

MITOCHONDRIAL AND NUCLEAR GENES FROM THE EXTINCT BALEARIC BOVID *MYOTRAGUS BALEARICUS*

Carles LALUEZA-FOX, Lourdes SAMPIETRO, Tomàs MARQUÈS, Josep Antoni ALCOVER & Jaume BERTRANPETIT

LALUEZA-FOX, C., SAMPIETRO, L., MARQUÈS, T., ALCOVER, J. A. & BERTRANPETIT, J. 2005. Mitochondrial and nuclear genes from the extinct Balearic bovid *Myotragus balearicus*. In ALCOVER, J.A. & BOVER, P. (eds.): *Proceedings of the International Symposium "Insular Vertebrate Evolution: the Palaeontological Approach"*. *Monografies de la Societat d'Història Natural de les Balears*, 12: 145-154.

Resum

Myotragus balearicus és un bòvid extingit de les Illes Balears Orientals o Gimnèsies (Mallorca, Menorca i illots que les envolten). *Myotragus* presenta nombroses novetats evolutives que enfosqueixen el seu emplaçament taxonòmic dintre dels Caprinae. A un projecte desenvolupat els darrers anys hem analitzat diferents mostres d'ossos de *Myotragus* i hem recuperat el gen cytb mtDNA complet, una seqüència del gen 12S mtDNA, una seqüència de la regió D-loop, i una seqüència d'un gen nuclear multi-còpia (el rDNA 28 S), emprant tècniques de DNA antic. Diferents controls experimentals, incloent-hi un laboratori dedicat, obtenció independent de rèpliques, solapament de fragments, extraccions múltiples i clonatge de productes PCR, donen suport a l'autenticitat de les seqüències. Els arbres filogenètics nous situen consistentment *Myotragus* a una posició basal al clade *Ovis+Budorcas*. Emperò, alguns gèneres de Caprinae, tals com *Oreamnos* i *Ammotragus*, presenten posicions inestables a tots els arbres. La branca llarga observada a *Myotragus* correspon a una taxa evolutiva elevada a aquesta línia que podria estar associada a la seva petita mida corporal. A més, la recuperació de gens nuclears per primera vegada d'una espècie extingida de la Mediterrània obre noves possibilitats de recerca sobre els trets genòmics i fenotípics.

Paraules clau: *Myotragus balearicus*, DNA antic, Caprinae, DNA mitocondrial, DNA nuclear, filogenia.

Abstract

Myotragus balearicus was an extinct endemic bovid from some of the Balearic Islands (Mallorca and Menorca). *Myotragus* had many evolutionary novelties that obscure its phylogenetic placement among the extant Caprinae. In a project developed during the last years, we have analyzed several *Myotragus* fossil bones, and have retrieved the complete mtDNA cyt b gene (1143 bp in length), a sequence from the mtDNA 12S gene, a sequence from the D-loop region, and a sequence from a multi-copy nuclear gene (the 28 S rDNA), using ancient DNA techniques. Different experimental controls, including a dedicated laboratory, independent replication, overlapping fragments, multiple extractions and cloning of PCR products, support the authenticity of the sequences. The new phylogenetic trees consistently place *Myotragus* in a position basal to the *Ovis+Budorcas* clade. However, some Caprinae species, such as *Oreamnos* and *Ammotragus*, show unstable positions in all trees, attributable to a quick initial radiation of the Caprinae lineages. Moreover, the retrieval of nuclear genes for first time from an extinct species from the Mediterranean area opens new possibilities of research on comparative genomics and genetic bases of phenotypic traits.

Key words: *Myotragus balearicus*, ancient DNA, Caprinae, mitochondrial DNA, nuclear DNA, phylogeny.

INTRODUCTION

DNA sequences have been retrieved from extinct species in the last two decades (see Hofreiter *et al.*, 2001). Almost all the ancient DNA studies, including the first complete extinct genome (Cooper *et al.*, 2001) have been based on the analysis of mitochondrial DNA (mtDNA), the genome of the organelles that provide energy to the cells. The reason of this identification between ancient DNA and mtDNA is due to the fact that the DNA is chemically degraded; since there are thousands of mtDNA copies in each cell, it is much more easier to retrieve mitochondrial than nuclear DNA. To date, only few studies have been able to soundly demonstrate the retrieval

of sequences from nuclear DNA genes from Siberian mammoths, the ground sloth and the cave bear (Greenwood *et al.*, 1999; Orlando *et al.*, 2002; Poinar *et al.*, 2003).

