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Anemias in Prehistoric Cyprus: Insights from Khirokitia

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Abstract

The site of Khirokitia, which illustrates the last phase of the Aceramic Neolithic in Cyprus, yielded a large series of human remains (MNI = 243) showing an unusual age distribution. The juvenile sample includes a high proportion of infants less than one year old, mostly perinates. Furthermore, porotic bone lesions, that might suggest anemia have been frequently observed on the juvenile sample. Taking into account the ancient origin of some β -thalassemias in the Mediterranean Basin, the hypothesis of prehistoric thalassemia in the Eastern Mediterranean, put forward by J. L. Angel in the 1960s, can be reconsidered, using the data from Khirokitia. For this purpose, we have undertaken an innovative interdisciplinary study combining anthropological and paleopathological analyses, digital imaging and isotopic studies. Promising preliminary results, regarding the microstructural analysis of bones through digital imaging techniques support the hemoglobinopathy hypothesis.

Introduction

Anemia is a major public health issue in many countries. It can be considered as »a general term for a variety of abnormalities of red blood cells that affect the ability of the circulatory system to exchange oxygen«¹. It is the result of a wide variety of causes, mainly iron-deficiency, although other conditions such as micronutrient deficiencies, chronic inflammation, parasitic infections or inherited disorders can all cause anemia. Anemia can occur at any age of life but its highest prevalence is observed in preschool age children (0–4.99 years)².

In Cyprus, anemia prevalence in children aged 6–59 months was estimated at 19 % on average in 2011³. The island is known to have one of the highest prevalence rates of thalassemsias in the world and was one of the first countries to develop, 40 years ago, a successful population-wide prevention program⁴.

According to J. L. Angel, who put forward the hypothesis of prehistoric thalassemia in the Eastern Mediterranean based on the frequency of porotic hyperostosis in Neolithic skeletal remains from this region, the mutations responsible for thalassemsias might have been present in the Eastern Mediterranean since the 7th millennium B.C. Porotic hyperostosis is described by J. L. Angel as »an overgrowth of the spongy marrow space of the skull«, with the possibility that other bones are affected in children⁵. The diagnostic value of such bone modifications has since been debated⁶ but analysis of the genetic background of thalassemsias mutations indicates the ancient origin of some β -thalassemsias in the Mediterranean Basin⁷.

Given the development of imaging techniques, allowing microstructural analysis of bones and advances in isotopic biogeochemistry, it is now possible to reconsider J. L. Angel's hypothesis, using a combined set of methods consisting of anthropological and paleopathological analyses, digital imaging and isotopic studies. The anthropological and paleopathological peculiarities observed at the Neolithic site of Khirokitia in Cyprus, which strongly suggest the presence of one or more forms of haemoglobinopathies and possibly of iron deficiency anemia, provide an

¹ Ortner 2003.

² WHO 2008.

³ WHO 2015.

⁴ Kountouris et al. 2016.

⁵ Angel 1966.

⁶ e.g. Stuart-Macadam 1985; Stuart-Macadam 1987; Stuart-Macadam 1991; Ascenzi et al. 1991; Hershkovitz et al. 1997; Holland – O'Brien 1997; Wapler 1998; Schultz 2001; Walker et al. 2009.

⁷ Filon et al. 1995; Le Mort et al. 2006; Cherry et al. 2016; Vigano et al. 2017.

excellent opportunity to undertake this type of study⁸, the preliminary results of which are presented here.

Materials and Methods

The Site of Khirokitia

The site of Khirokitia, situated on a hill, about 6 km as the crow flies from the present southern coast of the island, was first excavated by P. Dikaios between 1936 and 1946⁹. After a few soundings¹⁰, the excavations, directed by A. Le Brun, were renewed in 1977 and completed in 2009. The occupation of the site, which illustrates the last phase of the Aceramic Neolithic of Cyprus, took place during the 7th and early 6th millennium cal. B.C. The settlement area was determined to be approximately 3 ha, of which only a part has been explored. The village consists of houses composed of several round-shape buildings. It is divided into two sectors, east and west. During the course of recent excavations, nine stratigraphical levels have been recognized in the east sector (A to H, J) and three (I to III) in the west sector; it also appears that the boundaries of the village fluctuated during its occupation¹¹.

