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First results on the fossilization of Dwarf Hippo skeletal remains from Aghia Napa, Cyprus

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Resum

El 2001 la Universitat d'Atenes va començar l'excavació paleontològica d'un jaciment situat a Aghia Napa, Xipre, en col·laboració amb el Departament de Prospeccions Geològiques de Xipre. Aquest jaciment ha lliurat una rica col·lecció de restes esquelètiques d'hipopòtams nans del Quaternari Superior. L'objecte d'aquest treball rau en estudiar l'estat de conservació dels materials com a resultat de la seva fossilització. El material triat prové de les excavacions realitzades els dos darrers anys i consisteix en ossos i dents. La fossilització sembla haver afectat la histologia del material estudiat, mentre que la cristal·lització i la química sembla que l'han afectat menys. En general, els materials no s'han vist molt afectats pels processos causats per la interacció entre les restes del sistema esquelètic i el sòl.
Paraules clau: Fossilització, ossos, Hipopòtams nans, Aghia Napa, Xipre, Quaternari.

Abstract

During the year 2001, Athens University began palaeontological excavations at a site situated in Aghia Napa, Cyprus in collaboration with the Geological Survey Department of Cyprus. This site has given a rich collection of skeletal remains of Dwarf Hippos from the Upper Quaternary. The object of this paper is to study the state of preservation of the material as a result of fossilization. The material chosen was derived from the excavations realized during the last two years and consists of a number of bones and teeth. Fossilization seems to have mostly affected the histology of our material, while the crystallinity and the chemistry are less affected. In general our material has not been strongly affected by the procedures caused by the interaction of the system skeletal remain-soil.
Keywords: Fossilization, bones, Dwarf Hippopotamus, Aghia Napa, Cyprus, Quaternary.

Introduction

The site

The fossiliferous *Hippopotamus* locality of Aghia Napa was formed when the roof of a natural cave-shelter collapsed on a bone bearing layer. The first excavations at the Aghia Napa locality began in June 2001. It was the first scientifically controlled palaeontological excavation to be realized in Cyprus by a Greek-Cypriot palaeontological team. Since, 4 excavation periods of 2 weeks each have been completed and have brought to light more than 5000 fragmented or complete bones. Eventually, it became possible to uncover a very rich, mostly undisturbed fossiliferous layer that was more than one-meter thick. This layer was full of skeletal remains in excellent state of preservation.

The site includes bones from an endemic hippopotamus that lived on Cyprus during the Upper Quaternary. The first dating attempts by the method C^{14} were negative due to the lack of collagen. (Maniatis, 2003; pers. com.). Absolute dating by other methods is still expected.

More than 50 animals are already documented and a significant percentage of juveniles are present (6-7%). Biometrically the material belongs to one size group, which belongs to the known group of the endemic hippopotamus of Cyprus that is the *Phanourios minor* Group (Boekschoten & Sondaar, 1972; Houtekamer & Sondaar, 1979).

The accompanying fauna includes micromammals, birds and a small carnivore still not completely prepared and studied. A few deer phalanges are present but up to now they have only been found in the disturbed layers.

Disturbance of human origin is found in various areas of the shelter but is very recent. The site has been partly excavated without a scientific methodology about 60-70 years ago, causing significant and irregular disturbance. Otherwise, there is still no evidence to support an extinction of the hippopotamus due to humans during the Late Pleistocene or the earlier stages of Holocene, as it has been suggested from Aetokremnos (Simmons, 1999, 2001).

Fossilization

Fossilization, which is better described as diagenesis, is a highly complex phenomenon that depends on a number of geochemical parameters. It includes all post mortem alterations that eventually lead to the preservation of skeletal material through geological time. It has been intensely studied during the last decades (Piepenbrink, 1989; Hedges & Millard, 1995; Stathopoulou, 2000; Stathopoulou & Theodorou, 2001; Hedges, 2002).

Diagenesis can be recognized by a number of alterations that occur at different stages from the time of death and may concern the organic component of bone, the inorganic mineral fraction in means of recrystallization, uptake of certain cations, ionic exchange etc and the infilling with secondary mineral phases. Microbial activity is also very important, since it seems to lead to the gradual degradation of bone, which is easily interpreted by the alteration or the loss of histological detail.

