



Naturalis Repository

## Analysis of coprolites from the extinct mountain goat *Myotragus balearicus*

Frido Welker, Elza Duijm, Kristiaan J. van der Gaag, Bas van Geel, Peter de Knijff, Jacqueline van Leeuwen, Dick Mol, Johannes van der Plicht, Niels Raes and Jelle Reumer

Downloaded from:

<http://dx.doi.org/10.1016/j.ygres.2013.10.006>

### Article 25fa Dutch Copyright Act (DCA) - End User Rights

This publication is distributed under the terms of Article 25fa of the Dutch Copyright Act (Auteurswet) with consent from the author. Dutch law entitles the maker of a short scientific work funded either wholly or partially by Dutch public funds to make that work publicly available following a reasonable period after the work was first published, provided that reference is made to the source of the first publication of the work.

This publication is distributed under the Naturalis Biodiversity Center 'Taverne implementation' programme. In this programme, research output of Naturalis researchers and collection managers that complies with the legal requirements of Article 25fa of the Dutch Copyright Act is distributed online and free of barriers in the Naturalis institutional repository. Research output is distributed six months after its first online publication in the original published version and with proper attribution to the source of the original publication.

You are permitted to download and use the publication for personal purposes. All rights remain with the author(s) and copyrights owner(s) of this work. Any use of the publication other than authorized under this license or copyright law is prohibited.

If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the department of Collection Information know, stating your reasons. In case of a legitimate complaint, Collection Information will make the material inaccessible. Please contact us through email: [collectie.informatie@naturalis.nl](mailto:collectie.informatie@naturalis.nl). We will contact you as soon as possible.



## Analysis of coprolites from the extinct mountain goat *Myotragus balearicus*



Frido Welker<sup>a,b,\*</sup>, Elza Duijm<sup>a</sup>, Kristiaan J. van der Gaag<sup>c</sup>, Bas van Geel<sup>d</sup>, Peter de Knijff<sup>c</sup>,  
Jacqueline van Leeuwen<sup>e</sup>, Dick Mol<sup>f</sup>, Johannes van der Plicht<sup>g</sup>, Niels Raes<sup>a</sup>,  
Jelle Reumer<sup>f,h,i</sup>, Barbara Gravendeel<sup>a,j,\*\*</sup>

<sup>a</sup> Naturalis Biodiversity Center, Sylviusweg 72, 2333 BE Leiden, The Netherlands

<sup>b</sup> Faculty of Archaeology, Leiden University, Reuvensplaats 3, 2300 RA Leiden, The Netherlands

<sup>c</sup> Forensic Laboratory for DNA Research, Department of Human Genetics, Leiden University Medical Center, The Netherlands

<sup>d</sup> Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam, Science Park 904, 1090 GE Amsterdam, The Netherlands

<sup>e</sup> University of Bern, Institute of Plant Sciences, Altenbergrain 21, CH 3013, Bern, Switzerland

<sup>f</sup> Natuurhistorisch Museum Rotterdam, Westzeedijk 345, 3015 AA, Rotterdam, The Netherlands

<sup>g</sup> Energy and Sustainability Research Institute, Nijenborgh 4, 9747 AG, Groningen, The Netherlands

<sup>h</sup> Balearic Museum of Natural Sciences, Palma-Port de Sóller road, km 30, apartment 55, 07100 Sóller, Mallorca, Spain

<sup>i</sup> Department of Earth Sciences, Utrecht University, Budapestlaan 4, 3584 CD, Utrecht, The Netherlands

<sup>j</sup> University of Applied Sciences Leiden, J.H. Oortweg 21, 2333 CH Leiden, The Netherlands

### ARTICLE INFO

#### Article history:

Received 21 May 2013

Available online 13 November 2013

#### Keywords:

Ancient DNA

Balearic Islands

Diet

DNA barcoding

Ecological niche modelling

### ABSTRACT

Humans colonized the Balearic Islands 5–4 ka ago. They arrived in a uniquely adapted ecosystem with the Balearic mountain goat *Myotragus balearicus* (Bovidae, Antilopinae, Caprini) as the only large mammal. This mammal went extinct rapidly after human arrival. Several hypotheses have been proposed to explain the extinction of *M. balearicus*. For the present study ancient DNA analysis (Sanger sequencing, Roche-454, Ion Torrent), and pollen and macrofossil analyses were performed on preserved coprolites from *M. balearicus*, providing information on its diet and paleo-environment. The information retrieved shows that *M. balearicus* was heavily dependent on the Balearic box species *Buxus balearica* during at least part of the year, and that it was most probably a browser. Hindcast ecological niche modelling of *B. balearica* shows that local distribution of this plant species was affected by climate changes. This suggests that the extinction of *M. balearicus* can be related to the decline and regional extinction of a plant species that formed a major component of its diet. The vegetation change is thought to be caused by increased aridity occurring throughout the Mediterranean. Previous hypotheses relating the extinction of *M. balearicus* directly to the arrival of humans on the islands must therefore be adjusted.

© 2013 University of Washington. Published by Elsevier Inc. All rights reserved.

### Introduction

The Balearic Islands are an archipelago in the Western Mediterranean Sea (Fig. 1). Their isolated position may have probably played an important role in the late arrival of humans when compared to other Mediterranean islands (Bover et al., 2008). Both the time of arrival and the archaeological culture associated with the first human inhabitants have been debated since the start of archaeological studies on the Balearics (Waldren, 1982; Guerrero, 2001; Calvo and Guerrero, 2002; Ramis et al., 2002; Alcover, 2008). In the present study we follow a

conservative view of the available radiocarbon dates, and argue that human settlement on the island must have occurred between 5000 and 4050 <sup>14</sup>C yr BP (Fig. 2; Ramis et al., 2002; Alcover, 2008). This time span is based upon radiocarbon dates taken from charcoal samples, and has been established after reviewing the reliability of the available radiocarbon dates (Ramis et al., 2002). The established time span can be reduced to 4350–4150 <sup>14</sup>C yr BP (Alcover, 2008) when cultural indicators, or their absence, and comparison to the cultural chronologies of nearby mainland Spain and France are taken into consideration. This approach is not followed here, as this shorter time span, 4350–4150 <sup>14</sup>C yr BP, is based on relative dating, and therefore not supported by a confidence of  $p > 95.4\%$  (as is the case with the 5000–4050 <sup>14</sup>C yr BP interval). Also, the shorter time span is dependent on chronologies established in other regions, each with their own chronological problems (Alcover, 2008). This adds further uncertainties to the use of these shorter chronologies.

Some ecological information is available for the period when humans arrived on Mallorca. After the separation of the Balearics from

\* Correspondence to: F. Welker, Faculty of Archaeology, Leiden University, Reuvensplaats 3, 2300 RA Leiden, The Netherlands.

\*\* Correspondence to: B. Gravendeel, Naturalis Biodiversity Center, Sylviusweg 72, 2333 BE Leiden, The Netherlands.

E-mail addresses: [frido.welker@umail.leidenuniv.nl](mailto:frido.welker@umail.leidenuniv.nl) (F. Welker), [barbara.gravendeel@naturalis.nl](mailto:barbara.gravendeel@naturalis.nl) (B. Gravendeel).

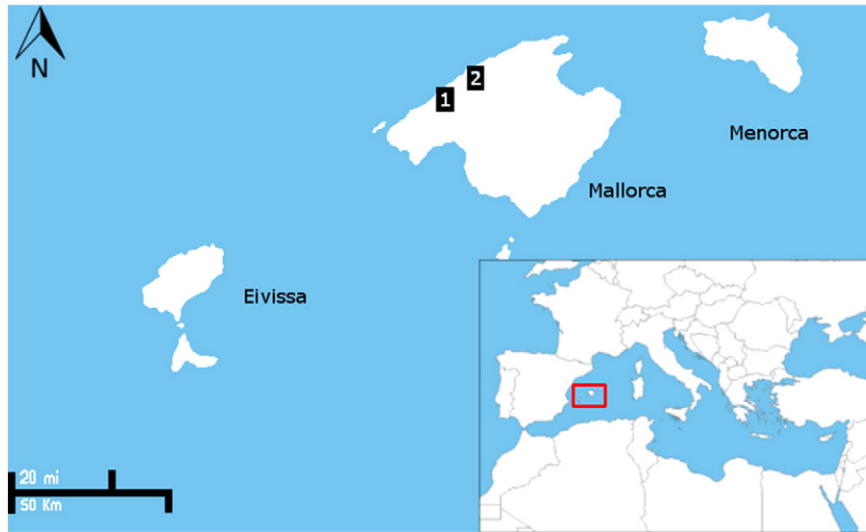


Figure 1. Location of the Balearic Islands in the Mediterranean Sea. Numbers refer to location of samples studied (1 = Balma de Son Matge; 2 = Cova de Muleta).

the mainland, about 5.3 Ma (Krijgsman et al., 1999), a unique combination of flora and fauna developed on the different islands (Bover et al., 2008, 2010; Quintana et al., 2011) for an overview of the faunal developments). Differences between the islands were present during parts of the last 5.3 Ma, although Mallorca and Minorca have shared the same biogeographic history since the beginning of the Holocene. Vertebrates present on Mallorca included mammals (*Myotragus balearicus*, *Hypnomys morpheus* and *Nesiotites hidalgo* and some bats), two amphibian species, a lizard and some birds (Bover et al., 2008). *Myotragus balearicus* is considered to be the key species in understanding Balearic paleoecology, mainly because of its size when compared to the other vertebrates present on the islands. Environmental conditions changed dramatically during the period of human colonization of the islands, leading to the extinction of endemic mammals (Bover and Alcover, 2003, 2008), the disappearance of existing vegetation composition (Yll et al., 1997), and the introduction of new mammals and plants by humans (Alcover, 2008) (Fig. 2).

