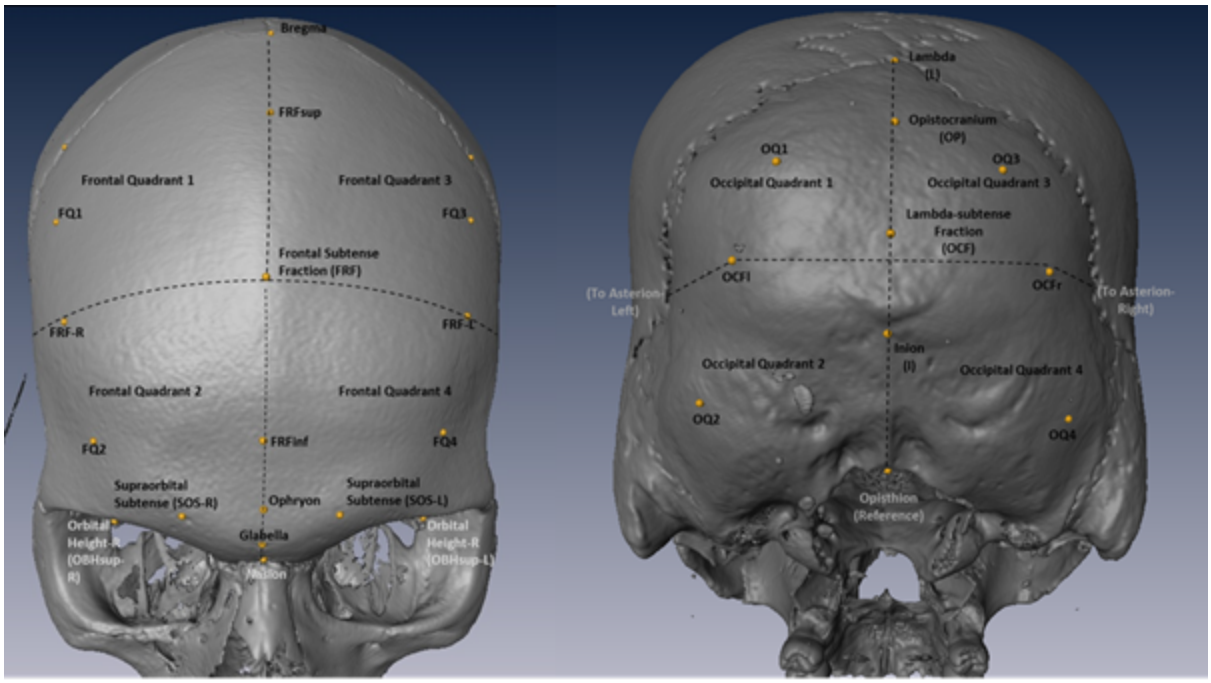


Figure 3: Bisecting and quadrant thickness measurement landmarks for the frontal, parietal and occipital bones



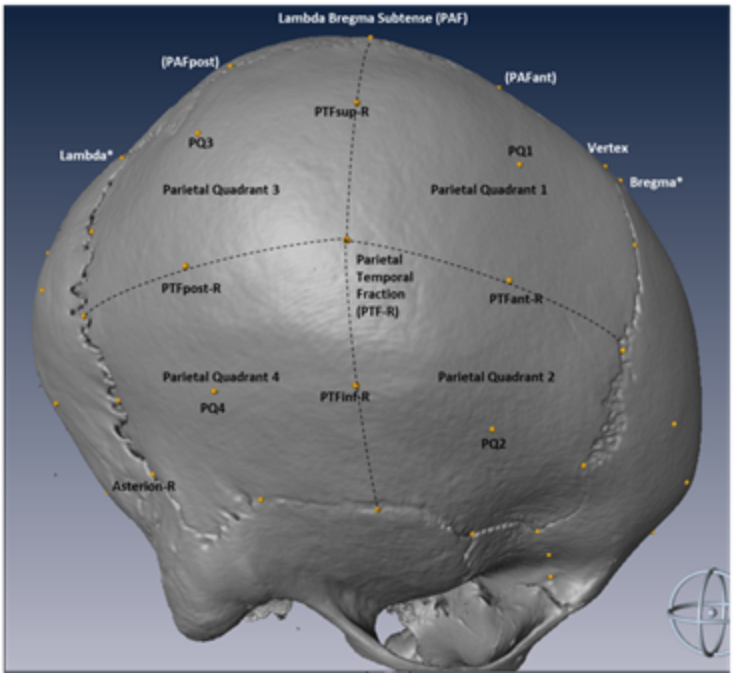


Figure 4: Independent T-test - total thickness at vertex between non-cribrotic and cribrotic individuals