In this study, we have retrieved mitochondrial and nuclear DNA sequences from the extinct *Myotragus balearicus*, a bovid that evolved in isolation in the Balearic Islands (Bate, 1909). *Myotragus* is a extremely modified Caprinae, characterized by a series of unusual morphological traits developed throughout more than three million years of evolution (Alcover *et al.*, 1981; Bover & Alcover 1999a; 1999b; Alcover *et al.*, 1999a; 1999b), that became extinct between 3,640 and 2,135 years cal BC (Ramis & Alcover, 2001), probably after the arrival of first humans to these islands. The morphologi-

cal peculiarities of *Myotragus*, including extreme size reduction, a single evergrowing lower incisor, modified limb bones and frontal eyes (Alcover *et al.*, 1981), makes it difficult to clarify its taxonomic position.

In previous studies (Lalueza-Fox *et al.*, 2000; 2002), we obtained bits of the mtDNA cytochrome *b* (*cyt b*) gene from two different *Myotragus* bone specimens from Mallorca island, one found in Cova Estreta (Pollença) and the other found in Cova des Gorgs (Escorca). The *cyt b* sequences indicated that *Myotragus* seems to be genetically close to the Takin (*Budorcas*) and the Sheep (*Ovis*). Moreover, the quick radiation of all the Caprinae, roughly 20 Mya (Vrba, 1985) and the short *cyt b* fragment retrieved made the previous analysis unable to fully resolve the phylogeny of this group. To try to fully resolve its phylogeny, we decided to retrieve the complete *cyt b* gene and some other genetic markers of *Myotragus*; to do this, we selected a third *Myotragus* sample, excavated in 2002 from Cova des Gorgs, that looked macroscopically very well preserved; we have designed different sets of overlapping primers for retrieving the complete *cyt b* gene, as well as a fragment from another mtDNA gene, the 12 S, and a fragment from the mtDNA HVR I. In addition, we retrieved a nuclear gene from the same extract, 28 S rDNA (located in the 10 chromosome), taking

advantage to the fact that there are some hundreds of copies of this gene in the vertebrate genome (Laudien Gonzalez *et al.*, 1985). Our future aims are to try to retrieve single copy nuclear genes from this species.

MATERIALS AND METHODS

A left tibiae (MNIB 60176) bone from *Myotragus balearicus* from Cova des Gorgs (Escorca, Mallorca), dated to 6,010-5,830 cal BC 2s (Beta-177239) was chosen for DNA analysis because of its excellent external preservation.

DNA was extracted following standard procedures described elsewhere (Lalueza-Fox *et al.*, 2002). The sample was demineralized overnight, incubated overnight with a lysis solution, extracted with phenol-chloroform and concentrated and desalinated with centricon columns. Several fragments of mtDNA *cyt b* gene were successfully amplified and sequenced until complete the whole gene (Table 1).

The *cyt b* sequences matched the sequence previously retrieved from another *Myotragus* bone from the

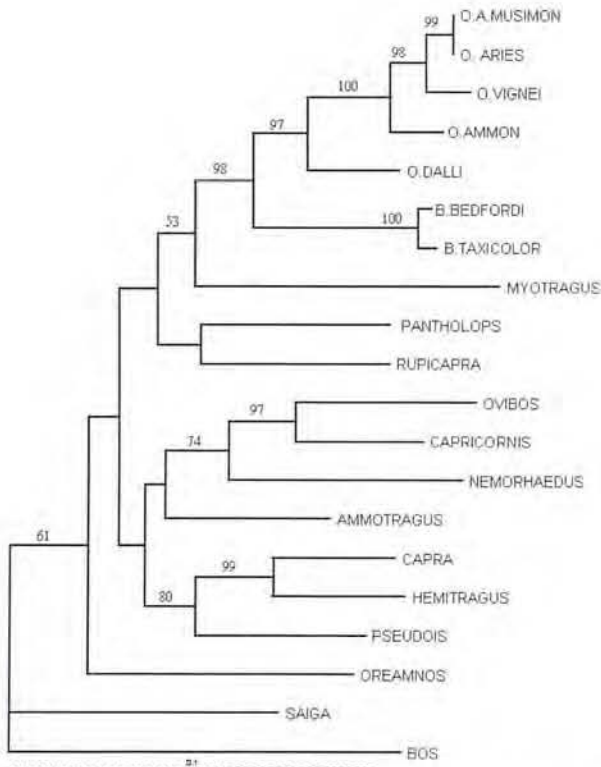


Fig. 1. Maximum likelihood tree of the entire *cyt b* (1143 bp) of all Caprinae species in databases. Nodes with bootstrap support greater than 50% are depicted.