All hard tissues found in fossils (bone, enamel, dentine) consist of a heterogeneous, complicated dynamic system which consists of a dense framework of organized fibrils of collagen and a type of biological apatite (poorly crystallized hydroxylapatite ($Ca_{10}(PO_4)_6(OH)_2$) which also contains a certain amount of carbonate and thus is called carbonate hydroxylapatite (Posner, 1985; Person *et al.*, 1995). This fact makes it obvious that in order to study diagenesis one must look into all the mentioned procedures in turn and in detail.

The object of this paper is to study the state of preservation of the material as a result of fossilization.

Material and Methods

The material chosen was derived from the excavations realized during the last two years and consists of ten long bones (parts or fragments of femora, tibias, humeri, etc.: AN-10 up to AN-19) and six teeth (two molars: AN-1, AN-4, two canines: AN-2, AN-5, two incisors: AN-6, AN-7). Some of the bone samples may be seen in Fig. 1.

Fig.1. Some of the dwarf hippo bones used in this study.

Fig. 1. Alguns dels ossos d'hipopòtams nans emprats a aquest estudi.

In this paper emphasis is given to the preservation of the internal microstructure of the material, the observation of which was realized via optical and scanning microscopy. Thin sections as well as small fragments of compact bone were prepared in order to be observed by polarizing light microscopy and a JEOL JSM-5600 scanning electron microscope.

Some first information concerning the mineralogical and chemical composition was also reached by X-ray Diffractometry (SIEMENS D500 Diffractometer with secondary graphite monochromator and CuKa radiation) and X-ray microanalysis (OXFORD LINK™ ISIS™ 300-Energy Dispersive X-Ray microanalysis -EDX) while the presence of secondary mineral phases was looked into as well.

The crystallinity index was determined by the XRD technique according to the method proposed by Person *et al.* (1995), in order to make some first speculations on how intense dissolution and recrystallization has been while other important ratios such as Ca/P were estimated to assist us conclude on the fossilization status of our material.

Results and Discussion

Histology

Studying the bone samples through a polarizing microscope, one may observe that the characteristic features of bone (osteons, intersistial and internal-external circumferential lamella, cement lines, lacunae and canaliculi) seem to be quite destroyed with areas of healthy osteons found mostly around the bone marrow cavity. Four zones seem to be present from the marrow cavity outwards. 1. Area of healthy osteons with all characteristic features present (Fig. 2). 2. Area of osteons with few obvious features (Fig. 3, 4). 3. Area of totally destroyed microstructure. 4. Area with remains of osteons. These areas are not present in all samples in this pattern but in general one could say that the histology is quite affected by diagenetic procedures.

The histology index measured according to Hedges & Millard (1995) varies from 1-2 (0: no original histology visible, 5: <5% affected, histology identical to fresh bone).

Observation with the SEM revealed further details concerning microstructure where it was still present, such as the lacunae and canaliculi and the structure of lamella, the network of the haversian canals, their internal surfaces, the crystals that are found within etc. We were able to observe generally few healthy areas of bone (Fig. 5, 6) and most areas revealed remnants of basic microstructure, mostly due to the very intense microbial activity. The usual image of these bones under the SEM was that of a complicated network of voids, tunnels and areas of intact bone.

The microstructure of dentine and enamel does not show any alterations due to diagenesis as expected and all characteristic features are present. One may observe the Hunter-Schreger lines in enamel (Fig. 7, 8), which characterize hippo teeth and also the enamel prisms and their characteristic patterns (Fig. 9). Dentinal tubules are seen in dentine with no evidence of diagenetic alterations concerning histology (Fig. 10, 11).

Fig. 2 Area of bone with well-preserved osteons, as observed via optical microscopy.

Fig. 2. Àrea d'os amb osteons ben conservats, tal com s'observen mitjançant microscopia òptica.

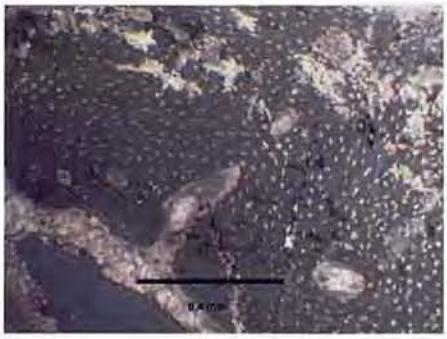

Fig. 3. Lacunae as seen in healthy osteons (optical microscopy).