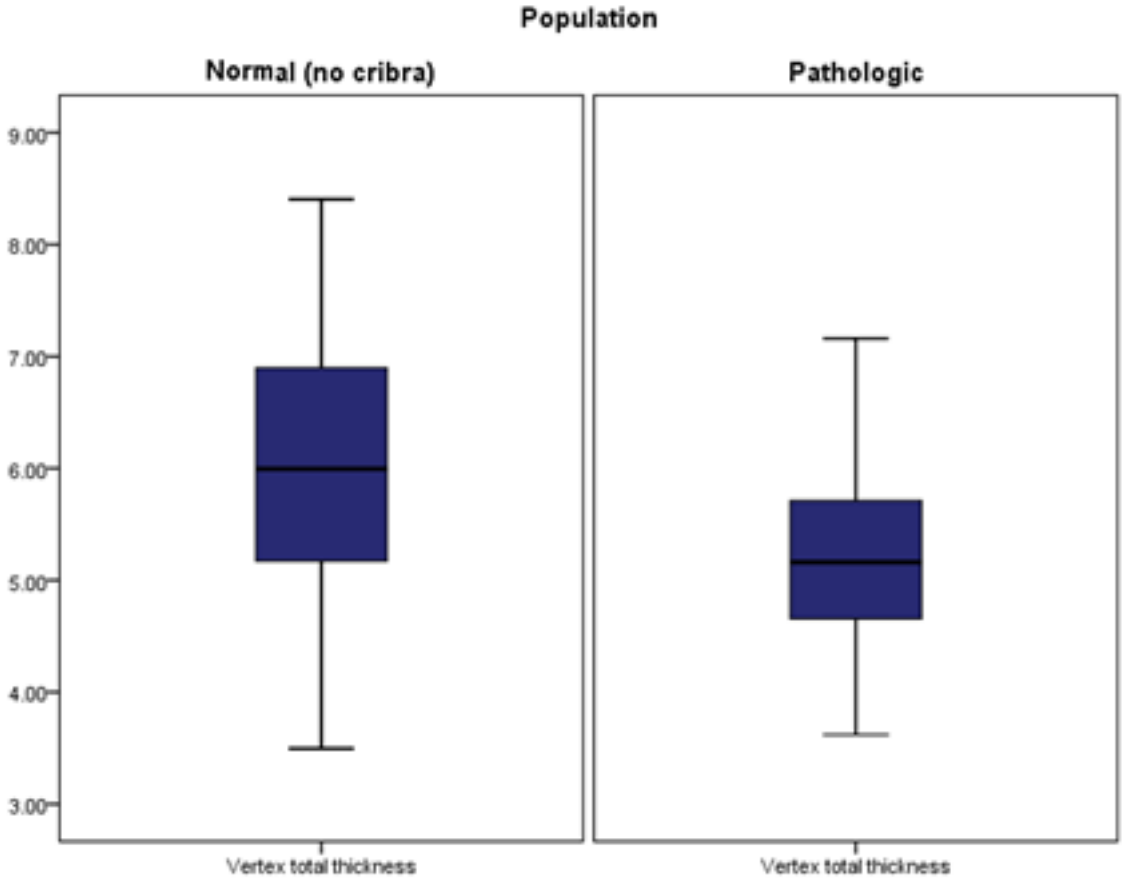


Figure 5: Independent T-test - inner table thickness of non-cribrotic and cribrotic individuals

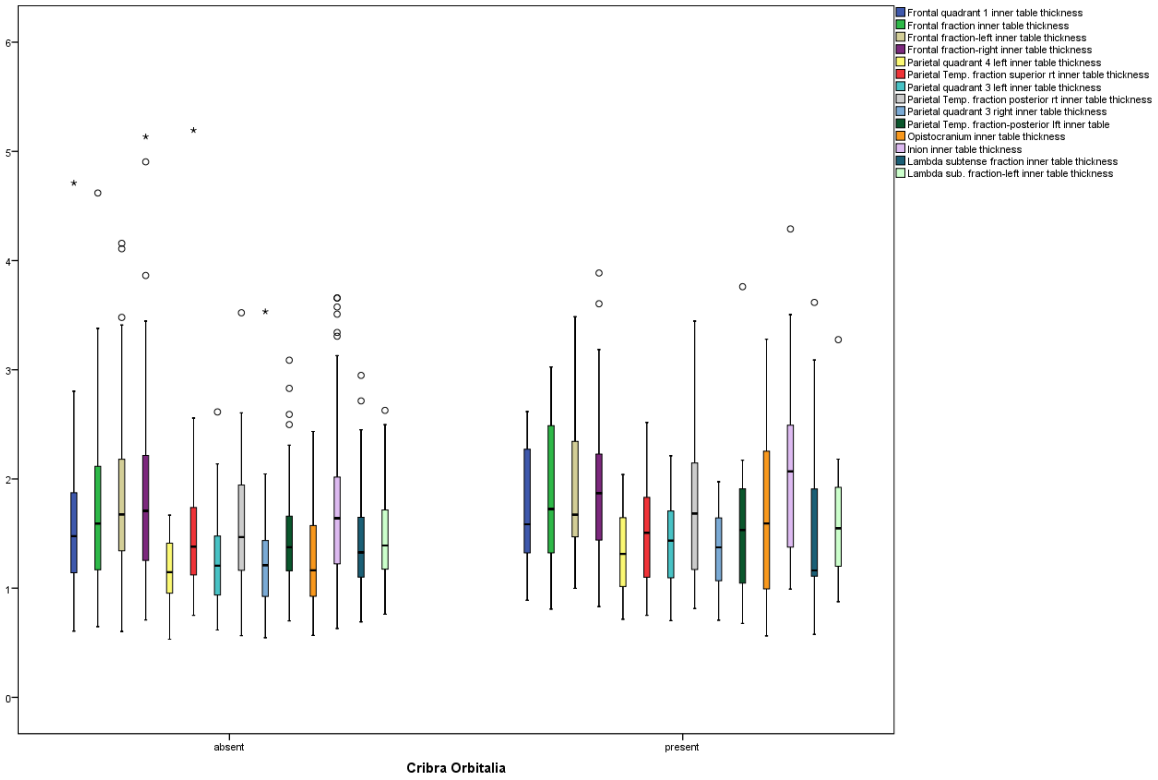


Figure 6: Independent T-test - diploic thickness of non-cribrotic and cribrotic individuals