The Burials

Many burials were discovered at the site, all of which are primary burials. The bodies lie in a contracted position in burial pits that were dug into the floors of houses while those houses were occupied (fig. 1). The dead are all buried in the same way, whatever their age-at-death. Most of the buildings yielded at least one, and as many as 18 burials, belonging either to the same or to various stratigraphical levels. Burials of a single age-at-death group (adults, infants or older juveniles¹²) may be found in one building; whilst in other cases, adult burials were found together with infant burials and/or older juveniles. There is no evidence that a special part of the house was reserved for graves. The buildings where infant burials have been found are distributed all over the investigated area¹³. Contrary to what is commonly observed in ancient populations¹⁴, no specific funeral treatment was devoted to infants who had been stillborn or had died shortly after birth and no reserved funeral area for these very young individuals seem to have existed at Khirokitia¹⁵.

Within the area explored by P. Dikaios, burials are distributed across all excavated areas¹⁶. In the recently excavated area, burials were found in all levels except for the oldest three (levels G, H, J), but it has to be noted that these levels have only been explored in a limited area¹⁷. It is difficult to establish the exact number of graves found during the older excavations for two reasons: first, some of them have not been published in the final report¹⁸; and second, the analysis of the published data leads to the hypothesis that the few burials considered by P. Dikaios to include more than one individual correspond to superimposed single burials¹⁹. Therefore, the total

⁸ Le Mort 2000; Le Mort 2003; Le Mort 2008.

⁹ Dikaios 1953.

¹⁰ Stanley Price – Christou 1973; Le Brun – Stanley Price 1977.

¹¹ Le Brun 1984; Le Brun 1989a; Le Brun 1994; Le Brun – Daune-Le Brun 2003; Le Brun – Daune-Le Brun 2009.

¹² In this text, the term »juvenile« is used for any stage of life that is not truly adult (until the final fusion of long bones epiphyses), according to the definition provided by Scheuer and Black (2000); the term infant is also used according to the definition recommended by these authors (from birth to the age of one year).

¹³ Dikaios 1953; Le Brun 1984; Le Brun 1989a; Le Brun 1989b; Le Brun 1994; Le Mort 1994; Le Mort 2003.

¹⁴ e.g. Dedet et al. 1991; Duday et al. 1995; Coqueugniot et al. 1998; Alpe 2008.

¹⁵ Le Mort 2000; Le Mort 2008.

¹⁶ Dikaios 1953.

¹⁷ Le Brun 1984; Le Brun 1989a; Le Brun 1994, as well as personal communication; Le Mort 1994.

¹⁸ Niklasson 1991.

¹⁹ Le Mort 2003.

number of individuals recovered from the Dikaios excavations is estimated to be 146 based on his published descriptions. During the soundings carried out in 1972 in the part of the site previously investigated by P. Dikaios, some new human remains were found but their burial treatment is not clear²⁰. Recent excavations (since 1977) have yielded at least 107 more individuals, resulting in a total burial sample of at least 253.

Skeletal Sample

The material from P. Dikaios' excavations was partly analysed by J. L. Angel²¹ and then by G. Kurth²² and R. P. Charles²³. The few remains found during the soundings were briefly reported on²⁴. A reappraisal of all accessible human remains has been carried out, in parallel to the study of the skeletons from recent excavations. A portion of the human remains unearthed by P. Dikaios are now missing and could not, therefore, be reconsidered. The reappraisal of the skeletons uncovered during the old excavations, and from the soundings, and the study of the human remains from recent excavations allowed us to identify at least 243 individuals,²⁵ of which 135 are juveniles.

Bone Preservation

At Khirokitia, there is a difference in preservation amongst burials. The preservation generally, is rather good in regard to the relative completeness of the skeleton. On the other hand, the bones themselves are often in a bad condition. Most of them are broken into numerous pieces and many are partial and/or eroded; but, in general, the remains of very young infants are well-preserved. Nearly all the remains are covered with concretions that could be partly dissolved in acid²⁶.