Fig. 3. Lacunae tal com s'observen als osteons sans (amb microscopia òptica).

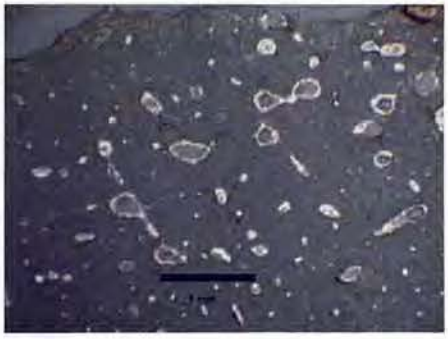

Fig. 4. Area of compact bone with only the haversian channels present as it was seen through the optical microscope.

Fig. 4. Àrea d'ós compacte amb només els canals haversians present, tal com s'observa mitjançant microscopia òptica.

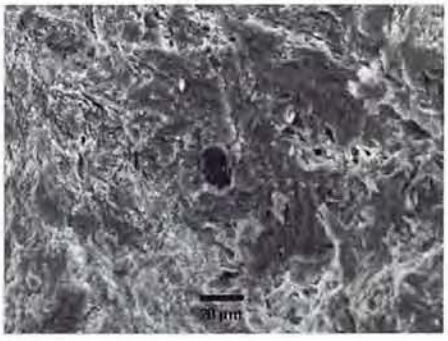

Fig. 5. One of the osteons to be seen under the SEM.

Fig. 5. Un dels osteons a veure mitjançant el SEM.

Fig. 6. Lamella as seen in healthy osteons under the SEM.

Fig. 6. Lamella tal com es veuen als osteons sans mitjançant el SEM.

Microbial attack is a common phenomenon in fossil bones and is produced by a variety of microorganisms (bacteria, cyanobacteria, fungi and protozoans of amoebic type) (Hackett, 1981; Soudry & Nathan, 2000). Bone tunneling is commonly considered as the result of the dissolution of the mineral and organic matrices of the bone by fungal metabolic acids (Piepenbrink, 1989).

Microbial activity is present in all bone samples but not in any tooth samples. This activity is found as voids and canals of relatively small diameter (about 0,5 microns). The activity is found in the form of small gatherings or total coverage of the samples (Figure 12-14), while one may see early stages of activity as well as totally destroyed areas or secondary activity amongst the voids of a previous occurrence. The organisms that have caused the characteristic images are still not well defined, but the size of the produced voids leads us to believe that they could be bacteria or fungi.

Infilling of voids

The only secondary mineral phase present in voids of our samples is Calcite. It is found in all bone samples, in some dentine samples (from canines and small amounts in molars) but in no enamel samples. Calcite is found in fractures, voids left by the decomposition of organic matter, such as the haversian canals, the lacunae etc, the marrow cavity. The crystals differ according to the size and shape of the void-fracture in which they are found.

Dissolution and recrystallization

Crystallinity refers to the degree of order within the crystal lattice. A quantitative estimation of crystallinity for bone apatites from their XRD spectra is given by the crystallinity index (C.I.), which was estimated according to Person *et al.* (1995). The C.I. of the biological apatites studied vary between 0,13 and 1,36 (Table 1, 2).

Fresh bone theoretically gives 0,00. The closest values to that are given by our dentine samples, while bone lies between the value of fresh bone and the C.I. of enamel samples. The bone values vary between 0,17 and 0.5 and give an average of 0,3, dentine does not seem to differ amongst the different types of teeth and gives an average of 0,15, while enamel seems to present higher values in canines than molars. Enamel consists of a totally crystalline apatite and gives an average of 1,2.