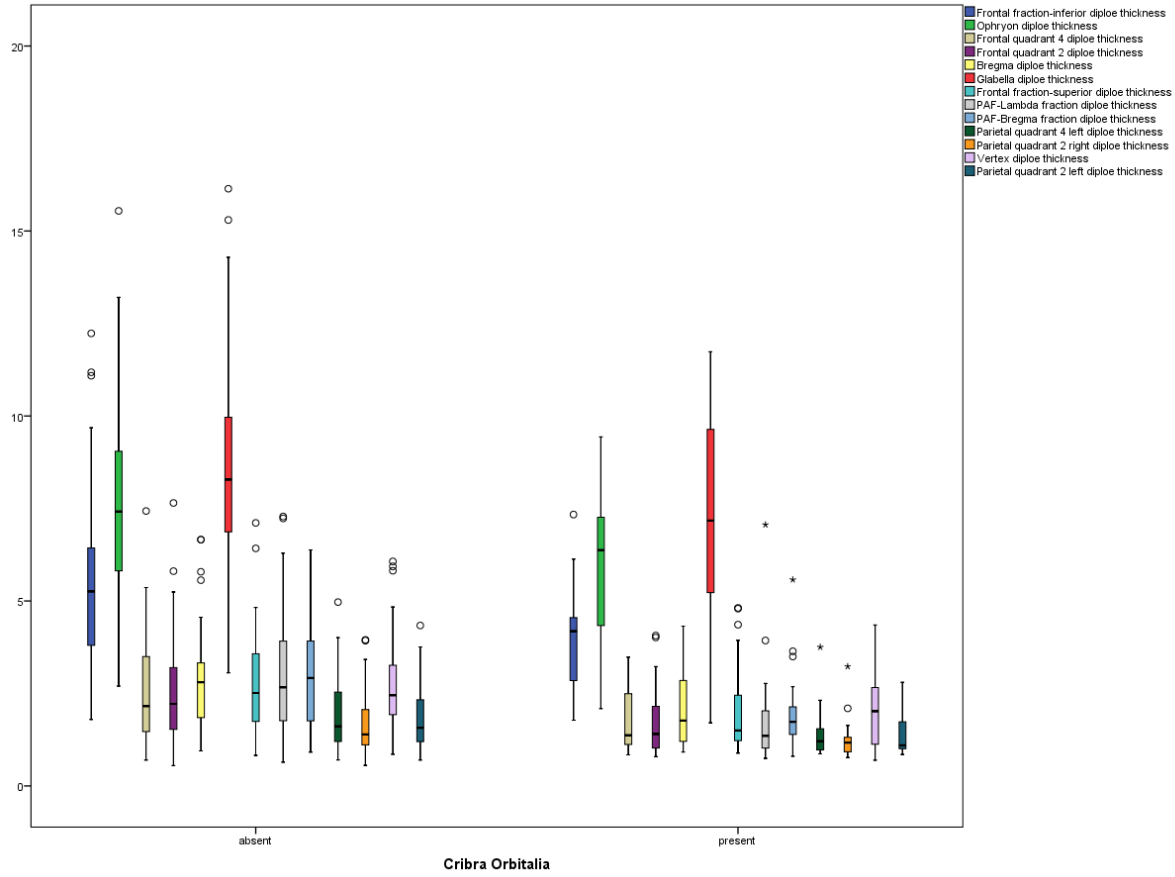


Figure 7: Discriminant function differentiation of cribrotic and non-cribrotic individuals