Estimation of Age-at-Death of Juvenile Individuals

For juvenile individuals, age-at-death was estimated according to dental development, applying the methods developed by C. Moorrees et al.²⁷ when the required teeth could be observed, or the chart of D. H. Ubelaker²⁸ in the other cases. For perinatal individuals, the bone size was, in many cases, the only available indicator of age-at-death; in these cases, age-at-death was thus estimated by measuring the length of limb bones²⁹. Because of the individual and interpopulation variability, these estimates should be considered with caution. That is the reason why we include in the perinate category fetuses more than six lunar months old, stillborns and infants under one month of age.³⁰

²⁰ Stanley Price – Christou 1973.

²¹ Angel 1953.

²² Kurth 1958.

²³ Charles 1962.

²⁴ Stanley Price – Christou 1973.

²⁵ In a few cases, when bones from several burials uncovered during P. Dikaios' excavations were mixed together, it was necessary to estimate the minimum number of individuals.

²⁶ Most of the skeletons unearthed in the course of P. Dikaios' excavations are still partly or totally covered with concretions; the way they were cleaned at the time of their discovery is unknown.

²⁷ Moorrees et al. 1963a; Moorrees et al. 1963b.

²⁸ Ubelaker 1978.

²⁹ Fazekas – Kosa 1978; Scheuer – Black 2000.

³⁰ According to paediatricians, the term perinatal means from 24 weeks gestation to 7 postnatal days and stillborn refers to an infant born after gestational period of 24 weeks who shows no sign of life (Scheuer and Black 2000).

Lesion Recording and 3D Imaging

All skeletal remains were examined macroscopically for porotic hyperostosis, cribra orbitalia and any abnormal porosity. Unfortunately, the state of preservation of the remains, the alterations due to taphonomic process, the concretions and the plaster used for reconstruction of skulls from P. Dikaïos' excavations, greatly limited macroscopical paleopathological observations. In order to have access to the inner structure of the skulls showing porosities, micro-computed tomography (μ -CT) imaging techniques, which permits nondestructive study of pathologies³¹ were applied to a selected set of 5 individuals. The bones were μ -CT scanned with a GE v|tome|x S at a resolution from 9.7 to 12 μ m (acquisition parameters: 120 kV, 120 μ A, 2550 projections, 360, 500 ms, frame averaging of 3, 0.1 mm copper filter).

Results

Age Distribution

The studied skeletal sample consists of 110 infants less than one year old, 25 other juveniles and 108 adults. The juveniles/adults ratio (56%) appears to be consistent with an ancient population³². On the other hand, the age distribution of juveniles is quite unusual³³; it reveals a high proportion of infants less than one year old (45% of the total sample and 81% of the juveniles), most of them (95%) deceased during the perinatal period, as well as a low proportion of juveniles more than one year old (fig. 2).

Bone Lesions

Cribra orbitalia (fig. 3) is the most prevalent pathology observed in the juveniles who did not die during the perinatal period, with a frequency of 78% in observable individuals (9); it was also identified in three adult individuals. Porotic hyperostosis involves pitting and porosity of the external surface of the cranial vault usually associated with a thickening of the diploë³⁴. Despite the alterations, the concretions and the plaster, porosities (fig. 4 a. 5 a) have been observed on the cranial vault of at least 21 juvenile individuals including perinates; a frequent thickening of the vault, up to 12 mm, was also noticed for the adults.

The study of these porosities using μ -CT, performed on selected samples, confirmed changes in cranial vault microstructure suggested by the appearance of the ectocranial surface. The μ -CT slice of a piece of occipital bone from a child, aged between 4.5 and 7 years-at-death, (locus 639, east sector, level A) showing porous lesions allows us to observe the widening of the diploë which presents large medullary sinuses, some of which are open on the external surface of the outer table as porosities (fig. 4 b). This trait indicates marrow hypertrophy very likely linked to anemia. This child also exhibits cribra orbitalia. The inner structure of a fragment of the right half of a frontal bone from a perinate (locus 434, west sector, level III) shows a vermiculated reorganization of the medullary space; locally the sinuses are oriented perpendicular to the outer table and are open at the external surface as numerous (fig. 5 b). This pattern is highly suggestive of porotic hyperostosis resulting from anemia.

³¹ Coqueugnot et al. 2010; Coqueugnot et al. 2015.

³² Ledermann 1969; Sellier 1996.

³³ Le Mort 2000.

³⁴ Stuart-Macadam 1985.