Remains of *M. balearicus* were first discovered in 1909 (Bate, 1909). This extinct mountain goat developed special adaptations, probably both as an evolutionary reaction to a resource limited environment as well as to within-species competition (Winkler, 2010; Jordana and Köhler, 2011). The adaptations found in *M. balearicus* evolved during a

successive series of six chronospecies (Moyà-Solà et al., 2007; Bover et al., 2008, 2010), starting after the separation of the Balearic Islands from the mainland of Spain (Krijgsman et al., 1999).

Three different sets of adaptations can be discerned. These adaptations initially included morphological changes. The first of these was a diminishing in size. Size estimates differ, but the largest individuals were not taller than 50 cm (Ramis and Bover, 2001). Body mass estimates range between an average of 30 kg to a maximum of 70 kg (Bover and Alcover, 1999a; Palombo et al., 2008). Second, a further series of morphological changes observed in the limbs suggests the animal “reduced the energy costs of locomotion by adopting a slow and powerful walking gait with reduced step length” (Palombo et al., 2008, 162). Third, additional information obtained from long bones showed that *M. balearicus* had a delayed maturity, with reproduction beginning late in the animal’s lifetime (Köhler and Moyà-Solà, 2009).

A relatively strong reduction of brain size when compared with the body size reduction has been observed in *M. balearicus* as well; especially a reduction of the cerebellum, including the vision, auditory and locomotory senses (Palombo et al., 2008, 2013). These relative reductions may be related to the predator-free environment, in which energy otherwise needed for predator avoidance can, by reducing the costs of locomotor abilities and vision, be used for other energy costs (Palombo et al., 2008). Adding to this, there was a more frontal positioning of the eye orbits compared with modern bovids. This reduced the width of the visual field (needed for predator avoidance), but may have given *M. balearicus* a greater form of visual depth (Bover, 2004).

A third adaptation observed in *M. balearicus*, but also evident in the earlier species of the lineage, is the evolution of its dentition. Dental evolution in *Myotragus* probably started with a dentition similar to that of extant continental bovids (the dental formula for *M. antiquus*, the first species for which a dental formula could be established) and resulted in a dental formula for *M. balearicus* unique for bovidae. The only present incisor in adult specimens of *M. balearicus* is the  $dl_2$  that replaces the  $dl_1$  by a horizontal process (Bover and Alcover, 1999b). Both teeth are present in juvenile specimens but located in different alveoli, separated by a bony septum (Bover and Alcover, 1999b). In juveniles, the  $dl_2$  is not yet erupted (Bover and Alcover, 1999b). Furthermore, the  $dl_2$  is ever-growing and, together with an overall increase in the degree of hypsodonty of the teeth in general, this has been related to an increased abrasiveness of the diet consumed (Bover and Alcover, 1999b; Winkler, 2010; Winkler et al., 2013). Crown formation times of molars indicate a slow rate of crown formation,

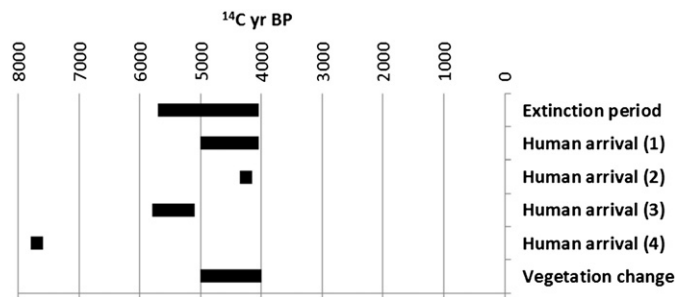


Figure 2. Overview of the most important periods in early Mallorcan prehistory. Extinction period of *Myotragus balearicus*, 5700–4050  $^{14}C$  cal yr BC, as established by Bover and Alcover (2003). Human arrival periods are shown according to different models: (1) 5000–4050  $^{14}C$  yr BP, the longer timespan as followed here, after Ramis et al. (2002) and Alcover (2008), (2) 4350–4150  $^{14}C$  yr BP, the shorter time span, after Alcover (2008), (3) 5800–5100  $^{14}C$  yr BP, after Calvo and Guerrero (2002), and (4) 7800–7600  $^{14}C$  yr BP, after Waldren et al. (2002). Vegetation change, 5000–4000  $^{14}C$  yr BP, after Yll et al. (1997). (See referenced articles for specific radiocarbon details).

**Table 1**  
Overview of the samples analysed in this study.

Sample	Site	Age of coprolite	Altitude (m)	State of preservation	Source	Collection
Sample 1	Area around Balma de Son Matge	Modern	250–300	Organic contents, fibres	Waldren, J.D.	Natural History Museum Rotterdam. Coprolites collected around Balma de Son Matge during excavations in the 70's/80's.
Sample 2	Cova de Son Muleta	4905 ± 40 <sup>14</sup> C yr BP	100–200	Organic contents	Waldren, W.H.	Personal collection of D. Mol, acquired through P. Sondaar.
Sample 3	Balma de Son Matge	Unknown	250–300	Oxidised	Waldren, J.D.	Deià Archaeological Museum.

which has been related to an increased hypsodonty of the teeth (Jordana and Köhler, 2011). This, together with the delayed growth patterns in long-bone histology, suggests that *Myotragus* developed a slow life history, caused by density-dependent resource limitation (Jordana and Köhler, 2011).

A relationship between *Myotragus* and mainland archaic Caprini such as the Vallesian Spanish Aragoral mudejar and the Turolian/Ruscian Greek species *Norbertia hellenica* is discussed in Bover et al. (2010) and Palombo et al. (2013). According to these authors several features are shared between *Myotragus* and these continental taxa. Genetic analysis revealed, in a successive series of papers (Lalueza-Fox et al., 2000, 2002, 2005; Ramirez et al., 2009) that *M. balearicus* is most probably related to *Ovis* (sheep).

The extinction of *M. balearicus*, and the other endemic fauna of the Balearics (Bover and Alcover, 2008) are a debated topic and relevant for the archaeological research on the islands. More knowledge about the drivers of extinction would allow an evaluation of the impact of prehistoric human arrival on their environment. Numerous theories have been proposed to explain the extinction mechanisms involved. These can be divided in two groups. The first theory involves climate and environmental changes. Several hypotheses have been postulated within this first group. First, gradual climatic changes observed throughout the Mediterranean caused increased desiccation and lead to extinction of *M. balearicus* (Yll et al., 1997; Lull et al., 1999). Second, vegetation changes influenced by human activity on the islands, such as the introduction of cattle and clearing of land for agriculture, lead to the extinction of *M. balearicus* (Bover and Alcover, 2003). The second theory involves direct human influence as the cause of these extinctions. Several hypotheses have been postulated within this second group. According to the first of these hypotheses, a so-called *Blitzkrieg* has been proposed wherein direct human predation may have caused the extinction of *M. balearicus* (Martin, 1984). Second, a 'hyperdisease' may have played a role as well, leading to the extinction of *M. balearicus* (also suggested for smaller mammal species present on these islands; Bover and Alcover, 2003, 2008). Finally, a so-called *Sitzkrieg* has been postulated that assumes a gradual extinction involving the co-occurrence of humans and *M. balearicus* over several millennia (Waldren, 1982; Patton, 2000). Different variants of this theory are possible, also including the introduction of competitors (domestic species such as cattle, sheep and goats; Bover and Alcover, 2008).

Current radiocarbon dates do not support a *Sitzkrieg* (Fig. 2; Guerrero, 2001; Ramis et al., 2002). In addition, indicators for possible domestication, related with a *Sitzkrieg* model and published as such elsewhere (Kopper and Waldren, 1967; Waldren, 1982), can be explained differently (Ramis and Bover, 2001; van der Geer et al., 2010; Winkler, 2010). The *Blitzkrieg* model can also be discarded because no evidence

currently exists of human predation on *M. balearicus* (Guerrero, 2001; Ramis et al., 2002). The climate-change models have to take into account published arguments for seasonal dependence of *Myotragus* on *Buxus* sp. (Alcover et al., 1999) and the decline of the *Buxus* species (*B. balearicus* is still marginally present on the island, *B. sempervirens* has disappeared, Lull et al., 1999). These climate models also have to take into account the long-term evolution and existence of the *Myotragus* lineage on the Balearics, a period which also includes major climatic changes in the Mediterranean region during glacial/interglacial periods (Bover and Alcover, 2003). The 'hyperdisease' hypothesis places the extinction close to the moment of, or immediately after, human arrival on these islands (Ramis et al., 2002; Bover and Alcover, 2003; Alcover, 2004, 2008; Bover et al., 2008). This is in accordance with evidence from numerous islands worldwide which show that coexistence of humans and endemic fauna is often limited in duration (Martin, 1984; MacPhee and Marx, 1997).

Limited dietary information from *M. balearicus* suggests how dependent the animal was on available plant species (Alcover et al., 1999). The present study focuses on two sites on Mallorca, and constitutes a wider geographical sample than previously studied. DNA research on coprolites from *M. balearicus* has not been carried out before and this approach may add important information to our understanding of its diet. The DNA results of the studied coprolites were compared with pollen and microfossil analysis carried out on the same samples, and with pollen analysis carried out during previous research (Alcover et al., 1999) to establish a more systematic dietary pattern. Furthermore, the information obtained was placed within a chronological framework and compared with hindcasted ecological niche models for *B. balearica* relating the extinction of the animal either to vegetation changes, or to changes within its environment that can be related to the arrival of humans on the island.

