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Bone lesions in a Late Pleistocene assemblage of the insular deer *Candiacervus* sp.II from Liko cave (Crete, Greece)

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ABSTRACT

Candiacervus sp.II is one of the deer species that inhabited the island of Crete during the Late Pleistocene. The species evolved on the island under a prolonged period of isolation and, as a consequence, developed a high degree of endemism. Fossils of this species have been discovered at many Cretan sites, including Liko cave (an attritional accumulation of several thousand fossils). In this paper, we present the results of a systematic analysis of the prevalence and anatomical distribution of bone lesions of *Candiacervus* sp.II, from that cave. We identified one metapodial with a healed fracture and nine (various) specimens with moderate to severe degenerative lesions of osteoarthritis. The lesions were evaluated macroscopically and radiographically, and they were classified as traumatic or degenerative. Degenerative lesions that affected adult individuals had prevalence rates below 5% and were attributed to environmental or nutritional causes. Representative bones were sampled for histological evaluation, to provide essential baseline data on possible underlying disorders. The aims of this study are to provide evidence for bone disease contributing to species morbidity, and to shed new light on causes and potential palaeoecological significance.

1. Introduction

During the Pleistocene, many Eurasian islands harbored at least one species of endemic ungulate (mostly deer or bovids) or subungulate (elephants). There are many important studies on the phylogeny, anatomy, life history, and development of insular ungulates (Van der Geer et al., 2010, an overview). However, studies of their palaeopathology are few (Maempel, 1993; Waldren, 1999; Jordana and Kohler, 2011; Palombo and Zedda, 2016). The study of ungulate bone lesions and their interpretation may help our understanding of island ecosystems, as well as elucidate life histories and behavior of extinct animals (Rothschild and Martin, 1993).

Particularly in the case of insular ungulates, Pleistocene island populations were quite different from those living on the same islands today. Most ungulates living on present-day islands are the result of relatively recent (usually Holocene) colonization or culture-related introduction (Long, 2003; Massetti, 2012). Although some of these new introductions exhibit genetic and morphologic divergence from mainland populations, they are still at initial stages of adaptation to insular conditions. The ungulates that inhabited the pre-Holocene islands evolved under prolonged isolation, and evolved significant morphological (and presumably genetic and behavioral) divergence from their mainland relatives.

Crete was an island throughout the entire Pleistocene. Mammals could reach it only via overseas dispersal (Van der Geer et al., 2015), thus profoundly affecting the faunal composition. Only a few mammalian species colonized Crete and evolved a high degree of endemism. During the Late Pleistocene, the mammalian fauna of Crete consisted only of a dwarf elephant (*Palaeoloxodon creutzburgi*), a mouse (*Mus minotaurus*), a shrew (*Crocidura zimmermanni*), an otter (*Lutrogale cretensis*), and several deer species (*Candiacervus* spp.) (Van der Geer et al., 2010).

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In this study, we present results of a systematic macroscopic, radiographic, and histologic analysis of the postcranial bones of the Cretan deer species, *Candiacervus* sp.II. The study is based on a collection from Liko cave. This species was chosen for being a typical insular form (Van der Geer et al., 2006) and because it has a very good representation in the fossil record. The material from Liko was chosen because is the largest available collection of *Candiacervus*. Given the limited knowledge of the role of diseases in insular ungulate populations, the aims of this study are to provide evidence of species morbidity and to shed new light on causes and potential palaeoecological significance.

2. Materials and methods

2.1. Taxonomic arrangement

The Cretan deer were represented by eight morphotypes, distributed over six size classes (sizes I through VI) (Fig. 1) of postcranial material (De Vos, 1979, 1984, 1996, 2000). In one taxonomic scheme, the two smallest size classes (I, II) are defined as species *Candiacervus ropalophorus* and *Candiacervus* sp.II (De Vos, 1979). In other taxonomies, the two smallest sizes are lumped together into *Megaceros ropalophorus* (Capasso Barbato, 1989) or *Megaceroides "ropalophorus"* (Caloi and Palombo, 1996). In this contribution, the taxonomy of De Vos (1996) is followed because 95.3% of the material from Liko cave belongs to size II. This size is commonly referred to as *Candiacervus* sp.II (De Vos, 1996; Van der Geer et al., 2006, 2010; Van der Geer, 2014; Kolb et al., 2015).

2.2. Liko cave and studied material

The study material comes from Liko cave, a small coastal cave 3 m above sea level, formed within breccia (Fig. 2). The cave was excavated systematically in the early 1970s by teams from the University of Utrecht. The material collected during 1973, 1974, and 1975 from Liko comes from the uppermost 75 cm of the cave deposits. Since 1975 material has been collected from trenches down to 1.50 m (De Vos, 1979). Because distinct layers could not be recognized stratigraphically at Liko, the collectors dug according to artificial units (A, B, C, D, and E) based on measured depths from surface. The majority of deer fossils came from units B and C (Van der Geer et al., 2014).