Discussion

The juvenile age distribution observed at Khirokitia is quite unusual. The proportion of infants who died before one year-of-age, as well as the proportion of perinates among the infants is very high. Historical demographic data reflects that the normal perinates/infants-less-than-one-year old frequency in pre-industrial populations ranges between 43% and 52% of the population³⁵. No infant remains have been reported from other Cypriot sites dating to the 7th or early 6th millennium B.C. Earlier Neolithic Cypriot sites, *Kalavastos-Tenta*, *Kissonerga-Mylouthkia* and *Parekklishia-Shillourokambos*, yielded a small number of individuals (up to 27 individuals at *Shillourokambos*) which included very few perinates³⁶. However, it is interesting to notice that a high proportion of the infant age group was also noted for the small skeletal series from the g site of *Kalavastos-Tenta* located not far from Khirokitia: at this site, infants make up 44 % of the total population (MNI=18). Comparisons with Neolithic Near Eastern sites dating from the 9th to the 7th millennium B.C. (Early Pre-Pottery Neolithic B (PPNB) to Pottery Neolithic) also point out the ~~specificity~~ exceptionality of the age distribution of the juveniles at Khirokitia (tab. 1).

Table 1 Mortality patterns at Khirokitia compared to earlier Cypriot sites and to some Syrian Neolithic sites

Site	Period	MNI	Proportion of juveniles	Proportion of infants among juveniles	Proportion of perinates among infants	References
Khirokitia (Cyprus)	7 th -early 6 th mill.)	243	56 %	81 %	95 %	
<i>Kalavastos-Tenta</i> (Cyprus)	8 th -7 th mill.	18	55.5 %	80 %	-	Moyer 2005
<i>Kissonerga-Mylouthkia</i> (Cyprus)	8 th mill.	6	50 %	33 %	100 %	Fox et al. 2003
<i>Shillourokambos</i> (Cyprus)	8 th mill.	27	29.6 %	37.5 %	66.7 %	Le Mort et al. (in press) b
Kerkh (Syria), Pottery Neolithic levels	7 th mill.	89	41.5 %	21.6 %	75 %	Chamel 2014
Abu Hureyra (Syria), phases 2A and 2B	9 th -7 th mill.	167	42.5 %	19.7 %	71.4 %	Chamel 2014
Dja'de el Mughara (Syria), Early PPNB levels	9 th mill.	111	54 %	18.3 %	81.8 %	Chamel 2014

Several hypotheses could account for the very high proportion of infants deceased as perinates at Khiriokitia. Firstly, it may be that this age distribution results from archaeological sampling bias. Nevertheless, when the remains from old excavations are compared to those from recent excavations in different areas of the site, or between the remains from the East and West sectors, a quite consistent picture appears: the perinatal group is over-represented in each case³⁷.

Another possibility could be the existence of age-related mortuary practices; it may be that the inhabitants of Khirokitia provided a specific treatment to the perinatal individuals leading to better preservation of their skeletal remains and allowing for the frequent discovery of their burials while excavating the houses. A consequence of this different treatment of perinatal individuals would mean that the other juvenile burials would be rare in the excavated portion, and imply that they were possibly buried in a, as yet, undiscovered location. Taking into account the homogeneity of mortuary practices at Khirokitia³⁸, such a hypothesis looks unlikely.

³⁵ Dupâquier 1979.

³⁶ Fox et al. 2003; Moyer 2005; Le Mort et al. 2011; Le Mort et al. (in press) a; Le Mort et al. (in press) b.

³⁷ Le Mort 2000.

³⁸ Dikaios 1953; Le Brun 1984; Le Brun 1989a; Le Brun 1989b; Le Brun 1994; Le Mort 1994; Le Mort 2003.

In a skeletal assemblage, a high number of perinates, in relation to other juveniles, can indicate infanticide³⁹; this hypothesis has been formulated to explain the high number of perinates uncovered at some archaeological sites⁴⁰. Further evidence for the sex ratio of the infant sample, and/or possible specific age-related mortuary practices, is yet needed to prove such a practice⁴¹. At Khirokitia, infanticide seems very improbable, taking into account again the homogeneity of mortuary practices at the site.