## Material and methods

Several different protocols and techniques were used and are described below.

### Sampling

Coprolite samples from two sites were available for destructive analysis. The samples were from sites located in the mountainous northwest region of Mallorca (Fig. 1; Table 1). The samples are from sites above 100 m asl. Dietary results can be influenced by geographic position; our samples are from relatively high-altitude sites, resulting in an elevational vegetation sampling bias.

**Table 2**  
Details of primers used in this study.

Primer name	Primer sequence 5'–3'	Target	Reference
rbcl Z1aF (F)	5'–ATGTCACCAACAGAGACTAAAGC–3'	Diet	Hofreiter et al. (2000)
rbcl-015 (R)	5'–TACAGTTGTCATGTACCAG–3'		Savolainen et al. (2000)
ITS1 (F)	5'–TCCGTAGGTGAACCTGCCG–3'	Diet, bacterial gut flora, fungi	White et al. (1990)
ITS2 (R)	5'–GCTGCGTCTTCATCGATGC–3'		White et al. (1990)
L599 (F)	5'–CTCAAAGGACTTGGCGGTGC–3'	Coprolite producer, bacteria	Lalueza-fox et al. (2005)
H673 (R)	5'–GAAGATGGCGGTATATAGAC–3'		Lalueza-fox et al. (2005)

**Table 3**

DNA barcoding 12S results from sample 2 (Cova de Muleta) obtained by Sanger sequencing. Query coverages and E-values are given as a support of the reliability of the DNA BLAST results. Accession numbers refer to the closest hit in NCBI Genbank.

Family	Genus/species	Query coverage (%)	E-value	Accession number
Ruminococcoceae	<i>Ruminococcus</i> sp.	100	5e–04	FP929052
Coriobacteriaceae	<i>Slackia heliotrinireducens</i>	98	7e–04	CP001684

These sites are important localities for understanding Balearic pre-human ecology and human arrivals on the islands. Both Balma de Son Matge and Cova de Son Muleta were excavated in the 1960/70's. Uncertainty exists regarding the chronology of the occupation of these sites by the first humans on Mallorca (Ramis et al., 2002; Alcover, 2008). Both studies present arguments against the reliability of the stratigraphies, the unclear position of dated material and the uncertain identification of the dated material. The authors conclude that these sites cannot play a role in establishing a chronology of the arrival of humans on the island. However, this does not mean that individual remains from these sites cannot be used when these are individually dated. Coprolites from these two sites can add valuable information to our understanding of Balearic pre-human ecology. Chronologic and stratigraphic problems were overcome by direct dating of the coprolites used in the present study, thus eliminating many problems associated with earlier executed radiocarbon dates from these two sites.

Organic fibres from the first coprolites studied (sample 1) from Balma de Son Matge proved to be modern after radiocarbon dating.

Sample 2 came from Cova de Son Muleta and consisted of a single coprolite. No stratigraphic information was provided for this sample (Waldren, 1982). The coprolite matrix was directly dated (Table 1). Sample 3 also came from Balma de Son Matge. No stratigraphic information was provided. When Balma de Son Matge was first published it was noted that preliminary research did not yield any identifiable organic fibres/seeds/pollen within the coprolites (Waldren, 1982). Previous research reported that the coprolites from this site were oxidised and were therefore not included in further research (Alcover et al., 1999). Multiple coprolites from this sample were included in the present study to test if it was possible to amplify their DNA.

#### Radiocarbon dating

Radiocarbon dating was carried out on sample 1 from Balma de Son Matge and on sample 2 from Cova de Muleta after DNA had successfully been retrieved. Radiocarbon dating (AMS) was carried out at the Groningen Centre for Isotope Physics. Dating of the material

**Table 4**

DNA barcoding and pollen results from the modern Balma de son Matge sample (sample 1). Query coverage is given as a support of the reliability of the DNA BLAST results (100% is the maximum and indicates full coverage).

Family	Genus/species	Pollen (n)				Sanger sequences (# clones)	Query coverage (%)
Anacardiaceae	<i>Pistacia lentiscus</i>	45	56	59	67		
Apiaceae	<i>Falcaria</i>	1		1			
Asteraceae	<i>Artemisia</i>	1	1		1		
	<i>Centaurea jacea</i>		1				
Betulaceae						1	100
	<i>Alnus</i> sp.		1				
	<i>Corylus</i> sp.				1		
Brassicaceae		1		1			
Chenopodiaceae		3					
Cistaceae	<i>Cistus/Helianthemum</i>	14	18	4	4		
Euphorbiaceae	<i>Euphorbia</i> sp.	13	15	3	7		
Ericaceae	<i>Erica arborea</i>	1		1			
Fabaceae			1				
	<i>Astragalus</i>		1				
	<i>Ceratonia siliqua</i>		1				
	<i>Trifolium repens</i>	1					
Fagaceae	<i>Quercus ilex</i>	11	32		2	1	98
Gentianaceae	<i>Centaureium</i>	1	3				
Lamiaceae	<i>Mentha</i>		1				
	<i>Teucrium</i> sp.	1	2				
Linaceae	<i>Linum catharticum</i>						
Malvaceae		1					
Myrtaceae	<i>Myrtus communis</i>	2	1				
Oleaceae	<i>Olea</i> sp.	16	15		1		
Pinaceae	<i>Pinus</i> sp.	425	445	853	987	5	100
Plantaginaceae	<i>Plantago lanceolata</i>		2				
Polygonaceae	<i>Rumex acetosella</i>	1					
Polypodiaceae	<i>Polypodium</i>	1	3	2			
Poaceae	<i>Hordeum</i>	311	320	4			
Ranunculaceae	<i>Ranunculus</i>			1			
Rhamnaceae	<i>Rhamnus</i>	6	5	1			
Rubiaceae		1	1				
Rutaceae	<i>Ruta</i>						
Vitaceae	<i>Vitis</i> sp.	1					
Mammalia	<i>Capra</i> sp.					1	99
Mycobacteriaceae	<i>Mycobacterium</i> sp.					1	82
Fungi						1	79
	<i>Gelasinospora</i>		1				
	<i>Sordaria</i>	1	5				
	<i>Sporormiella</i>	8				1	

was not only important for eliminating problems associated with earlier radiocarbon dating, but also for evaluating the dietary results in relation to the available paleoecological information. Sample 3 (the other sample from Balma de Son Matge) did not contain DNA or pollen, and was therefore not dated.

#### DNA methodology

##### Extraction, purification and PCR

Both CTAB and silica extractions were used to retrieve genomic DNA from the coprolites studied. Extractions were carried out in the Ancient DNA facility of the Leiden University using standard procedures to avoid contamination with modern DNA (Cooper and Poinar, 2000). Both extraction methods were used on all samples. Approximately 0.2 mg of air-dried dung was used per extraction. The outer surfaces of the coprolites were discarded to avoid the possibility of contamination, and only the innermost part of the coprolite was used for DNA extraction. Extraction procedures followed known protocols (Doyle and Doyle, 1987; Rohland and Hofreiter, 2007). After extraction, DNA was further purified with the QIAquick™ PCR purification kit. Extraction blanks were included in all steps to monitor for contamination.

Special ancient DNA primers were used to amplify parts of the chloroplast *rbcl* gene and nrITS1 region in 25 µl reactions to generate 161 and 230-bp fragments respectively. A small part of the 12S mitochondrial DNA, 73-bp long, was amplified as well (Table 2). The chloroplast *rbcl* gene was chosen because sequences from this gene are the most abundant in DNA barcoding reference databases (NCBI GenBank). Sequences obtained from this gene normally provide information up to family level, but genus/species level is in most cases not possible (Schlumbaum et al., 2008). The nrITS1 region is longer and has a higher identification level, mostly up to genus level (Schlumbaum et al., 2008). The obtained 73-bp fragment of the 12S mtDNA gene would make it possible to differentiate between *Myotragus* and *Ovis/Capra* and was used as proof of the authenticity of the coprolite samples.

PCR's were carried out with *Phire* Hot Start DNA Polymerase (Finnzymes) in 25 µl aliquots. PCR products were loaded on 2% agarose gels for electrophoresis in an aliquot containing 5 µl PCR product and 1 µl loading dye. Gels were run for 30 min, together with GeneRuler. Afterwards the gels were soaked in ethidiumbromide for 15 min and photographed.

#### Cloning

All PCR products that were obtained were tailed and cloned using the TOPO TA Cloning Kit (Invitrogen). From each plate the highest possible amount of clones was picked for Sanger sequencing. Cloning was done to enable direct Sanger sequencing of mixed PCR products, containing sequences of multiple species, and to detect the possibility of C to G and G to A changes due to de-amination, a process causing substitutions during the ageing of DNA (Cooper and Poinar, 2000; Pääbo et al., 2000).

**Table 5**

DNA barcoding results obtained for sample 2 (Cova de Muleta) by Sanger, Roche-454 and Ion Torrent sequencing. Query coverages and E-values are given as a support of the reliability of the DNA BLAST results. Accession numbers refer to the closest hit in NCBI Genbank.