Fig. 1. Arbre de màxima probabilitat del *cyt b* (1143 parells de bases) de totes les espècies de Caprinae. S'indiquen els nodes amb un suport de bootstrap superior al 50%.

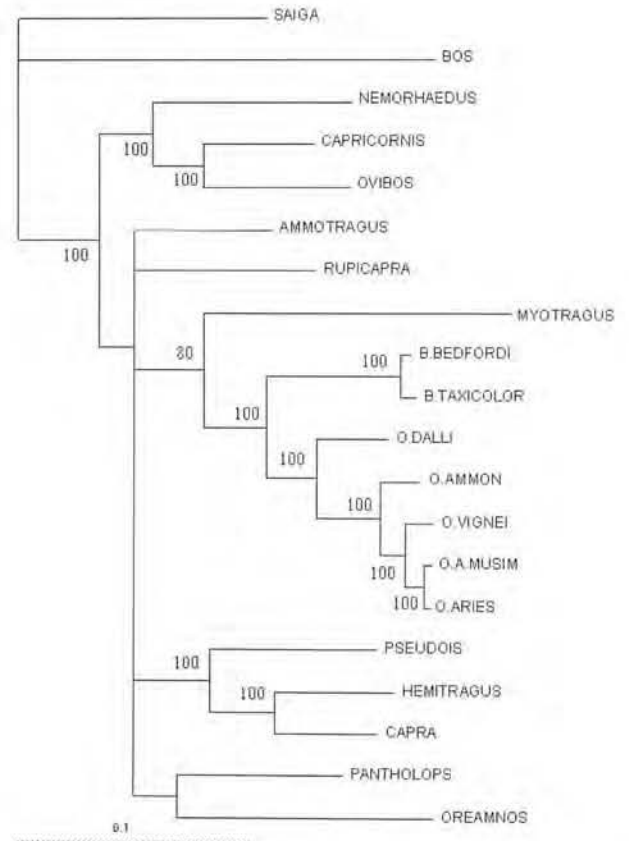


Fig. 2. Bayesian tree of the entire *cyt b* (1143 bp) of all Caprinae species. Nodes with bootstrap support greater than 50% are depicted.

Fig. 2. Arbre Bayesià del *cyt b* (1143 parells de bases) de totes les espècies de Caprinae. S'indiquen els nodes amb un suport de bootstrap superior al 50%.

same site (Lalueza-Fox *et al.*, 2000); multiple controls, such as independent replication of the results in two different laboratories and cloning of different, overlapping PCR products, contributed to the authentication of the sequences. All the work was carried out in a dedicated ancient DNA laboratory, with UV lights, air-positive pressure and regular bleaching of the working surfaces. No signs of contamination with exogenous DNA were observed along the study. For the 12 S gene, some primers were designed to match the previous Caprinae sequences published by Gatesy *et al.* (1997).

Due to the exceptional quality of this extract, a pilot project was launched for trying to retrieve a fragment of a nuclear gene. The 28 S rDNA was chosen because it is present in some hundreds of copies in the genome. The primers used to amplify the 28 S rDNA were those designed by Greenwood *et al.* (1999) to be vertebrate-specific (Table 1). 5 µl of extract were added to 20 µl PCR reactions, containing 1x reaction buffer, 1 unit of taq DNA polymerase, 2.5 mM of MgCl₂, 25 pmol of each primer. Forty cycles of 1 min at 94°C, 1 min at 50°C and 1 min at 72°C were performed. PCR products were resolved in 1% low-melting agarose gels in a TA buffer; bands were excised from the gel and subjected to a second 30 cycles of PCR with limiting reagents. PCR product was cloned with the SureClone Ligation Kit (Pharmacia, Upsala, Sweden) following supplier's instructions. Inserts were sequenced with 3100 Gene Analyzer (Applied Biosystems).