The high proportion of perinates at Khirokitia coupled with the high frequency of porotic bone lesions in the juvenile sample may suggest poor maternal/fetal health. A number of aetiologies, such as anemias (both inherited and nutritional), scurvy, rickets, infections, etc. might induce porotic bone lesions of the skull⁴². 3D imaging techniques has allowed us to show that, at least in some cases, the porosities observed on the cranial vault of the juveniles from Khirokitia were associated with an enlargement of the diploë, as well as with changes in its microstructure, including sinuses merging at the external table, which is highly suggestive of anemia. According to P. L. Walker et al.⁴³, the iron-deficiency-anemia hypothesis, which has been largely accepted during the past decades, cannot explain the marrow hypertrophy that produces porotic hyperostosis (including cribra orbitalia). These pathologies would be most likely caused by hemolytic anemias and megaloblastic anemias, mainly due to vitamin B12 deficiency or folate deficiency. In possible conjunction with this, in the island of Cyprus there is a high prevalence of β -thalassemia and also high frequencies of Hemoglobin H disease ($\beta\beta$ - α) causing severe anemia and serious health problems⁴⁴.

Conclusion and Perspectives

The very high perinatal mortality and the bone modifications observed at Khirokitia might be consistent with a hereditary anemia such as thalassemia. During the 7th millennium B.C., the inhabitants of the island of Cyprus develop an original culture, indicating a relative isolation from the mainland. It seems that the island was inhabited by a homogeneous population whose traits (i.e. brachycrany, practice of cranial deformation, funeral practices) already existed at sites of the 8th millennium B.C.⁴⁵ This population, which is likely to have resulted from the local evolution of communities that arrived much earlier on the island, might have favored endogamy, leading to increased risk of autosomal diseases.

To explore, in-depth, the anemia hypothesis, we are performing a multi-factorial approach, combining micro-computed tomography and isotopic analysis at a large scale in order to reconstruct the diet and health status of the population of Khirokitia, alongside the previously completed anthropological analyses. In total, 248 bone samples, including pieces with and without porous lesions from all age categories, have been collected. Within this ongoing study, carbon, nitrogen and calcium isotopic analyses are in progress and preliminary results are promising. This project could, further, be extended to other Neolithic populations from Cyprus and the Near East in order to contribute to a more complete understanding of the early history of anemia from the Neolithic period onwards.

³⁹ Molleson 1991.

⁴⁰ E.g. Faerman et al. 1998; Mays – Eyers 2011.

⁴¹ Faerman et al. 1998.

⁴² E.g. Ortner 2003; Lewis 2018.

⁴³ Walker et al. 2009.

⁴⁴ Kountouris et al. 2016.

⁴⁵ Le Mort 2003; Le Mort 2017.

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Figures



Fig. 1 Khirokitia. Infant burial 830, structure 122, level C, East sector (© French Archaeological Mission at Khirokitia)

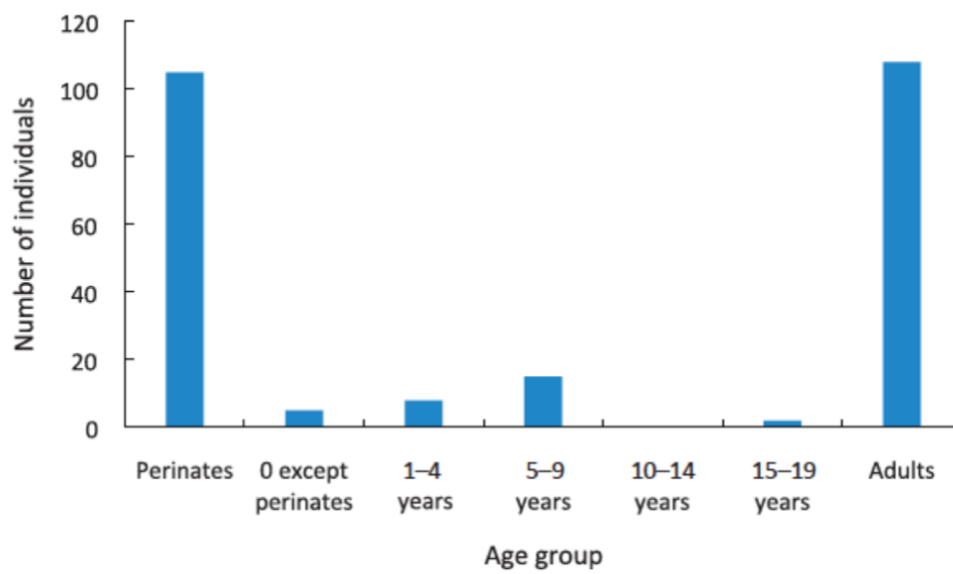


Fig. 2 Age distribution of the juvenile skeletal sample from Khirokitia (© F. Le Mort)