Family	Genus/species	Barcoding marker	Query coverage (%)	E-value	Method	Accession number
Araliaceae	<i>Hedera helix</i>	rbcl	99	7e–100	Roche-454	FJ395559
Poaceae		rbcl	98	1e–94	Roche-454	HQ713389
		nrITS1	95	6e–126	Sanger	AF171186
Rosaceae	<i>Mespilus</i> sp.	rbcl	97	4e–16	Ion Torrent	JQ391239
	<i>Prunus</i> sp.	rbcl	99	2e–70	Ion Torrent	HE963614
	<i>Prunus</i> sp.	rbcl	100	8e–78	Sanger	HQ619779
Trichocomaceae	<i>Aspergillus</i> sp.	nrITS1	100	4e–107	Sanger	HQ997377
	<i>Penicillium</i> sp.	nrITS1	100	1e–22	Sanger	EU427285
Gymnoascaceae		nrITS1	77	2e–12	Sanger	AF129853
Tremmelaceae	<i>Cryptococcus macerans</i>	nrITS1	100	1e–85	Sanger	JQ070110

**Table 6**

Results of pollen analysis for sample 2 (Cova de Muleta). *Buxus* sp. dominates the assemblage by more than two-thirds. Comparison with Carrión (2012) shows that *Juniperus* sp., *Pinus* sp. and *Corylus* sp. are most likely *Juniperus phoenicea*, *Pinus halepensis* and *Corylus avellana*.

Family	Genus/species	Number of pollen grains
Asteraceae	<i>Artemisia</i> sp.	1
Buxaceae	<i>Buxus</i> sp.	Dominant
Ephedraceae	<i>Ephedra fragilis</i>	1
Ericaceae	<i>Erica arborea</i>	1
Betulaceae	<i>Corylus</i> sp.	2
Euphorbiaceae	<i>Euphorbia dendroides/E. chracias</i>	23
Thymelaeaceae		2
Cupressaceae	<i>Juniperus</i> sp.	5
Pinaceae	<i>Pinus</i> sp.	26
Poaceae		1
	<i>Lygeum spartum</i>	1
Rosaceae	<i>Cheilanthes</i> sp.	2

#### DNA sequencing and analysis

Sanger sequencing was performed by Macrogen, Amsterdam, on an ABI 3730xl (Applied Biosystems). The Sanger sequences produced were checked and aligned with Sequencher v. 4.10.1. Pyrosequencing was carried out on a Roche 454-FLX Titanium sequencer at the Leiden University Medical Center. Ion Torrent analyses were done on a PGM machine at Naturalis Biodiversity Center with the Ion PGM 200 sequencing kit and an Ion-314 chip (520 cycles per run). Reads obtained were trimmed with CLC Workbench Genomics v. 4.5 and clustered using the Octopus pipeline. Representative consensus sequences of each cluster were compared with reference data present in NCBI GenBank using Blast Search for taxonomic identification. Only hits with >97% coverage and >99% similarity were accepted.

#### Hindcasted ecological niche models

Ecological Niche Models (ENMs) identify the relationship between species presence records and (a)biotic conditions at those sites (<http://pmip2.lscce.ipsl.fr/pmip2/>; Peterson et al., 2011). When this relationship is projected in geographic space it allows one to predict habitat suitability for a species in geographic time and space. When projected in time, it allows one to predict habitat suitability under past or future climatic conditions. Distributions of *Buxus balearica* (Buxaceae) were modelled using presence-only data retrieved from 133 geo-referenced herbarium specimens, stored in the Botanical Research And Herbarium Management System (BRAHMS) database of Naturalis Biodiversity Center and the GBIF portal ([www.gbif.org](http://www.gbif.org)). Replicate records per 1-km spatial raster cell were removed. To prevent niche truncation we modelled the full ecological niche of *B. balearica* using presence records from its entire Mediterranean range, and not just collections from the Balearic Islands (Sánchez-Fernández et al., 2011; Raes, 2012). Data for 19 bioclimatic predictors at 1 km<sup>2</sup> spatial resolution for the present and Last Glacial Maximum climatic conditions were retrieved from WorldClim ([www.worldclim](http://www.worldclim)).

org; Hijmans et al., 2005). The spatial data covering the 19 bioclimatic variables at 1 km<sup>2</sup> for the Mid-Holocene were derived from the Paleoclimate Modelling Intercomparison Project Phase II database (<http://pmip2.lsce.ipsl.fr/pmip2/>; Braconnot et al., 2007). Furthermore, the ENM for *B. balearica* was trained using bioclimatic data of raster cells within 1 degree buffers around collection sites; this was done to prevent a decrease in discriminatory power and calibration of the model in the core area of the species distribution (Acevedo et al., 2012). After calibration of the ENM for present climatic conditions (within buffered Mediterranean collection sites) it was projected to the geographical extent of the Balearic Islands for present, Mid-Holocene, and LGM climatic conditions. The ENM was developed using the non-parametric maximum entropy algorithm implemented in MaxEnt (version 3.3.3 k; Phillips et al., 2006; Elith et al., 2011). We allowed MaxEnt to use linear, quadratic and hinge features only, and we used all 133 records and the 19 bioclimatic variables for model training. We report the model AUC for comparative reasons only because when AUC values are based on presence-only records and a background sample (instead of true absences) the maximum AUC value is no longer 1, and the maximum value cannot be determined (Phillips et al., 2006; Raes and ter Steege, 2007; Lobo et al., 2008).

## Results

### Radiocarbon dating

The dating of coprolite Sample 1 from Balma de Son Matge resulted in <sup>14</sup>C activity of 108 ± 0.4% (GrA-49936) meaning that the sample is younger than 1950 (AD 1950 is 100%). Therefore, this sample cannot be from *Myotragus balearicus*.

The radiocarbon dating carried out on Sample 2 from Cova de Son Muleta resulted in an age of 4905 ± 40 <sup>14</sup>C yr BP (GrA-52253, Table 1). This places the age of the sample at the edge of the established extinction period of *Myotragus balearicus*, 5700–4050 <sup>14</sup>C yr BP (Bover and Alcover, 2003), and also before the vegetation transition period, approximately 5000–4000 <sup>14</sup>C yr BP (Fig. 2). The radiocarbon date of this coprolite is the youngest date obtained for *M. balearicus* from Cova de Muleta, when compared with Pérez (2005).

### Zoological DNA results

12S mitochondrial DNA was used to identify the producer of the modern Balma de Son Matge coprolite which was identified as *Capra* rather than *Ovis* (Table 4) further supporting a recent origin. Unfortunately, no mammalian DNA was retrieved with the 12S primer pair from any of the other coprolites, presumably because of DNA degradation in a thermally unfavourable environment for DNA preservation (Pääbo et al., 2000) and our unfamiliarity with using N-phenacylthiazolium bromide (PTB). This compound has been demonstrated to cleave Maillard products, thus freeing up DNA for PCR amplification (Poinar et al., 1998). PTB was used to successfully analyse mitochondrial and nuclear DNA extracted from a 20,000-yr-old coprolite produced by a giant ground sloth in Gypsum Cave in Nevada as well as mtDNA from human coprolites from the Paisley Caves in Oregon (Jenkins et al., 2012). We encourage others to try the addition of this reagent in future ancient DNA studies on Balearic coprolites.

Bacterial DNA sequences retrieved from Sample 2 (Cova de Son Muleta) are reported in Table 3. Both genera identified relate to the digestion and decomposing of organic material, and are therefore not surprising to be found in a coprolite. All of the bacterial DNA sequences reported have relative low maximum identities when compared with the sequences that match with them. This means that their identification is not strongly supported, and that it is likely that future genetic research will change the taxonomic identifications provided.

### Herbaceous DNA results

#### Modern sample

The results of the modern sample from Balma de Son Matge are presented in Table 4. Of interest are the sequences from *Pinus* sp. No species identification within the *Pinus* genus could be made. In addition, one sequence from *Quercus ilex* was found, and one sequence from the Betulaceae family. It must be noted that *Pinus* sp., which forms the majority of the DNA sequence identified, is also the largest portion of the pollen counted in the sample (71.1%).

#### Ancient samples (S2 and S3)

No DNA sequences were obtained for Sample 3. All three DNA sequencing methods used resulted in DNA barcodes for Sample 2 (Cova de Muleta; Table 5). Three different plant families could be identified (Araliaceae, Poaceae and Rosaceae). Two different genera were retrieved from the Rosaceae family (*Mespilus* and *Prunus*). No pollen grains of these genera are present in the pollen counted from Sample 2. The Poaceae family (grasses) was identified by both Sanger and Roche-454 sequencing. No detailed taxonomic identifications were possible for this family. The Poaceae are also present in the pollen count from the same sample with one pollen grain attributed to genus level, and one grain to the genus *Lygeum*. Noteworthy is the absence of *Buxus* sp. in the DNA sequences in comparison with the dominance of *Buxus* pollen.

The nrITS1 DNA barcodes obtained contained some fungal sequences (all retrieved by Sanger sequencing). These are reported in Table 5. All fungal sequences are from genera living in, at or near coprolites. The identification of these genera is therefore not surprising, but noteworthy in the light of DNA survival in a thermally unsuitable environment for DNA preservation.