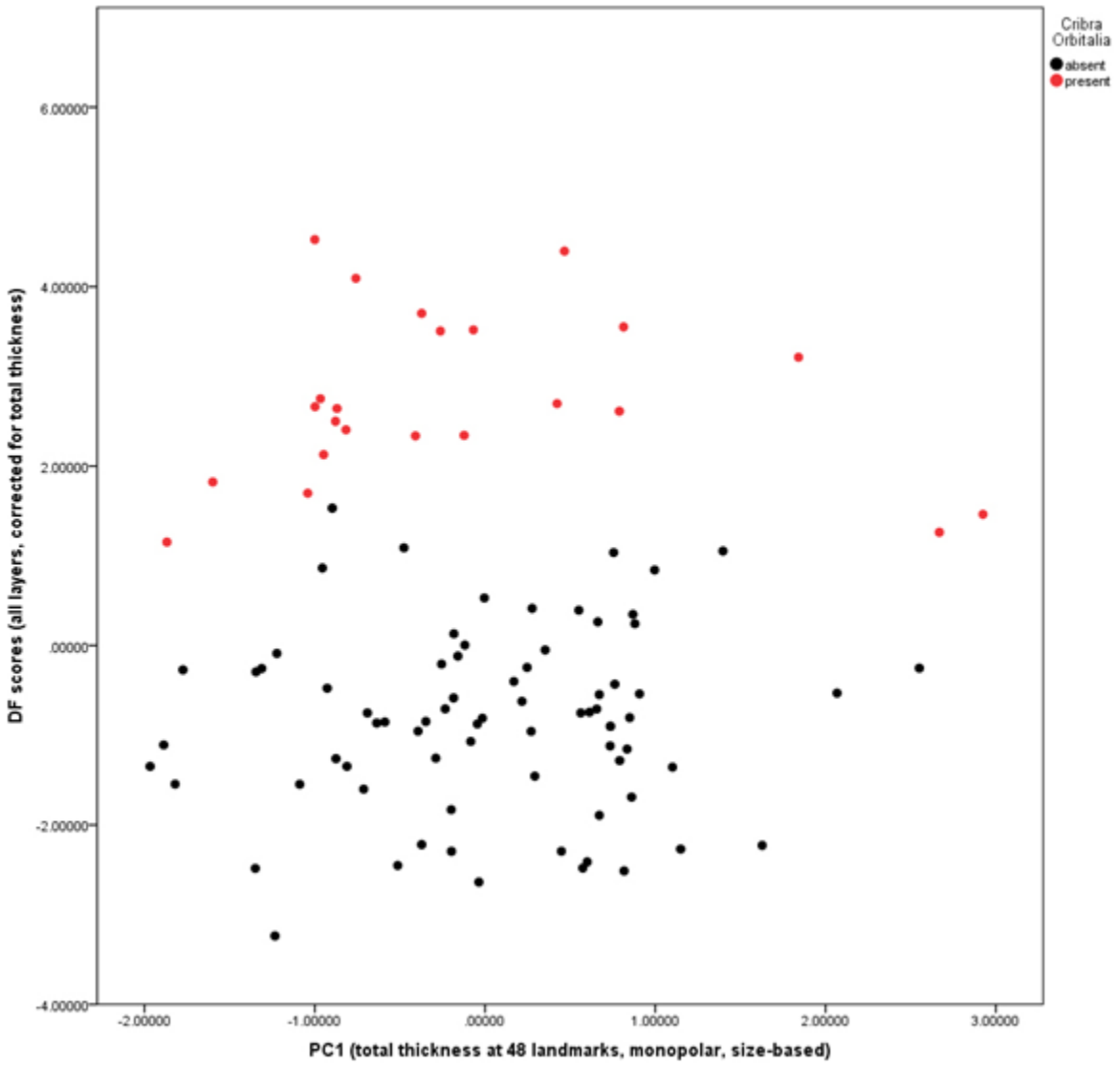


Figure 8: Discriminant function cranial thickness distributions of cribrotic and non-cribrotic individuals

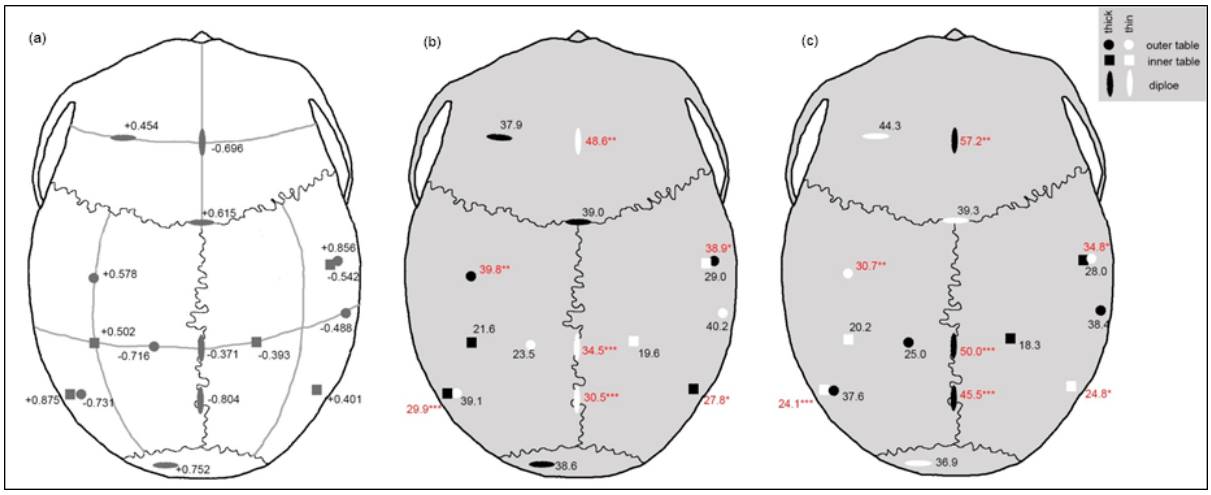


Figure 8: Pattern of cranial thickness in individuals with and without *cribra orbitalia* as revealed by a Discriminant Function analysis based on the thickness of the three cranial layers across 48 points on the vault. (a) Degree of correlation of the 16 thickness variables that contribute to the Discriminant Function; (b) pattern of relative thickness of the sixteen variables in individuals with *cribra orbitalia* (asterisks mark univariate significance levels); (c) pattern of relative thickness of the sixteen variables in individuals without *cribra orbitalia* (asterisks mark univariate significance levels).