Fig. 3 a Cribra orbitalia on a child aged between 6.5 and 10 years (locus 277, west sector, level I) (Photo by B. Chamel)

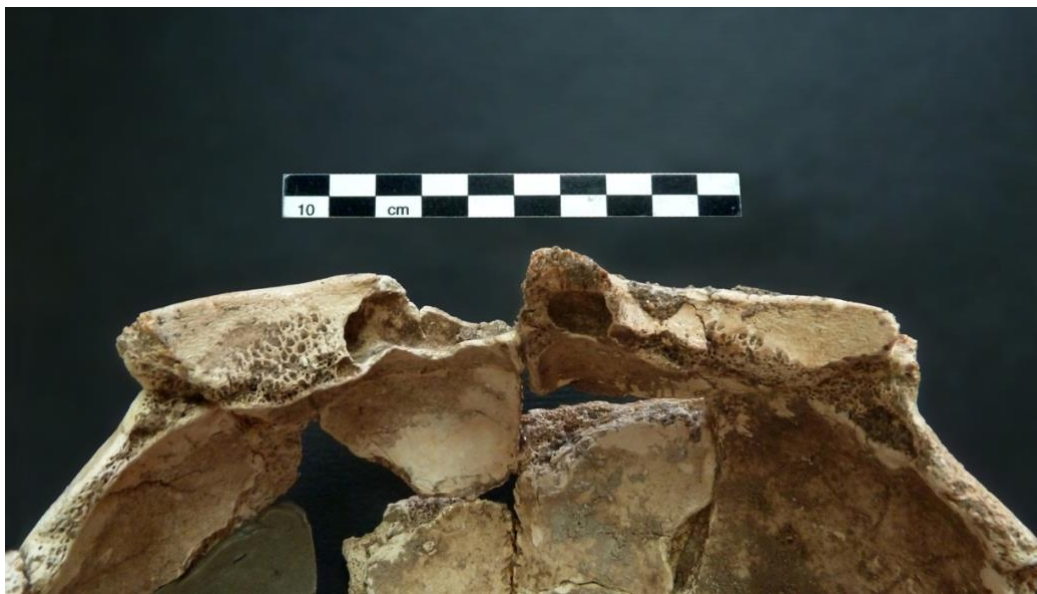


Fig. 3 b Cribra orbitalia on a young adult (locus 638, east sector, level A) (Photo by B. Chamel)



Fig. 4 a Piece of occipital bone from a child aged between 4.5 and 7 years (locus 639, East sector, level A) showing porous lesions (Photo by B. Chamel)