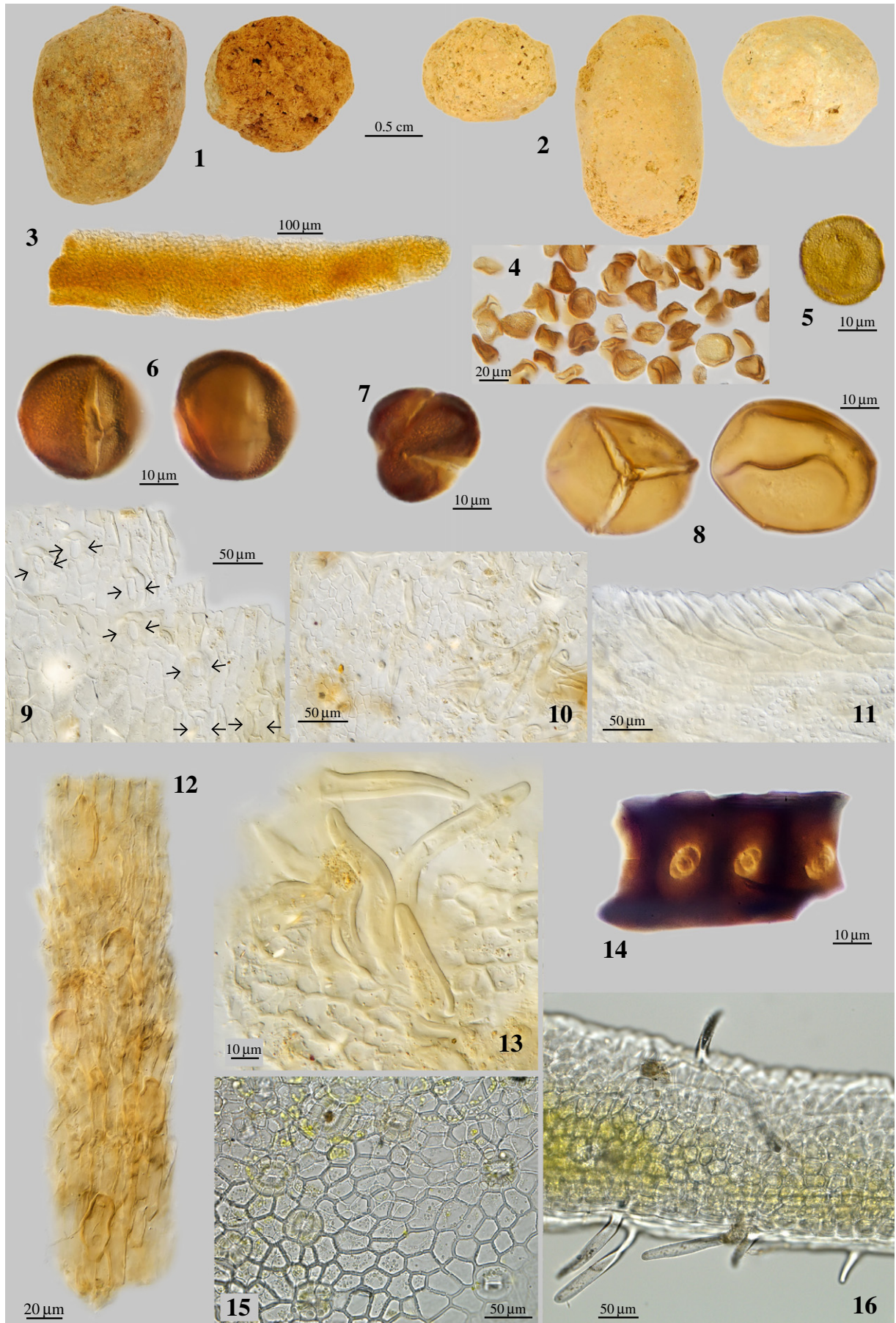
#### Pollen and macrofossils

No percentages can be given for the pollen count from Sample 2 (Cova de Son Muleta) (Table 6). *Buxus* sp. (box or boxwood) is very common, more than two-thirds of the pollen grains recorded. Macrofossils of box were also found in the Cova de Muleta coprolite (Fig. 3). These comprise leaf epidermis fragments (with stomata), different parts of the inflorescence and very clear parts of the anther (part of the stamen) that still contain compacted, not dispersed pollen grains. The high percentage of unripe pollen grains in the pollen samples also suggests that *M. balearicus* ate flowers of box.

Two species of *Buxus* were present on the island, *B. balearica* and *B. sempervirens*. It is assumed that *Buxus balearica* once was the most abundant species of the two (Lázaro and Traveset, 2006). Pollen grains of *Pinus* and *Euphorbia* were also common in the pollen assemblage and the presence of unripe grains in both the coprolites suggests that these taxa may also have been actively consumed.

#### Hindcasted models

For the period between 8.5 and 6.0 ka, pollen diagrams obtained from the Balearics indicate that *B. balearica* dominated the vegetation (Yll et al., 1997; Carrión, 2012). From 6.0 ka onwards the species almost entirely disappeared in the pollen records. The MaxEnt model trained under present climatic conditions (AUC = 0.967) shows that only small sections in the northern part of Mallorca represent suitable habitat at present (Fig. 5A; warm colours). When this model is hindcasted to the Mid-Holocene (Fig. 5B) a larger section of the Balearic Islands is predicted as suitable habitat for *B. balearica*. However, when the model is projected to the LGM (Fig. 5C) almost the entire area of the Balearic Islands is predicted as suitable habitat. The jackknife test of variable importance indicated that bioclimatic variable 5 – maximum temperature in the warmest month – was the best single predictor with a strong optimum response around 31 °C.



## Discussion

Data generated from the Cova de Muleta coprolite were combined with a previous pollen analysis carried out on coprolites from *M. balearicus*. This previous study by Alcover et al. (1999), was performed on eleven different coprolites from Cova de Estreta, dated ca. 6.0 ka. That study, like ours, showed a dominance of *Buxus balearica* in the pollen assemblages. Alcover et al. report an average of 98.3% of *Buxus balearica* pollen (with 94.1% being the minimum) and, on average, no other pollen percentages higher than 0.62% in the remaining pollen spectra.

Four plant families have been identified in the research presented here which were not recorded in the study by Alcover et al. (1999) (Table 7). These are Euphorbiaceae, Araliaceae, Rosaceae and Pteridaceae. Of these, one was solely identified by DNA analysis (Araliaceae). DNA sequences also revealed the presence of Rosaceae (*Prunus* sp.), which was not recorded as pollen. *Prunus* sp. were not identified previously in any pollen core taken from sediments on Mallorca (Burjachs et al., 1994). This is the first indication of this particular family and genus being present on Mallorca before human arrival and/or vegetation changes. Araliaceae and the genus *Euphorbia*, found in DNA and pollen analysis, respectively, have been found once previously (Burjachs et al., 1994).

DNA analysis does not provide a clear picture about *M. balearicus* being a browser or a grazer. Both sequences of trees (*Prunus* sp.) and grasses (Poaceae) were retrieved. Other identifications concern families that contain both trees and shrubs (Rosaceae). When compared with the pollen analysis and macrofossils only strong evidence for the consumption of *Buxus* can be found. This would indicate that *M. balearicus* was a browser or seasonal mixed feeder. This is in agreement with conclusions from research carried out on the hypsodonty of its teeth and the abrasiveness of its diet (Winkler, 2010; Winkler et al., 2013), its general cranial morphology (Alcover et al., 1999) and  $\delta^{13}\text{C}$  values obtained from coprolites (Alcover et al., 1999).

In addition to plant species, our DNA research provides some insight into bacteria and fungi present on the island. The *Ruminococcus* identified probably played an important role in the digestion of cellulose, and must have been present within the gastrointestinal tract of *M. balearicus*. *Ruminococcus* is an important microorganism in the gastrointestinal tract of all ruminants, as mammals are normally not able to digest cellulose themselves, but rely on their gut flora (including *Ruminococcus*) for this digestion process (Withers, 1992). *Slackia heliotrinireducens* might play a similar role within the gastrointestinal tract. The presence of this species in the coprolites we studied is noteworthy, as it has so far only been isolated from the rumen of sheep (Wade et al., 1999). Our finding supports the hypothesis of a close relationship between *M. balearicus* and *Ovis* as suggested earlier (Lalueza-Fox et al., 2005).

Four different fungal genera were identified, of which *Penicillium* and *Aspergillus* are common worldwide.

### Diet and vegetation changes on Mallorca

All available pollen data show a high dominance of *Buxus balearica*, indicating that this species was an important component of the diet of *M. balearicus* during at least part of the year. The macrofossils encountered in the coprolite from Cova de Muleta show remains of the flowers and leaves of *Buxus balearica*, and suggest that *M. balearicus* consumed the tips of twigs with flowers of box. *Buxus balearica*, the most abundant *Buxus* species on the island, has a flowering time with

**Table 7**

Plant families identified in this study by pollen and DNA barcoding analysis of the Cova de Muleta (sample 2) coprolite as compared with Alcover et al. (1999).

Family	Cova de Muleta				Alcover et al. (1999)
	Pollen	Sanger	Roche-454	Ion Torrent	Pollen
Anacardiaceae					x
Araliaceae			x		
Asteraceae	x				x
Betulaceae	x				x
Buxaceae	x				x
Chenopodiaceae					x
Convolvulaceae					x
Cupressaceae	x				x
Cyperaceae					x
Dipsacaceae					x
Ephedraceae	x				x
Ericaceae	x				x
Euphorbiaceae	x				
Fabaceae					x
Fagaceae					x
Lamiaceae					x
Malvaceae					x
Oleaceae					x
Pinaceae	x				x
Plantaginaceae					x
Poaceae	x	x	x		x
Polygonaceae					x
Pteridaceae	x				
Rosaceae		x		x	
Sapindaceae					x
Thymelaeaceae	x				x

a maximum of two months, nowadays placed between April to May on Mallorca (the flowering time is slightly earlier on the mainland of Spain, occurring between February and March, Lázaro and Traveset, 2006). This would mean that all coprolites studied so far could have been deposited during the same two-month period.