After excavation, the fossils were transferred to the University of Utrecht (Netherlands), where they were prepared and catalogued; in 2002 they were returned to Greece. Today, the collection is curated at the Museum of Palaeontology and Geology of the University of Athens (AMPG). It consists of more than 6000 specimens, making it the largest collection of Cretan deer from a single site.

Practically all material consisted of disarticulated bones; no complete skeletons were found (Van der Geer et al., 2006). The *in situ* presence of some articulated limbs and the absence of sedimentary abrasion on the bones suggests limited translocation prior to deposition. Based on the fauna, the time period of the deposition is considered to be Late Pleistocene (Mayhew, 1977; Van der Geer et al., 2013). There are only two absolute dates, both based on Amino Acid Racemization (AAR). AAR age estimates range from 105,000 yr ± 20% (units B and C) to 87,000 yr ± 20% (unit D) (Reese et al., 1996).

According to De Vos (1979), it is possible to distinguish 4 size groups within the material from Liko, namely the size groups II, III, IV, and VI, of which size II is by far the most common (95.3%). In size group III three specimens have been found. Group IV is represented by 14 specimens, while from the largest deer (size VI) only 2 fragments have been found. Size groups I and V are not represented in Liko (De Vos, 1979), and almost all the studied material is of size group II (Fig. 1). For this study, we included only specimens belonging to fully grown individuals, as indicated by epiphyseal closure. In total, 3955 bones and bone fragments were examined macroscopically. Further assessment and diagnosis of bone lesions was performed by additional direct observation, radiography, and histology.

2.3. Paleohistology of Candiacervus sp.II adult bones

Eleven representative long bones from adult individuals, having the same macroscopic morphometric characteristics as pathological bones, were examined histologically. These bones were two femora, three antebrachii (radium and ulna fused), two metacarpi, and four metatarsi. Bones were sectioned transversely at mid-shaft and were coated and impregnated with acrylic resin (Technovit 7200 VLC, Heraeus Kulzer, Friedrichsdorf, Germany) prior to cutting and grinding (Chinsamy-Turan and Hurum, 2005; Lamm, 2013; Kolb et al., 2015).

Bone sections were observed in normal transmitted light using a Nikon upright microscope D-Eclipse 80i C1, equipped with a Nikon digital camera DS-Fi1, at magnifications: × 4 Plan objective (numerical aperture [NA] 0.10); × 10 Plan objective (numerical aperture [NA] 0.25); × 20 Apo Plan objective (numerical aperture [NA] 0.75); and × 40 Plan Fluor objective (NA 1.30). Sections were assessed for: (1) bone tissue type; (2) skeletochronology, based on growth mark analysis; (3) specific anatomical features, such as micro-fractures; and (4) bone histomorphometry. For skeletochronology, we used quantification of the growth rate [annual growth rate with an estimated mean growth period of 260 days] as explained by Kolb et al. (2015) and based on other reports (Kohler et al., 2012; Marin-Moratalla et al., 2012). Only femoral growth rate will be discussed because Kolb et al. (2015) considered the femur to be the most informative bone in cervids. Growth zone measurements were taken in the cranial region of the bones, to remain consistent with Kolb et al. (2015).

Bone histomorphometry was performed according to Parfitt et al. (1987) and Dempster et al. (2012). The following parameters were estimated by the computer-assisted image analysis system Image pro plus software (Media Cybernetics, Inc., Silver Spring, Maryland 20910, USA): (1) bone diameter (B.Dm); (2) cortical width (Ct.Wi); (3) marrow diameter (Ma. Dm); (4) width of outer (OCF) and inner circumferential lamellae (ICF) and middle cortex, and their relative proportions; (5) vascular canal orientation and type; (6) relative cortical bone porosity (or vascular density); (7) vascular canal size; (8) diameter of primary and secondary osteons; (9) void osteocytic lacunae density or index (number of void osteocytic lacunae/bone area); and (10) void osteocytic lacunae surface (% bone area).

All data were analyzed using the SPSS version 20.0 statistical software (IBM Corp., Armonk, NY). Quantitative data were pooled to obtain the mean ± SE for each parameter, and independent samples *T*-test were used for comparisons between groups. Differences were deemed statistically significant at $P < 0.05$.

3. Results

3.1. Lesion prevalence rates

Bones with pathology all belonged to adult individuals, denoted by complete epiphyseal fusion. Pathological findings were classified as traumatic or degenerative. We identified one metatarsal with a healed fracture (AMPG 543-Li929) and six bones with severe degenerative changes: two acetabula (AMPG 544 and AMPG AMPG 1045), two femurs (AMPG 540-Li356 and AMPG 541-Li1331), one metacarpal (AMPG 542-Li816) and one calcaneus (AMPG 549).