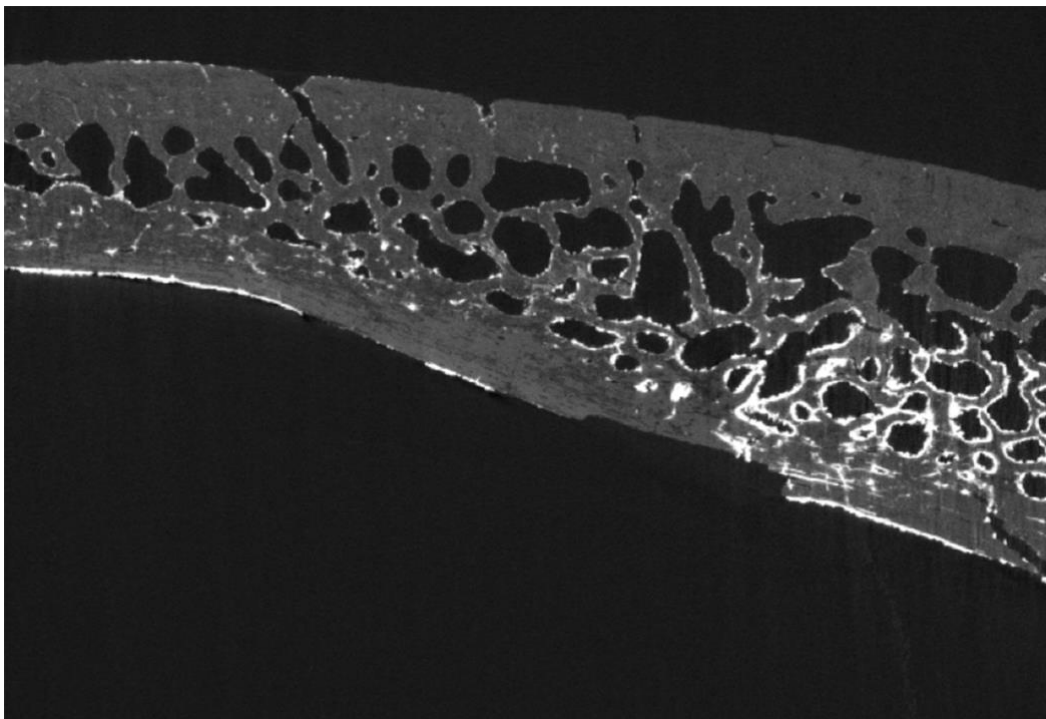


Fig. 4 b μ -CT slice (resolution: 12 μ m) revealing the widening of the diploë which presents large medullary sinuses, some of which are opened at the surface of external table as porosities. This pattern reflects *hyperostosis spongiosa cranii* (© H. Coqueugniot)



Fig. 5 a Piece of the right half of the frontal bone of a perinate (locus 434, West sector, level III) exhibiting porosities (photo by B. Chamel)

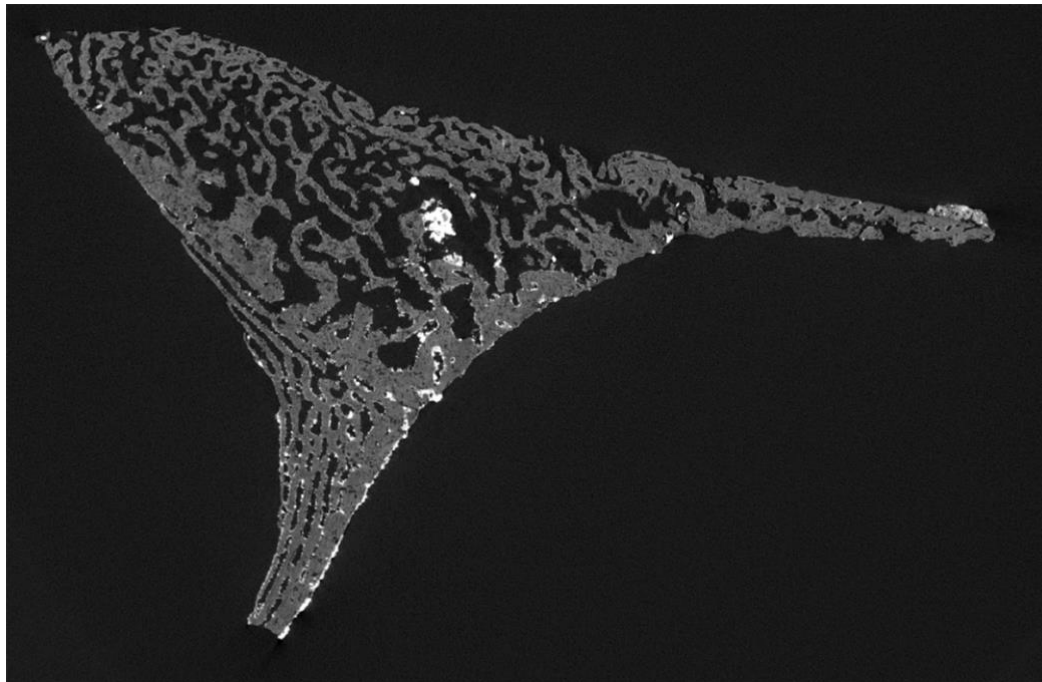


Fig. 5 b μ -CT slice (resolution: 9.7 μ m) showing a vermiculated reorganization of the medullary space; locally the sinuses are oriented perpendicular to the external table, with opening at the external surface as numerous porosities. This pattern reflects *hyperostosis spongiosa cranii* (© H. Coqueugniot)