Chemical Composition changes

The bone and teeth material consist of Carbonate hydroxylapatite and there are no significant signs of F present. There is a differentiation of the Ca/P ratios, and possible ionic exchanges with the environment have also occurred. The average chemical composition of all samples can be found in Table 3 & 4. One may observe that the most common elements apart from Ca and P are Si, S and Na, K. Enamel is also richer in Cl. The Ca/P ratio for fresh bone is 2,14 and for the mineral Hydroxylapatite 2,16. The ratios measured in our bone samples are quite higher than 2,14; varying between 2,38 and 2,80 and giving an average of 2,5. Dentine gave a Ca/P of 2,39 and enamel an average of 2,13, which is very close to fresh bone.

	AN-10	AN-11	AN-12	AN-13	AN-14	AN-15	AN-16	AN-17	AN-18	AN-19	AVERAGE
C.I. (bones)	0.50	0.30	0.35	0.30	0.29	0.26	0.17	0.32	0.27	0.25	*0.3*

Table 1. Average Crystallinity indexes of studied bone samples.

Taula 1. *Indexs de cristal·linitat promig de les mostres estudiades d'ossos.*

Conclusions

Bone shows serious diagenetic changes, especially in terms of histology. Microbial activity is abundant and has caused the partial destruction of its structure. Enamel is the hardest tissue and shows great endurance during fossilization. On the other hand, dentine is more like bone and more vulnerable to diagenetic procedures. Often there is microbial activity in dentine, similar to bone, though not in our case (Stathopoulou *et al.*, in press). Enamel has not shown any such forms.

In means of crystallinity enamel is closest to the mineral hydroxylapatite. On the contrary dentine has behaved more like bone, since it is closer to bone concerning its mineral and organic contents and crystal size.

Contamination of bone in the ground takes both physical and chemical forms. The porous structure of bone tissue is susceptible to infiltration by foreign materials. The contaminants result from either precipitation from groundwater or physical incorporation of materials in the bone. Ca, for example can be introduced by the precipitation of Calcite in ground water. Inclusions, such as quartz are included in bone as solid grains. Na and Mg are an associated pair that is present due to metabolic and dietary factors and tend to leach out through time while Al, Mn, Fe, Mg may come from enrichment from soil contaminants (Price *et al.*, 1992). The average Ca and P values in most of our bone samples are higher and lower than those in fresh bone respectively and their ratio could indicate an increase in Ca due to the intrusion of Calcite in the bone voids or/and a loss of P perhaps due to its depletion.

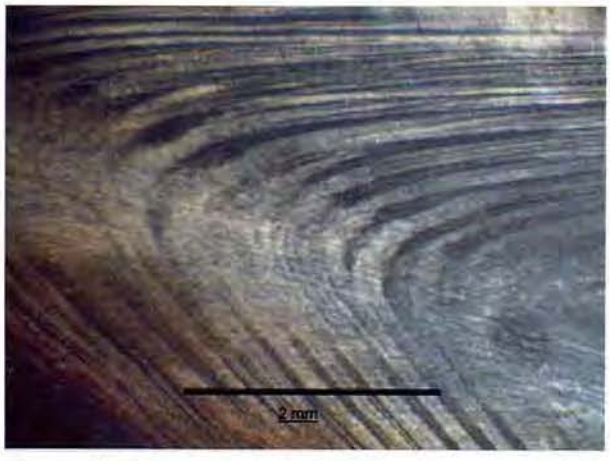

Fig. 7. Longitudinal section of an incisor, observed through optical microscopy. One may observe the Hunter-Schreger lines.

Fig. 7. *Secció longitudinal d'una incisiva, observada mitjançant microscopia òptica. Es poden observar les línies de Hunter-Schreger.*

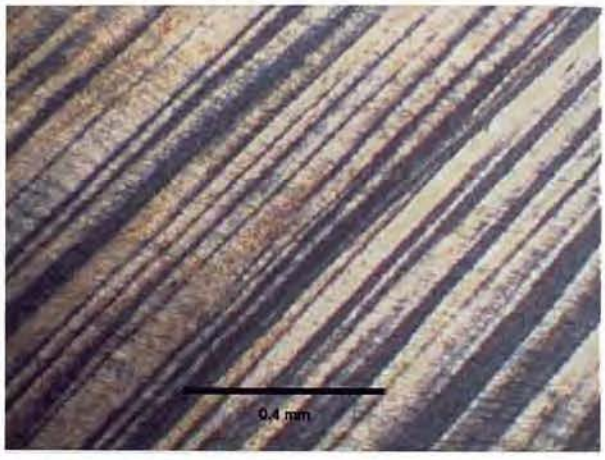

Fig. 8. Transverse section of an incisor (optical microscopy). One may observe the Hunter-Schreger lines in the enamel.