The dependence of *M. balearicus* on *Buxus balearica* during (at least part of) the year can be seen as a confirmation of the density-dependent resource limitation inferred from crown formation times and long bone histology in *M. balearicus* (Jordana and Köhler, 2011). This would especially be the case if *M. balearicus* preferentially ate the flowers and pollen of *Buxus balearica* (pollen is a highly energetic food source). Variations within pollen and flower production of *Buxus* would be a determining factor in the amount of food available. *Buxus balearica* is known to display significant annual variation of numbers of flowers, pollen, and fruit production (Lázaro and Traveset, 2006; Lázaro et al., 2006). In addition, it has been shown that the species distribution is highly influenced by three different climatic variables: average temperature in the warmest month, spring rainfall, and average temperature in the coldest month (Navarro-Cerrillo et al., 2011). Both minimum and maximum temperatures, as well as minimum annual rainfall, show that the species tolerance to climatic change is relatively low (Navarro-Cerrillo et al., 2011; Fig. 5). Climate changes in the past should therefore have had large-scale influences on the distribution of *Buxus balearica* and other plant species on the Balearic Islands and surrounding areas.

Pollen diagrams of the Balearic Islands give information on the vegetation history of the islands. All of the cores have been taken from coastal areas because these are the only places where sufficient sediments have been deposited (Pérez-Obiol and Sadori, 2007). This means that the pollen records of these diagrams show a bias for the vegetation in coastal environments. However, a general overview about the vegetation history of the Balearics, and changes therein, is visible (Yll et al., 1994, 1997;

**Figure 3.** Pollen and macrofossils analyzed. 1. Ancient Cova de Son Muleta coprolite (4905 ± 40 <sup>14</sup>C yr BP). 2. Recent Cova de Son Muleta coprolite. 3. Fragment of anther of *Buxus balearica* containing pollen. 4. Pollen of *Buxus balearica*. 5. Single pollen grain of *Buxus balearica*. 6. Pollen of *Euphorbia*, equatorial view. 7. Pollen of *Euphorbia*, polar view. 8. Trilete spore in high and low focus. 9. Leaf epidermis of *Buxus balearica* with stomata (indicated with arrows). 10. Leaf epidermis of *Buxus balearica* with hairs. 11. Leaf epidermis of *Buxus balearica* with crystals. 12. Peduncle of *Buxus balearica*. 13. Detail of hairs on leaf epidermis of *Buxus balearica*. 14. Coniferous wood fragment with pits. 15. Modern leaf epidermis of *Buxus balearica* (voucher Gravendeel 05052013 stored in the herbarium of Naturalis Biodiversity Center in Leiden) with stomata. 16. Modern leaf epidermis of *Buxus balearica* with hairs.

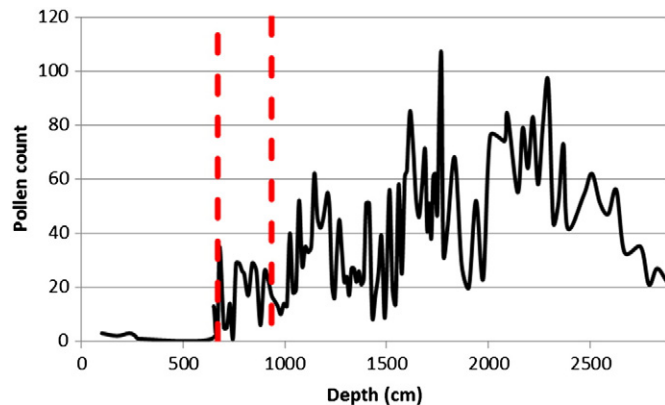
Carrión, 2012). Similar vegetation processes took place on Minorca and Mallorca (Palmer et al., 1999; Pérez-Obiol, 2007; Pérez-Obiol and Sadori, 2007; Di Domenico et al., 2012). Below, a review in relation to the data generated is given for the time interval relevant for the present study.

#### Vegetation prior to 5.0 ka

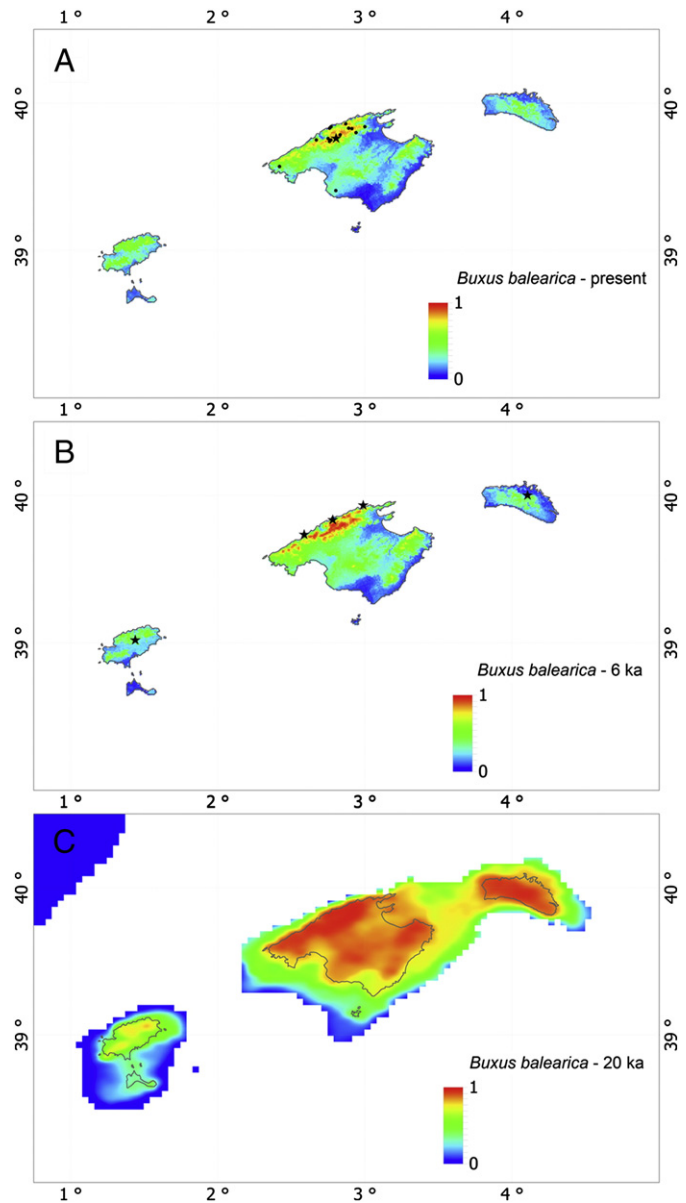
Before 5.0 ka the vegetation on the islands was dominated by *Buxus* (box) and *Corylus* (hazel). Furthermore, *Juniperus* (juniper), *Ephedra* (jointfir) and *Quercus* (oak) were important (Yll et al., 1997; Pérez-Obiol and Sadori, 2007; Carrión, 2012). It is important to note that after this period arboreal pollen shows a decline, indicating a progressive opening of the landscape (Burjachs et al., 1994; MacPhee and Marx, 1997). The dietary information retrieved in the present study corresponds to the vegetation present during this period. Further, the dietary information available shows that *M. balearicus* was depended on box, which was an important plant species on the island during this period (Fig. 5C).

#### The transitional phase, 5.0–4.0 ka

During this period the vegetation changed drastically. Mesophyllous taxa, including *Buxus*, *Pinus* (pine), *Juniperus*, *Fagus*, *Ephedra* and *Corylus* decreased in abundance or disappeared. This so-called “old” vegetation was replaced by maquis, dominated by *Olea* (olive). Taxa like *Plantago* (plantain), Ericaceae (heaths) and *Polygonum* (knotweed) started to appear (Yll et al., 1994, 1997; Carrión, 2012). This transition is not documented for the other Mediterranean Islands (Yll et al., 1997; Carrión et al., 2010). The higher values of *Plantago* and Ericaceae are related to human impact on the island (Yll et al., 1994, 1997). During this transition phase there was a considerable drop in the amount of rainfall in the Mediterranean, influencing vegetation and leading to an overall decline of biomass (Magny et al., 2002; Pérez-Obiol and Sadori, 2007). There are indications that the drop in precipitation was less apparent in the mountainous regions of Mallorca, making it possible for mesophyllous species to remain as relics in this area of the island up to today (Ninyerola et al., 2007; Di Domenico et al., 2012). The present day distribution is also highly influenced by historical effects of human activity on the island. This is especially important with regards to the diet reconstructed by DNA and pollen content of the coprolites studied. While *Buxus balearica* diminished in abundance on the island, this decline was probably less apparent in the mountainous regions of Mallorca, where nowadays *Buxus balearica* can still be found. The species disappeared from the other parts of the island, nonetheless, and *Buxus sempervirens* disappeared completely. If more sites become available with *Myotragus* fossils in future, a possible spatial



**Figure 4.** Decrease of *Buxus* on the Balearic Islands. Absolute *Buxus* pollen count from the Algendar core, showing that the decreasing relative amount of *Buxus* pollen is not due to the rise of other pollen taxa included in the count. The amounts are displayed in accordance to the depth of the core. The vegetation change is situated at a depth between 935 and 670 cm (indicated with dashed lines). Direct radiocarbon dates of the core are 5000 to 4050  $^{14}\text{C}$  yr BP. Algendar data and  $^{14}\text{C}$  data modified after Yll et al. (1997).



**Figure 5.** Distributions of *Buxus balearica* on the Balearic Islands according to a MaxEnt model trained on environmental data from raster cells within 1-degree buffered collection localities and subsequently projected to the present climatic conditions of the Balearic Islands (upper graph), hindcasted to mid-Holocene (6 ka; middle graph) and hindcasted to the Last Glacial Maximum (20 ka; lower graph). The total area of favourable local climate conditions for this plant species (indicated with an orange and red colours) decreased over time and was replaced by unfavourable local conditions (indicated with green and blue colours). Collection localities of herbarium specimens used for the modelling are indicated with black circles, localities of (macro)fossils with stars.

correlation could be investigated between the last remains of this animal found and remaining *B. balearica* pockets on Mallorca.