Several phylogenetic analyses were performed with the *Myotragus* sequences; the whole *cyt b* of all Caprinae genera (including subspecies of *Ovis* and *Budorcas*), were obtained from the GenBank, and a maximum likelihood analysis (ML) was performed using PHYLIP v3.6 (Felsenstein, 1993) package. The analysis considered *Bos* as outgroup, although it could be observed that *Saiga* behaved also as a perfect outgroup. To test the robusticity of the tree clades, a 1,000 bootstrap repeats were performed. A maximum parsimony (MP) and NJ tree from a distance matrix (using Kimura two parameters model)

were also generated for the *cyt b* using PHYLIP v3.6. A Bayesian inference of phylogeny was also performed with MrBayes v2.01 programme (Huelsenbeck & Ronquist, 2001), using general time reversible (GTR) model, partition defined for each base position within codons, sites specific rates estimated for each partition and estimated proportion of base types from the data. 500,000 trees were generated, with burning completed by the 5,000th tree; thus, the first 5,000 trees were discarded.

RESULTS

The complete *cyt b* gene (Table 2) was retrieved in 11 overlapping fragments (see Table 1), including the 338 section already retrieved in Lalueza-Fox *et al.* (2002). All *cyt b* fragments were routinely cloned (not shown), although very few heteroplasmies were detected in the direct sequencing, attributable to both the exceptional preservation of the sample and the short lengths of the fragments; consequently, the error rates (number of substitutions/1,000 bp) are very low (<2).

The 12 S gene sequence (Table 4) was retrieved in three overlapping fragments (Table 1), and the short HVRI sequence (Table 3) in a single amplification (Table 1). The 12 S sequence displays a substitution diagnostic of all Caprinae, a G in np 732 of the *Ovis* mtDNA genome (Gatesy *et al.*, 1997). There are fewer sequences available for the 12 S and HVRI region than for the *cyt b* gene; therefore, we focused the analysis mainly in the latter gene.

The PCR of the 28 S nuclear gene yielded a very faint band around 140 bp and was subsequently cloned and sequenced (Table 1). The 96 bp sequence obtained from *Myotragus* is similar to that found in other Caprinae (Table 5), and clearly different to the human one. Moreover, no other bovids, extinct or living, had been analyzed in the same laboratory; therefore, the 28 S sequence seems to be endogenous of *Myotragus*. The

Cyt b	12 S
L14136 5'-GCTTGATATGAAAAACCAATCGTTG-3'	L599 5'-CTCAAAGGACTTGGCGGTGC-3'
H14313 5'-IGTGTCCGGATGTATAGTGTATTG-3'	H673 5'-GAAGATGGCGGTATATAGAC-3'
L14310 5'-ATCCTAACAGGCCTATTCCT-3'	L671 5'-TCACCAATCCTTGCIAATAC-3'
H14481 5'-CCGATGTTTCATGTTTCTAGGA-3'	H805 5'-AATGGCTTTCGTATTAAT-3'
L14475 5'-CGAGGCTGTACTACGGATC-3'	L733 5'-AACAAAGAGTAAGCTCAATCA-3'
H14650 5'-AACTGAGAATCCGCCTCAG-3'	H891 5'-CGGTGTGTGCGTGTCTTCATG-3'
L14631 5'-GCTATCCCATACATGGAAC-3'	
H14813 5'-GTATARTARGGGTGAAATGG-3'	HVRI
L14792 5'-TCCAACAACCCTCAGGAATTC-3'	L16,125 5'-CCTTCTTCTCGCTCCGGGCC-3'
H14987 5'-TTGATCGTARGATTGCGTATGC-3'	H16,227 5'-AATTAGTCCATCGAGATGTC-3'
L14973 5'-CCTCACATCAAACCCGAATG-3'	
H15159 5'-TCCTCCAATTCATGTGAGTG-3'	28 S
L15152 5'-TTCTGAATCCTAGTAGCCGACC-3'	L28S 5'-GGTCGTCCGACCTGGGTATA-3'
H15327 5'-TGCAGTCATCTCCGGTTTACAAGAC-3'	H28S 5'-TCTAATCATTCGCTTTIACCGGAT-3'

Table 1. Primer sequences used in this study for different mtDNA and nuclear genes; L and H refer to light and heavy strand, respectively, and numbers refers to the 3' position of the *Ovis* mtDNA sequence.

Taula 1. Seqüències de cebadors emprades a aquest estudi per a diferents gens de DNA mitocondrial i nuclear; L i H es refereixen a filaments lleugers i pesats, i els nombres es refereixen a la posició 3' de la seqüència de DNA mitocondrial d'*Ovis*.