Figure 9: Pattern of cranial layer thickness correlating with the Discriminant function

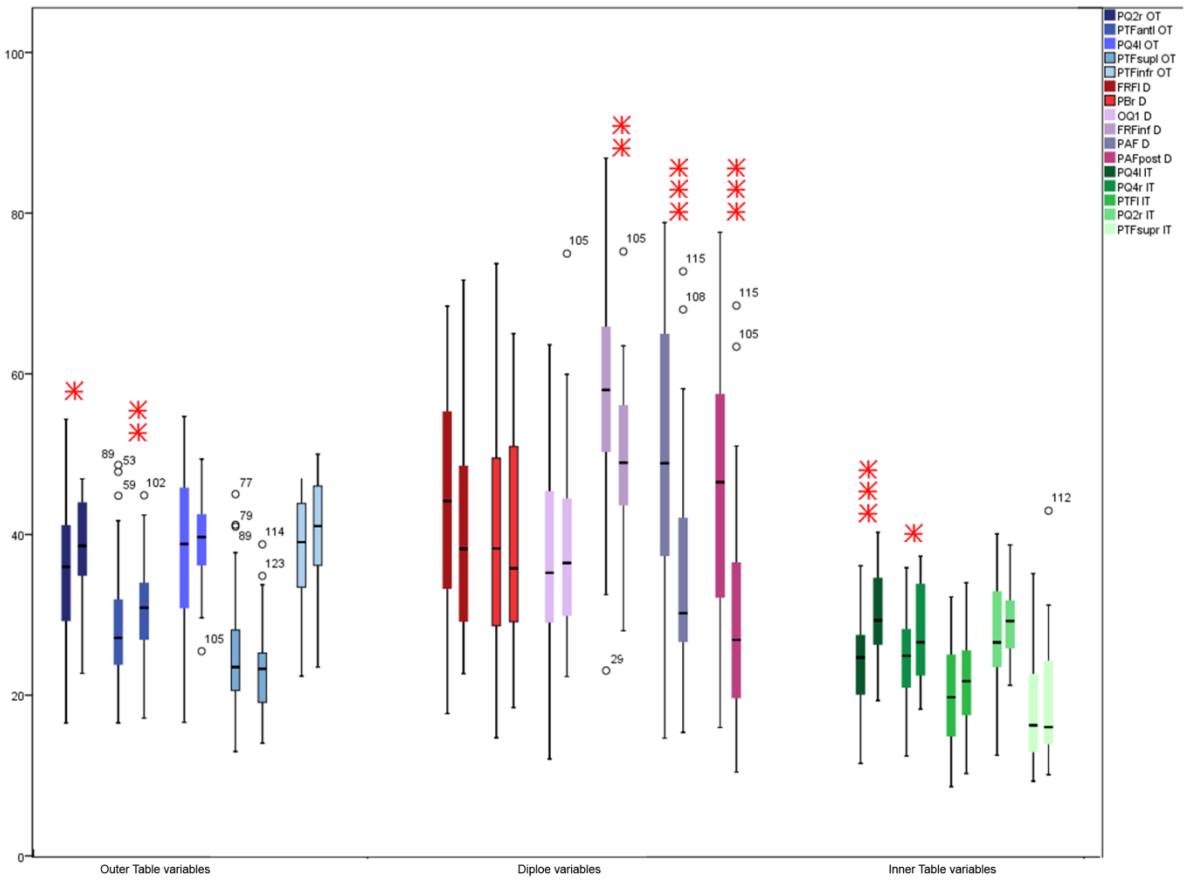


Figure 9: Boxplot of the 16 variables that correlate with the Discriminant Function, organized by cranial layer, and clustered by absence (left) and presence (right) of *cribra orbitalia.* Asterisks mark level of significance.