To summarise, we can state that (i) the total arboreal pollen count gradually dropped, in accordance with the above statement of the evolving opening of the landscape, and a vegetation replacement between 5.0 and 4.0 ka (Pérez-Obiol and Sadori, 2007); (ii) the absolute *Buxus* pollen count gradually dropped as well, until the disappearance of *Buxus* pollen around 4.0 ka. (Fig. 4), and; (iii) the relative *Buxus* pollen count also gradually dropped until the species regional disappearance in all cases (Fig. 5; Ninyerola et al., 2007). The disappearance of the mesophyllous vegetation and the appearance of a maquis, the abrupt regional disappearance of *Buxus balearica*, and the continuing opening of the landscape since 5.0 ka will have had profound impact on the survival of populations of *M. balearicus*.

## Conclusions

Ancient DNA, pollen analysis and macrofossils provided evidence that *M. balearicus* was a browser or a seasonal mixed feeder, and that it was relying heavily on *Buxus balearica*, at least during part of the year. The drastic decrease in abundance of *Buxus balearica* on the Balearic Islands will have posed a serious density-dependent constraint on the fitness of the populations of *M. balearicus*, and may have had a significant influence on the final extinction of the species. This can be related to an increasing aridity occurring during the period under consideration in the Mediterranean region, substituting the local mesophyllous vegetation with a more open maquis. The plant species composition may have changed too rapidly for *M. balearicus*. This does not exclude a possible interaction with humans. The lack of compelling archaeological evidence documenting contact between humans and *M. balearicus* cannot be explained by mutual avoidance because of *M. balearicus*'s limited agility in escaping. We conclude that more than one factor was probably involved in the extinction of this species. Previous hypotheses relating the extinction of *M. balearicus* directly to the arrival of humans on the islands must therefore be adjusted.

## Acknowledgments

T. van Kolfschoten and I. van der Jagt are thanked for their valuable comments on earlier versions of this manuscript. J. Waldren and the late W. Waldren are thanked for providing coprolite material. F. Burjachs is thanked for providing information about the vegetation on the Balearic Islands. J. van Arkel and B.J. van Heuven are thanked for providing photographs. N. Raes was supported by the Netherlands Research Council NWO-ALW with grant 819.01.014.

## References

- Acevedo, P., Jiménez-Valverde, A., Lobo, J.M., Real, R., 2012. Delimiting the geographical background in species distribution modelling. *Journal of Biogeography* 39 (8), 1383–1390.
- Alcover, J.A., 2004. Disentangling the Balearic first settlement issues. *ENDINS* 26, 143–156.
- Alcover, J.A., 2008. The first Mallorcan: prehistoric colonization in the Western Mediterranean. *Journal of World Prehistory* 21 (1), 19–84.
- Alcover, J.A., Perez-Obiol, R., Yll, E.L., Bover, P., 1999. The diet of *Myotragus balearicus* Bate 1909 (Artiodactyla: Caprinae), an extinct bovid from the Balearic Islands: evidence from coprolites. *Biological Journal of the Linnean Society* 66 (1), 57–74.
- Bate, D.M.A., 1909. Preliminary note on a new artiodactyle from Mallorca *Myotragus balearicus*, gen. et. sp. nov. *Geological Magazine* 6, 385–389.
- Bover, P., 2004. Noves aportacions al coneixement del gènere *Myotragus* Bate 1909 (Artiodactyla, Caprinae) de les Illes Balears. *Universitat de les Illes Balears, Palma de Mallorca*.
- Bover, P., Alcover, J.A., 1999a. Estimating physical characteristics of neonate *Myotragus balearicus* (Artiodactyla, Caprinae). *Deinsea* 7, 33–54.
- Bover, P., Alcover, J.A., 1999b. The evolution and ontogeny of the dentition of *Myotragus balearicus* Bate, 1909 (Artiodactyla, Caprinae): evidence from new fossil data. *Biological Journal of the Linnean Society* 68 (3), 401–428. <http://dx.doi.org/10.1111/j.1095-8312.1999.tb01178.x>.
- Bover, P., Alcover, J.A., 2003. Understanding Late Quaternary extinctions: the case of *Myotragus balearicus* (Bate, 1909). *Journal of Biogeography* 30 (5), 77–81.
- Bover, P., Alcover, J.A., 2008. Extinction of the autochthonous small mammals of Mallorca (Gymnesic Islands, Western Mediterranean) and its ecological consequences. *Journal of Biogeography* 35 (6), 1112–1122.
- Bover, P., Quintana, J., Alcover, J.A., 2008. Three islands, three worlds: Paleogeography and evolution of the vertebrate fauna from the Balearic Islands. *Quaternary International* 182 (1), 135–144.
- Bover, P., Quintana, J., Alcover, J.A., 2010. A new species of *Myotragus* Bate, 1909 (Artiodactyla, Caprinae) from the Early Pliocene of Mallorca (Balearic Islands, western Mediterranean). *Geological Magazine* 147 (6), 871–885.
- Braconnot, P., Otto-Bliessner, B., Harrison, S., Joussaume, S., Peterchmitt, J.Y., Abe-Ouchi, A., Crucifix, M., Driesschaert, E., Fichefet, T., Hewitt, C.D., Kageyama, M., Kitoh, A., Lañé, A., Loutre, M.F., Marti, O., Merkel, U., Ramstein, G., Valdes, P., Weber, S.L., Yu, Y., Zhao, Y., 2007. Results of PMIP2 coupled simulations of the Mid-Holocene and Last Glacial Maximum – Part 1: experiments and large-scale features. *Climate of the Past* 3, 261–277.
- Burjachs, F., Pérez-Obiol, R., Roure, J.M., Julià, R., 1994. Dinámica de la vegetación durante el Holoceno en la isla de Mallorca. In: Mateu, I., Dupré, M., Güemes, J., Burgaz, M.E. (Eds.), *Trabajos de Palinología básica y aplicada*. Universitat de València, Valencia, pp. 199–201.
- Calvo, M., Guerrero, V.M., 2002. Los inicios de la metalurgia en las Baleares. El Calcolítico (c. 2500–1700 cal BC). *Edit El Tall, Palma de Mallorca*.
- Carrión, J.S., 2012. Paleoflora y paleovegetación de la península Ibérica e Islas Baleares: Plioceno-Cuaternario. Ministerio de Economía y Competitividad, Madrid.
- Cooper, A., Poinar, H.N., 2000. Ancient DNA: do it right or not at all. *Science* 289 (5482), 1139.
- Di Domenico, F., Lucchese, F., Magri, D., 2012. *Buxus* in Europe: Late Quaternary dynamics and modern vulnerability. *Perspectives in Plant Ecology, Evolution and Systematics* 14, 354–362.
- Doyle, J.J., Doyle, J.L., 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemistry Bulletin* 19, 11–15.
- Elith, J., Phillips, S.J., Hastie, T., Dudík, M., Chee, Y.E., Yates, C.J., 2011. A statistical explanation of MaxEnt for ecologists. *Diversity and Distributions* 17, 43–57.
- Hijmans, R.J., Cameron, S.E., Parra, J.L., Jones, P.G., Jarvis, A., 2005. Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology* 25, 1965–1978.
- Hofreiter, M., Poinar, H.N., Spaulding, W.G., Bauer, D., Martin, P.S., Possnert, G., Pääbo, S., 2000. A molecular analysis of ground sloth diet through the last glaciations. *Molecular Ecology* 9 (12), 1975–1984.
- Jenkins, D.L., Davis, L.G., Stafford, T.W., Campos, P.F., Hockett, B., Jones, G.T., Cummings, L.S., Yost, C., Connolly, T.J., Yohe, R.M., Gibbons, S.C., Raghaven, M., Rasmussen, M., Pajjmans, J.L., Hofreiter, M., Kemp, B.M., Barta, J.L., Monroe, C., Gilbert, M.T., Willerslev, E., 2012. Clovis age Western Stemmed projectile points and human coprolites at the Paisley Caves. *Science* 337 (6091), 223–228.
- Jordana, X., Köhler, M., 2011. Enamel microstructure in the fossil bovid *Myotragus balearicus* (Majorca, Spain): implications for life-history evolution of dwarf mammals in insular ecosystems. *Palaeogeography, Palaeoclimatology, Palaeoecology* 300 (1), 59–66.
- Köhler, M., Moyà-Solà, S., 2009. Physiological and life history strategies of a fossil large mammal in a resource-limited environment. *Proceedings of the National Academy of Sciences* 106 (48), 20354–20358.
- Kopper, J.S., Waldren, W.H., 1967. Balearic prehistory. A new perspective. *Archaeology* 20 (2), 108–115.
- Krijgsman, W., Hilgen, F.J., Raffi, I., Sierro, F.J., Wilson, D.S., 1999. Chronology, causes and progression of the Messinian salinity crisis. *Nature* 400 (2001), 652–655.
- Lalueza-Fox, C., Bertranpetit, J., Alcover, J.A., Shailer, N., Hagelberg, E., 2000. Mitochondrial DNA from *Myotragus balearicus*, an extinct bovid from the Balearic Islands. *Journal of Experimental Zoology* 288 (1), 56–62.
- Lalueza-Fox, C., Shapiro, B., Bover, P., Alcover, J.A., Bertranpetit, J., 2002. Molecular phylogeny and evolution of the extinct bovid *Myotragus balearicus*. *Molecular Phylogenetics and Evolution* 25 (3), 501–510.
- Lalueza-Fox, C., Castresana, J., Sampietro, L., Marqués-Bonet, T., Alcover, J.A., Bertranpetit, J., 2005. Molecular dating of caprines using ancient DNA sequences of *Myotragus balearicus*, an extinct endemic Balearic mammal. *BMC Evolutionary Biology* 5, 70. <http://dx.doi.org/10.1186/1471-2148-5-70>.
- Lázaro, A., Traveset, A., 2006. Reproductive success of the endangered shrub *Buxus balearica* Lam. (Buxaceae): pollen limitation, and inbreeding, and outbreeding depression. *Plant Systematics and Evolution* 261, 117–128.
- Lázaro, A., Traveset, A., Méndez, M., 2006. Masting in *Buxus balearica*: assessing fruiting patterns and processes at a large spatial scale. *OIKOS* 115, 229–240.
- Lobo, J.M., Jiménez-Valverde, A., Real, R., 2008. AUC: a misleading measure of the performance of predictive distribution models. *Global Ecology and Biogeography* 17, 145–151.
- Lull, V., Micó, R., Rihuette, C., Risch, R., 1999. La Cova des Càrritx y la Cova des Mussol. *Ideología y Sociedad en la Prehistoria de Menorca*. Consell Insular de Menorca, Menorca.
- MacPhee, R.D.E., Marx, P.A., 1997. The 40,000 year plague: humans, hyperdisease and first-contact extinctions. In: Goodman, S.M., Patterson, B.D. (Eds.), *Natural Change and Human Impact in Madagascar*. Smithsonian Institution Press, Washington, pp. 169–217.
- Magny, M., Miramont, C., Silvan, O., 2002. Assessment of the impact of climate and anthropogenic factors on Holocene Mediterranean vegetation in Europe on the basis of palaeohydrological records. *Palaeogeography, Palaeoclimatology, Palaeoecology* 186 (1/2), 255–290.
- Martin, P.S., 1984. Prehistoric overkill: the global model. In: Martin, P.S., Klein, R.G. (Eds.), *Quaternary Extinctions. A Prehistoric Revolution*. University of Arizona Press, Tucson, pp. 354–403.
- Moyà-Solà, S., Köhler, M., Alba, D., Pons-Moyà, J., 2007. El significado de *Myotragus batei* y *M. binigasensis* en la evolución del género *Myotragus* (Bovidae, Mammalia) en la isla Balearica. In: Pons, G.X., Vicens, D. (Eds.), *Geomorfología litoral I Quaternari. Homenatge a Joan Cuerda Barceló*. Monografies de la Societat d'Historia Natural de les Balears, 14. Universitat de València, Valencia, pp. 155–180.
- Navarro-Cerrillo, R.M., Hernández-Bermejo, J.E., Hernández-Clemente, R., 2011. Evaluating models to assess the distribution of *Buxus balearica* in southern Spain. *Applied Vegetation Science* 14, 256–267.
- Ninyerola, M., Sáez, L., Pérez-Obiol, R., 2007. Relating postglacial relict plants and Holocene vegetation dynamics in the Balearic Islands through field surveys, pollen analysis and GIS modelling. *Plant Biosystems* 141 (3), 292–304.
- Pääbo, S., Poinar, H., Serre, D., Jaenicke-Després, V., Hebler, J., Rohland, N., Kuch, M., Krause, J., Vigilant, L., Hofreiter, M., 2000. Genetic analysis from ancient DNA. *Annual Review of Genetics* 38, 645–679.
- Palmer, M., Pons, G.X., Cambefort, I., Alcover, J.A., 1999. Historical processes and environmental factors as determinants of inter-island differences in endemic faunas: the case of the Balearic Islands. *Journal of Biogeography* 26 (4), 813–823.
- Palombo, M.R., Bover, P., Valli, A.M.F., Alcover, J.A., 2008. The Plio-Pleistocene endemic bovids from the Western Mediterranean islands: knowledge, problems and perspectives. *Hellenic Journal of Geosciences* 41, 153–162.