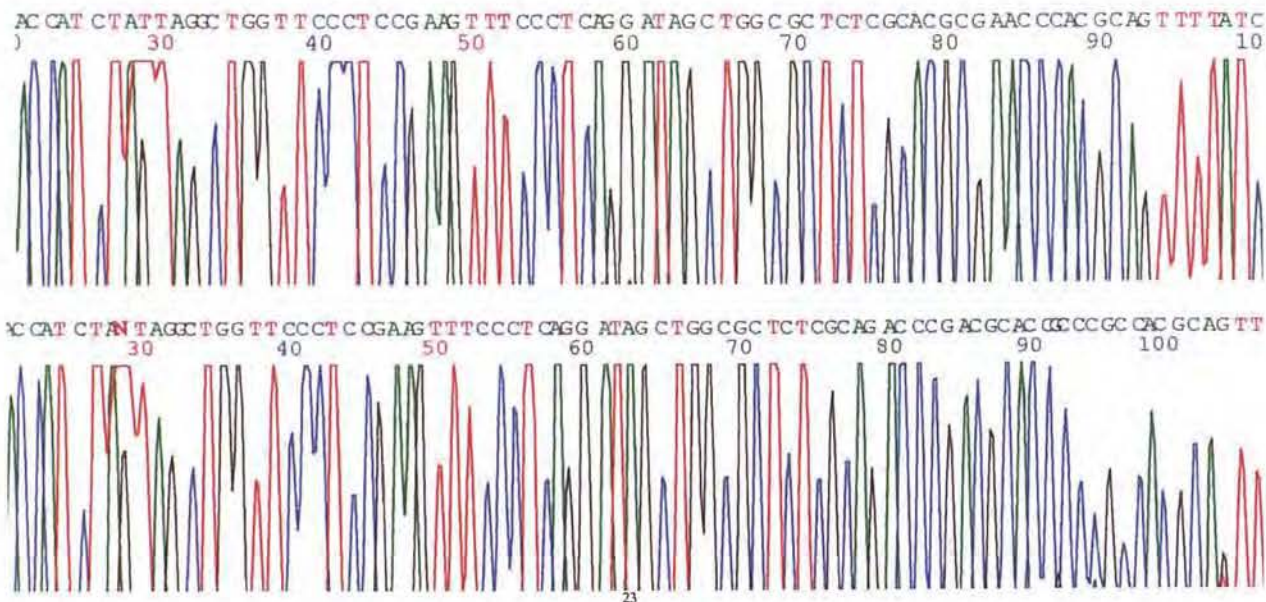


Fig. 3. Sequence chromatogram of the diagnostic fragment of the *Myotragus* 28 S nuclear gene (top), as compared to human (bottom).

Fig. 3. Cromatograma de la seqüència del fragment diagnòstic del gen nuclear 28 S de *Myotragus* (a dalt) comparat amb l'humà (a sota).

cloning of the 28 S PCR product showed that two of the sequences (about 20% of the clones) are human contaminants; this accounts for the noise observed in the direct sequencing of the PCR product. Most likely, the human DNA comes from handling of the *Myotragus* bones during its excavation and posterior morphological study.

The *cyt b* ML tree (Fig. 1) shows a topology roughly similar to that found in previous studies on Caprinae phylogeny (e.g., Hassanin & Douzery, 1999; Lalueza-Fox *et al.*, 2002); some of the clades, that were already supported by high bootstrap figures, are present, specially *Capricornis+Ovibos+Nemorhaedus* (74,3%) and *Hemitragus+Pseudois+Capra* (79,8%). Some species show unstable positions in the trees, especially *Ammotragus*, *Pantholops*, *Rupicapra* and *Oreamnos* (with *Saiga* in a basal position and likely to be not a Caprinae); again, the problematic position of these taxa have been already reported (e.g., Groves & Shields, 1996; Hassanin & Douzery, 1999). However, our tree has been finally able to resolve the phylogenetic position of *Myotragus*, which it is now basal to the *Budorcas+Ovis* clade; the bootstrap value for the *Myotragus+Budorcas+Ovis* grouping is 53,1%. The MP and NJ trees produced an identical tree topology for *Myotragus*, *Budorcas* and *Ovis*, although the already mentioned unstable species differ in their placement among different trees. In the Bayesian tree (Fig. 2), the three stable Caprinae clades (*Capricornis+Ovibos+Nemorhaedus*, *Hemitragus +Pseudois+Capra* and *Ovis+Budorcas*) have bootstrap values of 100; the position of *Myotragus* basal to the latter species is supported by a lower bootstrap figure of 80.