Figure 10: Severity degrees and differing patterns of cranial vault thickness

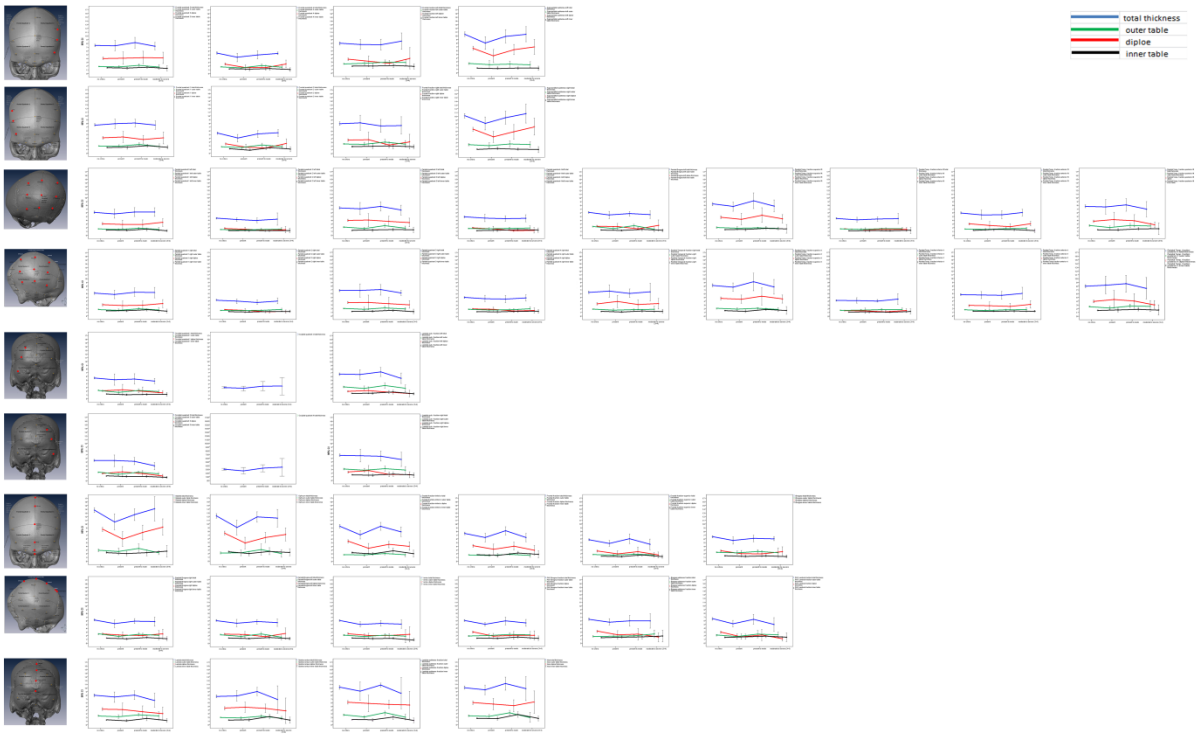


Figure 10: Severity of *CO* per variable across the cranial vault

Figure 11: Discriminant function showing differentiation among cribrotic individuals with mild, moderate and pronounced severity

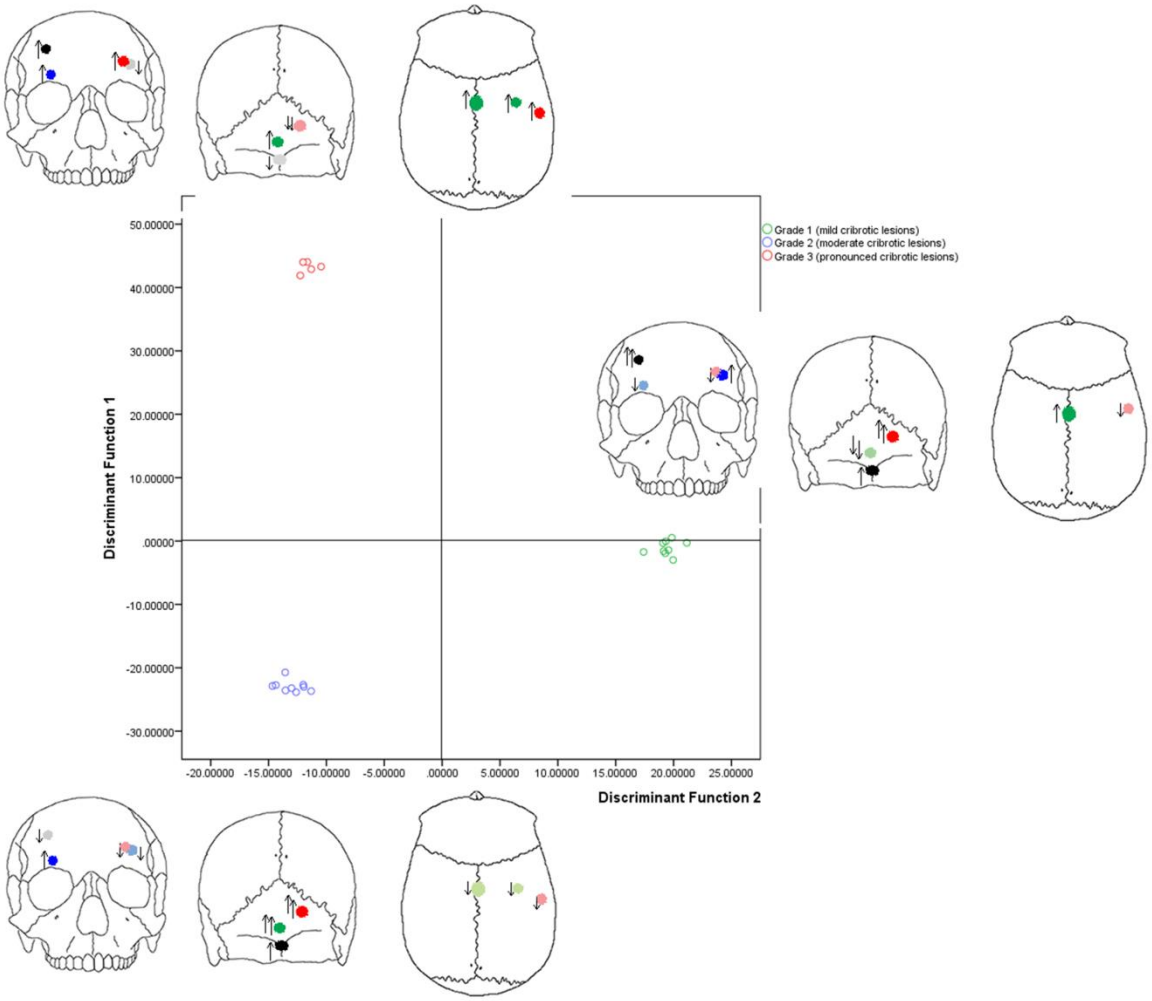


Figure 11: Figure 11 Shows the results of a discriminant function (15 variables), where cases with moderate (Grade 2) and pronounced (Grade 3) cribrotic lesions separate along Function 1, and where mild cases (Grade 1) separate from cases of moderate and pronounced CO (Grades 2 and 3) along Function 2