- Palombo, M.R., Rozzi, R., Bover, P., 2013. The endemic bovids from Sardinia and the Balearic Islands: the state of the art. *Geobios* 46, 127–142.
- Patton, M., 2000. Blitzkrieg or Sitzkrieg: the extinction of endemic faunas in Mediterranean island prehistory. In: Nicholson, R.A., O' Connor, T.P. (Eds.), *People as an Agent of Environmental Change*. Oxbow Books, Oxford, pp. 117–124.
- Pérez-Obiol, R., Sadori, L., 2007. Similarities and dissimilarities: synchronisms and diachronisms in the Holocene vegetation history of the Balearic Islands and Sicily. *Vegetation History and Archaeobotany* 16 (4), 259–265.
- Peterson, A.T., Soberón, J., Pearson, R.G., Anderson, R.P., Martínez-Meyer, E., Nakamura, M., Araújo, M.B., 2011. *Ecological Niches and Geographic Distributions*. Princeton University Press, Princeton and Oxford.
- Phillips, S.J., Anderson, R.P., Schapire, R.E., 2006. Maximum entropy modeling of species geographic distributions. *Ecological Modelling* 190, 231–259.
- Poinar, H.N., Hofreiter, M., Spaulding, W.G., Martin, P.S., Stankiewicz, B.A., Bland, H., Evershed, R.P., Possnert, G., Paabo, S., 1998. Molecular coproscopy: dung and diet of the extinct ground sloth *Nothrotherops shastensis*. *Science* 281 (5375), 402–406.
- Quintana, J., Kohler, M., Moya-Sola, S., 2011. *Nuralagus rex*, gen. et sp. nov., an endemic insular giant rabbit from the Neogene of Minorca (Balearic Islands, Spain). *Journal of Vertebrate Paleontology* 31 (2), 231–240.
- Raes, N., 2012. Partial versus full species distribution models. *Natureza & Conservação* 10, 127–138.
- Raes, N., ter Steege, H., 2007. A null-model for significance testing of presence-only species distribution models. *Ecography* 30, 727–736.
- Ramirez, O., Gigli, E., Bover, P., Alcover, J.A., Bertranpetit, J., Castresana, J., Lalueza-Fox, C., 2009. Paleogenomics in a temperate environment: shotgun sequencing from an extinct Mediterranean Caprine. *Public Library of Science One* 4 (5), e5670. <http://dx.doi.org/10.1371/journal.pone.0005670>.
- Ramis, D., Bover, P., 2001. A review of the evidence for domestication of *Myotragus balearicus* Bate 1909 (*Artiodactyla*, *Caprinae*) in the Balearic Islands. *Journal of Archaeological Science* 28 (3), 265–282.
- Ramis, D., Alcover, J.A., Coll, J., Trias, M., 2002. The chronology of the first settlement of the Balearic Islands. *Journal of Mediterranean Archaeology* 15 (1), 3–24.
- Rohland, N., Hofreiter, M., 2007. Ancient DNA extraction from bones and teeth. *Nature Protocols* 2, 1756–1762.
- Sánchez-Fernández, D., Lobo, J.M., Hernández-Manrique, O.L., 2011. Species distribution models that do not incorporate global data misrepresent potential distributions: a case study using Iberian diving beetles. *Diversity and Distributions* 17, 163–171.
- Savolainen, V., Fay, M.F., Albach, D.C., Backlund, A., van der Bank, M., Cameron, K.M., Johnson, S.A., Lledó, M.D., Pintaud, J.C., Powell, M., Sheahan, M.C., Soltis, D.E., Soltis, P.S., Weston, P., Whitten, W.M., Wurdack, K.J., Chase, M.W., 2000. Phylogeny of the eudicots: a nearly complete familial analysis based on rbcL gene sequences. *Kew Bulletin* 55, 257–309.
- Schlumbaum, A., Tensen, M., Jaenicke-Després, V., 2008. Ancient plant DNA in archaeobotany. *Vegetation History and Archaeobotany* 17 (2), 233–244.
- van der Geer, A., Lyras, G., de Vos, J., Dermizakis, M., 2010. *Evolution of Island Mammals. Adaptation and Extinction of Placental Mammals on Islands*. Wiley-Blackwell, Chichester.
- Waldren, W.H., 1982. Balearic prehistoric ecology and culture. The excavation of certain caves, rock shelters and settlements. BAR International Series, 149. Archaeopress, Oxford.
- Waldren, W.H., Ensenyat, J., Orvay, J., 2002. New coals on old fires: the question of early Balearic settlement. In: Waldren, W.H., Ensenyat, J.A. (Eds.), *World Islands in Prehistory: International Insular Investigations: V Deia International Conference of Prehistory*. BAR International Series, 1095. Archaeopress, Oxford, pp. 68–90.
- White, T.J., Bruns, T., Lee, S., Taylor, J., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, M.A., Gelfand, D.H., Sninsky, J.J. (Eds.), *PCR Protocols, A Guide to Methods and Applications*. Academic Press, San Diego, pp. 315–322.
- Winkler, D.E., 2010. *Dentition and Diet: New 3D-Methods help to discover Dental Adaptations in the Extinct Bovid Myotragus*. MSc Thesis Hamburg University, Hamburg.
- Winkler, D.E., Schulz, E., Calandra, I., Gailer, J.P., Landwehr, C., Kaiser, T.M., 2013. Indications for a dietary change in the extinct Bovid genus *Myotragus* (Plio-Holocene, Mallorca, Spain). *Geobios* 46, 143–150.
- Withers, P.C., 1992. *Comparative Animal Physiology*. Saunders, London.
- Yli, E.I., Pérez-Obiol, R., Juliá, R., 1994. Vegetational change in the Balearic Islands (Spain) during the Holocene. *Historical Biology* 9 (1/2), 83–89.
- Yli, E.I., Pérez-Obiol, R., Pantaleon-Cano, J., Roure, J., 1997. Palynological evidence for climatic change and human activity during the Holocene on Minorca (Balearic Islands). *Quaternary Research* 48 (3), 339–347.