Fig. 8. *Secció transversal d'una incisiva, observada mitjançant microscopia òptica. Es poden observar les línies de Hunter-Schreger a l'esmalt.*

Fig. 9. Enamel prisms as they appear in a transverse section of a molar (SEM images).

Fig. 9. *Prismes d'esmalt tal com apareixen a una secció transversal d'un molar (imatges del SEM).*

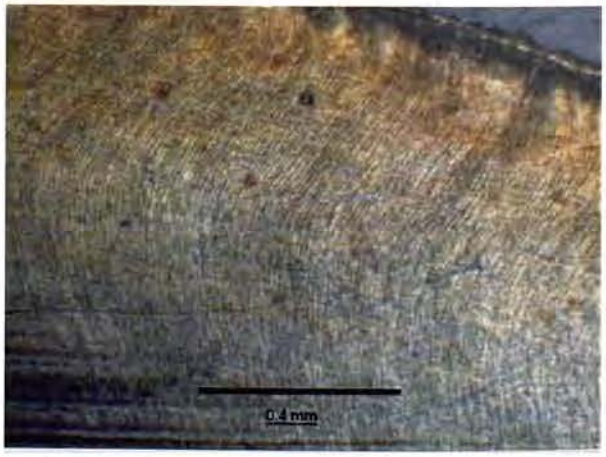

Fig. 10. Longitudinal section of a canine, one may observe the primary curvatures of the dentinal tubules (optical microscopy).

Fig. 10. *Secció longitudinal d'una canina. Es poden observar les corbatures primàries dels túbuls dentinals (mitjançant microscopia òptica).*

C.I. *average*	molars	canines	incisors	Average value
dentine	0.16	0.16	0.13	*0.15*
enamel	1.05	1.36	Not measured	*1.2*

Table 2. Average Crystallinity indexes of studied dentine and enamel samples.

Taula 2. *Indexs de cristal-linitat promig de les mostres d'esmalt i dentina estudiades.*

Most bone and dentine voids are filled with Calcite, making the material compact and strong.

As we have already mentioned this is only a preliminary study and further work is to be realized in the near future. Based on these first results, one may say that diagenesis in Aghia Napa appears mostly as a destruction of histology in bones, and a few changes in the chemistry of our material and the crystallinity. Compared to bone material from various Greek sites one may observe that the C.I. and the Ca/P are quite lower. This means that the material from Aghia Napa is less crystalline and closer to fresh bone, and less affected by Ca increase and P depletion. In few words, less affected by the procedures caused by the interaction of the system skeletal remain-soil.

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Fig. 11. Dentinal tubules as they are seen in a fragment of tooth dentine (SEM image).

Fig. 11. *Túbuls de dentina com es veuen a un fragment de dentina de dent (miatge SEM).*

Fig. 12. Microbial activity in bone (SEM image).

Fig. 12. *Activitat microbiana a un os (imatge SEM).*

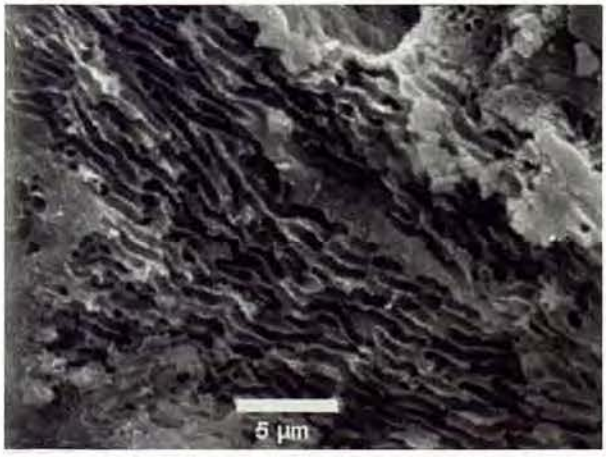

Fig. 13. Microbial activity, found as canals (SEM image).