Figure 12: Discriminant function distribution of individuals with and without CO according to severity grades

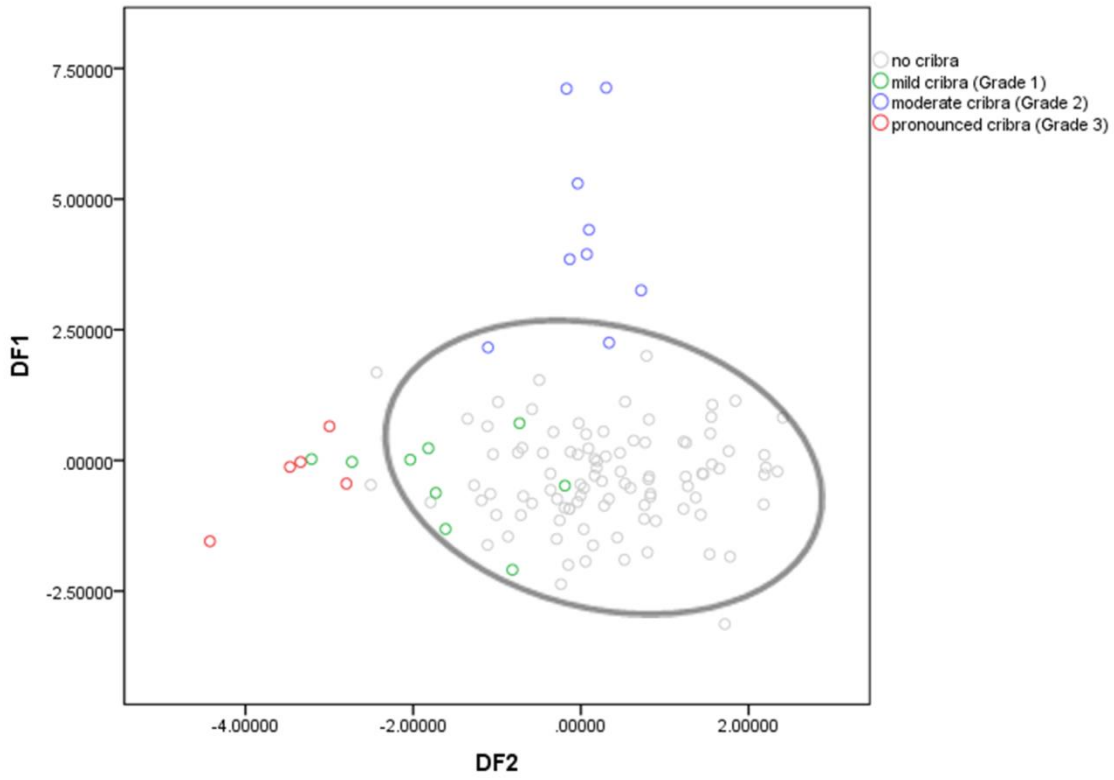
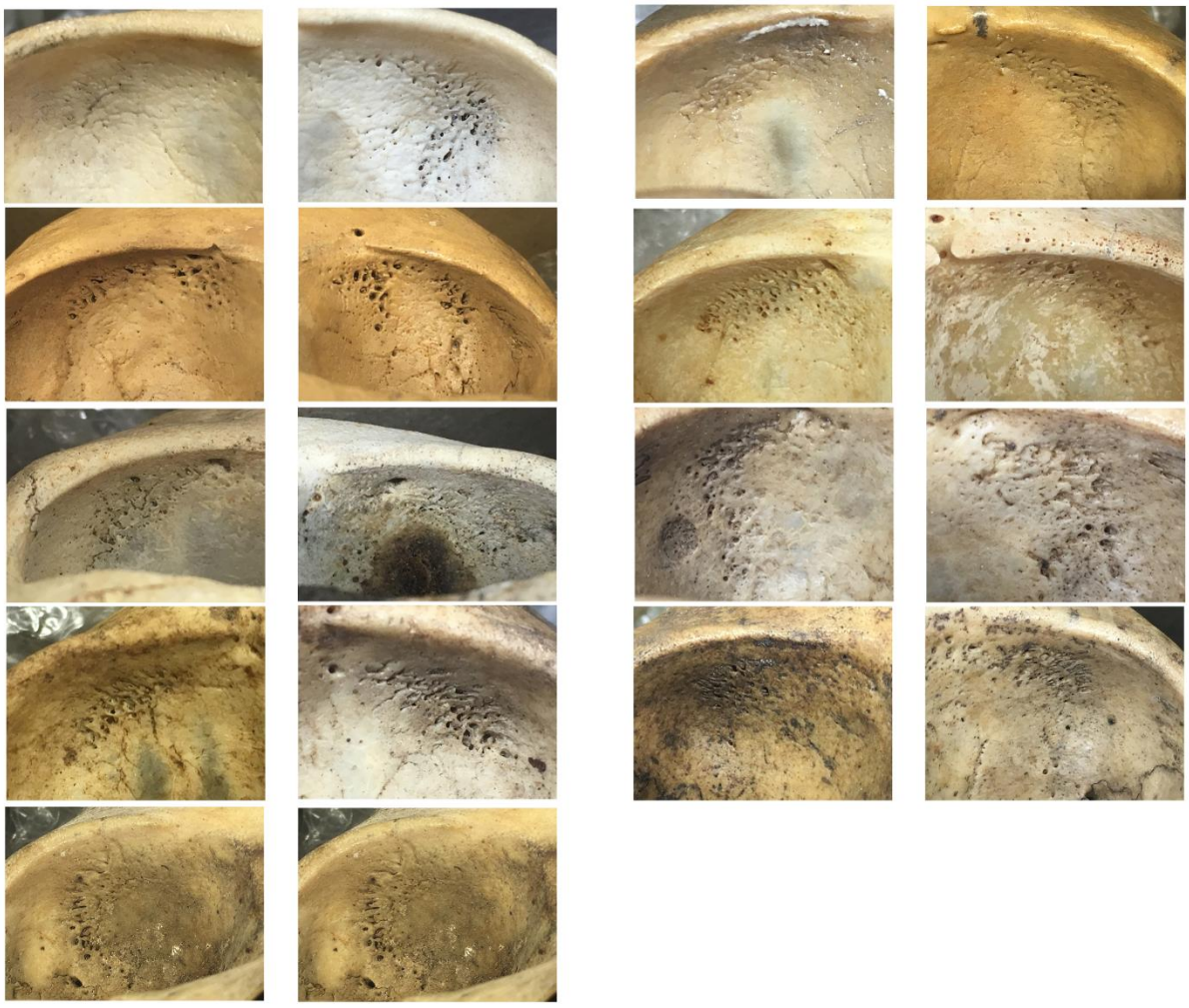