The ML tree with the 12 S fragment produced a slightly different topology (not shown); the *Capricornis+Ovibos+Nemorhaedus* and *Hemitragus+Pseudois+Capra* clades are well supported by bootstrap analysis (85.4% and 70.8%, respectively), while *Myotragus* tend to

cluster basal to the *Capricornis+Ovibos+Nemorhaedus* clade instead of with *Ovis*. This discrepancy most likely can be attributed to its basal placement in the *Budorcas+Ovis* clade in the *cyt b* tree, which cause instability in the *Myotragus* placement when fewer species and shorter DNA sequences are consider, as it is the case with the 12 S gene. In the HVRI sequence (not shown), *Myotragus* shows the highest homology with *Budorcas* (85%).

DISCUSSION

Different genetic trees support the phylogenetic position of *Myotragus* as closely related to *Ovis* and *Budorcas*. From its basal position at the *Ovis+Budorcas* clade, it can be deduced that both taxa diverged after the divergence of the *Myotragus* lineage, likely to have occurred around 5.35 million years ago, when the *Myotragus* ancestors became isolated in the emerging Balearic Islands. However, the complete resolution of the Caprinae phylogeny cannot be achieved with the *cyt b* gene alone, as some species of the subfamily (specially *Oreamnos*) still have an unstable position. This is likely to be due to the initial evolutionary radiation of the Caprinae.

A fairly long genetic branch of *Myotragus* in the ML and NJ trees was previously detected in the short *cyt b* fragment (Lalueza-Fox *et al.*, 2002) and was attributed to a faster substitution rate in this species with respect to other Caprinae. This different rate was initially explained by a putative early age of first reproduction and a shorter generation time in *Myotragus* than in other Bovids (Lalueza-Fox *et al.*, 2002). In mammals, the generation and gestation time are usually related to the body size (see, for instance, Martin & MacLarnon, 1985).

Myotragus is the smallest Caprine known; some adult specimens reached only 22 cm in height. The ratio of neonate to adult *Myotragus* weight has been estimated, from recently found neonate bone remains, to be only 2%, again the smallest ratio ever described in Bovids (Bover & Alcover, 1999a). Although the reproductive strategy and gestation time in *Myotragus* are currently unknown, it seems clear that the extreme reduction in body size affected both biological processes. From the correlation between generation time and body size recorded in mammals (Martin & MacLarnon, 1985) a reduction both in the age of the first reproduction and in the generation time would seem to be the expected result of such a dwarfism process. The long-term isolated evolution towards dwarfism not only occurred in the Balearic Islands, but also in other Mediterranean Islands, where endemic mammals like dwarf deers, dwarf hippos and dwarf elephants were present until the arrival of humans (Burness *et al.*, 2001). Therefore, this an evolutionary hypothesis that could be tested with the retrieval of DNA from other dwarf endemics and the comparison of evolutionary rates of related extant species. However, several tests we applied with the complete *cyt b* data, based on the ratio of synonymous (*Ks* parameter) and non synonymous substitutions (*Ka* parameter) between *Myotragus* and related Caprinae (*Budorcas* and *Ovis*), failed to find statistically significant differences in the *Myotragus* branch length.

An alternative explanation for the long branch of *Myotragus* is that the sub-structuring of its evolutionary lineage is missing; in this sense, there were other Caprinae endemics in Europe and in other Mediterranean islands (e.g. *Nesogoral* and *Gallogoral*) that could be closely related to *Myotragus*. The inclusion of genetic data from these lineages, if technically possible, might significantly alter the *Myotragus* branch pattern we found.

Finally, this study is one of the few instances where ancient nuclear DNA has been recovered, and the first one in which nuclear DNA has been retrieved from an extinct species from the Mediterranean area. The possibility of retrieving nuclear sequences, with a wide range of genetic markers available, could finally allow to clarify the phylogenetic position of *Myotragus* as well as the extant Caprinae with a problematic position. Also, our research demonstrates the potential interest of this approach in comparative genomics; our future goals are to retrieve single copy nuclear genes that can be informative of phenotypic aspects of *Myotragus*.