Fig. 13. *Activitat microbiana, trobada com canals (imatge SEM).*

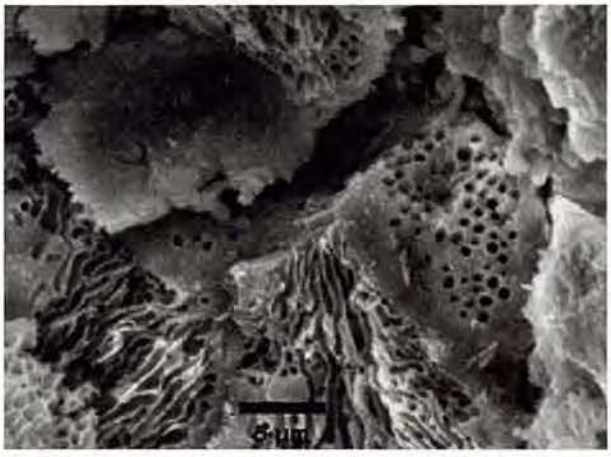

Fig. 14. Microbial activity as a combination of round voids and canals (SEM images).

Fig. 14. *Activitat microbiana com una combinació de buits arrodonits i canals (imatges SEM).*

	AN-10	AN-11	AN-12	AN-13	AN-14	AN-15	AN-16	AN-17	AN-18	AN-19	AVERAGE
O	38.7	37.7	37.0	36.5	38.9	39.3	39.6	39.8	38.8	39.3	*38.6*
Na	0.1	0.4	0.1	-	0.3	0.6	0.5	0.1	0.5	0.5	*0.3*
Mg	0.1	-	-	-	-	0.1	0.2	0.1	0.2	-	*0.1*
Al	-	-	-	-	0.2	0.1	0.2	0.1	0.3	0.1	*0.1*
Si	0.3	0.2	0.4	0.2	0.3	0.2	0.3	0.2	0.6	0.3	*0.3*
P	17.7	17.5	17.8	17.6	17.3	16.4	16.9	17.2	15.3	16.2	*17.0*
S	0.2	0.4	0.2	0.4	0.3	0.2	0.1	0.1	0.3	0.3	*0.3*
K	0.1	0.1	0.2	-	-	-	0.1	0.1	0.1	0.1	*0.1*
Cl	-	-	-	-	-	-	-	-	0.1	0.1	-
Ca	41.9	41.8	43.0	42.8	41.3	42.7	42.0	41.9	43.1	42.7	*42.3*
Ca/P	2.38	2.39	2.42	2.44	2.39	2.6	2.49	2.44	2.8	2.64	*2.5*

Table 3. Average Chemical composition and Ca/P ratios of studied bone samples. Values in percentages.

Taula 3. *Composició química promedi i proporcions Ca/P de les mostres estudiades d'ossos. Dades expressades en percentatges.*

	dentine				enamel		
	molars	*canines*	*incisors*	*AVERAGE*	*molars*	*canines*	*AVERAGE*
O	42.24	41.8	41.5	*41.8*	42.5	40.2	*41.7*
Na	0.4	0.5	0.3	*0.4*	0.5	0.5	*0.5*
Mg	<0.1	-	-	*<0.1*	0.1	-	*<0.1*
Al	<0.1	-	0.1	*<0.1*	0.1	-	*<0.1*
Si	0.2	-	0.1	*0.1*	0.2	0.2	*0.2*
P	16.4	16.1	16.6	*16.4*	17.9	18.0	*18.0*
S	0.3	0.3	0.3	*0.3*	0.2	0.1	*0.2*
Cl	-	0.2	-	*<0.1*	0.3	0.3	*0.3*
K	-	-	<0.1	*<0.1*	0.1	0.2	*0.2*
Ca	39.3	38.3	39.6	*39.2*	37.7	39.4	*38.0*
Fe	-	-	-	-	0.1	-	*<0.1*
Ca/P	2.4	2.39	2.39	*2.39*	2.1	2.18	*2.13*

Table 4. Average Chemical composition and Ca/P ratios of studied tooth samples. Values in percentages.

Taula 4. *Composició química promig i proporcions Ca/P de les mostres estudiades de dents. Dades expressades en percentatges.*

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