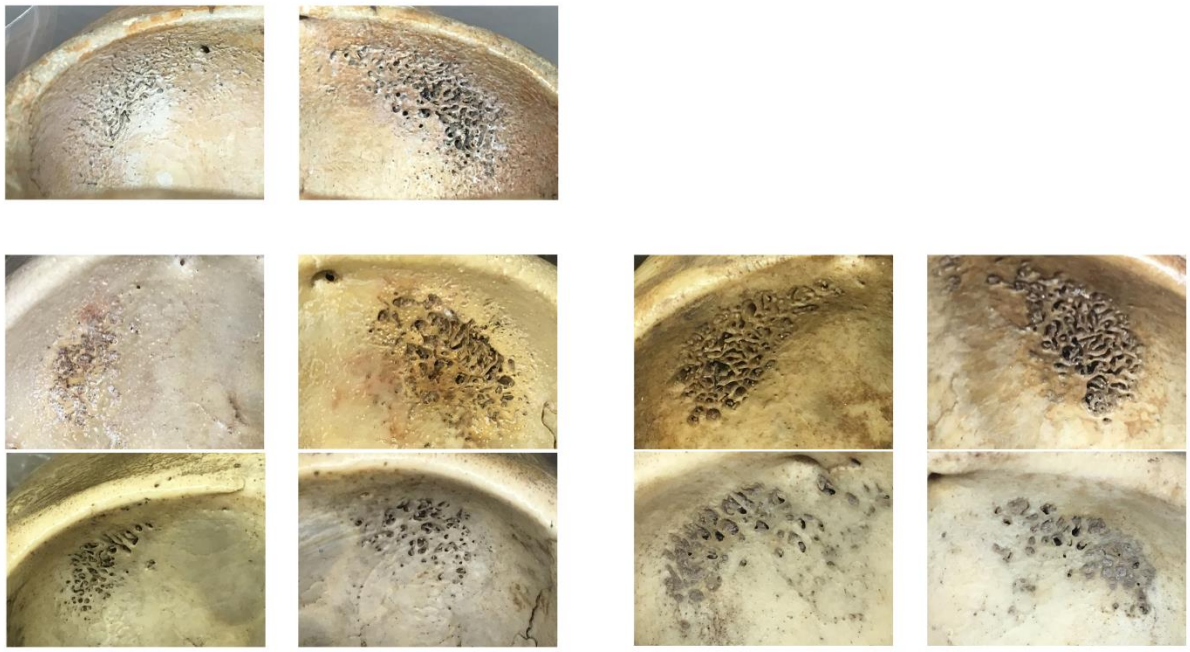
Figure 12: Shows the cranial thickness distribution of non cribrotic individuals and those with varying severity of the lesions, where mild cases of *CO* do not differentiate from those without *CO*

Supplimentary Figure (S1): Severity categories 1 - 3 from mild, moderate and pronounced

Cribra grades: (a) grade 1, presence of pores



b) grade 2, present to moderate



(c) grade 3 and 4, moderate and moderate to severe, respectively