ACKNOWLEDGEMENTS

This research was supported by the Dirección General de Investigación Científica y Técnica in Spain (project). We are grateful to Alan Cooper and Beth Saphiro (Ancient Biomolecules Center, Oxford), for his technical support in the partial replication of some ancient sequences and to Alexandre Hassanin (Muséum National d'Histoire Naturelle, Paris) for advice in the designing of the 12 S *Myotragus* primers.

REFERENCES

- Alcover, J.A., Moyà-Solà, S. & Pons-Moyà, J. 1981. *Les quimeres del passat. Els vertebrats fòssils del Plió-Quaternari de les Balears i les Pitiüses*. Ed. Moll. Palma de Mallorca. 261 pags.
- Alcover, J.A., Perez-Obiol, R., Yll, E.-I. & Bover, P. 1999a. The diet of *Myotragus balearicus* Bate 1909 (Artiodactyla: Caprinae), an extinct bovid from the Balearic Islands: evidence from coprolites. *Biol. J. Linn. Soc.*, 66: 57-74.
- Alcover, J.A., Seguí, B. & Bover, P. 1999b. Extinctions and Local Disappearances of Vertebrates in the Western Mediterranean Islands. In MacPhee, R.D.E. (ed.), *Extinctions in near time*. 165-188. Kluwer Academic/Plenum Publishers, New York.
- Bate, D.M.A. 1909. A new artiodactyle from Majorca. *Geol. Mag. N.S.*, 6: 385-388.
- Bover, P. & Alcover, J.A. 1999a. Estimating physical characteristics of neonate *Myotragus balearicus* (Artiodactyla, Caprinae). In Reumer, J.W.F. & De Vos, J. (eds.), *Elephants have a snorkel! Papers in honour of Paul Y. Sondaar*. Natuurmuseum Rotterdam. Rotterdam. *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. *Biol. J. Linnean Soc.*, 68: 401-428.
- Burness, G.P., Diamond, J. & Flannery, F. 2001. Dinosaurs, dragons, and dwarfs: the evolution of maximal body size. *Proc. Nat. Acad. Sci. USA*, 98 (25): 14518-14523.
- Cooper, A. & Poinar, H.N. 2000. Ancient DNA: Do it right or not at all. *Science*, 289: 1139.
- Cooper, A., Lalueza-Fox, C., Anderson, S., Rambaut, A., Austin, J. & Ward, R. 2001. Complete mitochondrial genome sequences of two extinct moas clarify ratite evolution. *Nature*, 409: 704-707.
- Felsenstein, J. 1993. PHYLIP (Phylogeny Inference Package) version 3.6. Distributed by the author. Department of Genetics, University of Washington, Seattle.
- Gatesy, J., Amato, G., Vrba, E., Schaller, G. & DeSalle, R. 1997. A cladistic analysis of mitochondrial ribosomal DNA from the Bovidae. *Mol. Phylogenet. Evol.*, 7 (3): 303-319.
- Greenwood, A., Capelli, C., Possnert, G. & Pääbo, S. 1999. Nuclear DNA sequences from Late Pleistocene megafauna. *Mol. Biol. Evol.*, 16 (11): 1466-1473.
- Hassanin, A. & Douzery, E.J.P. 1999. The tribal radiation of the family Bovidae (Artiodactyla) and the evolution of the mitochondrial Cytochrome b gene. *Mol. Phylogenet. Evol.*, 13 (2): 227-243.
- Hofreiter, M., Serre, D., Poinar, H.N., Kuch, M. & Pääbo, S. 2001. Ancient DNA. *Nature Reviews*, 2: 353-359.
- Huelsenbeck, J.P. & Ronquist, F. 2001. MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics Application Notes*, 17: 754-755.
- 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. *J. Exp. Zool. (Mol. Dev. Evol.)*, 288: 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*. *Mol. Phylogenet. Evol.*, 25(3): 501-510.
- Laudien Gonzalez, I., Gorski, J.L., Campen, T.J., Dorney, D.J., Erickson, J.M., Sylvester, J.E. & Schmickel, R.D. 1985. Variation among human 28 S ribosomal RNA genes. *Proc. Natl. Acad. Sci. USA*, 82: 7666-7670.
- Orlando, L., Bonjean, D., Bocherens, H., Thenot, A., Argant, A., Otte, M., & Hanni, C. 2002. Ancient DNA and the population genetics of cave bears (*Ursus spelaeus*) through space and time. *Mol. Biol. Evol.*, 11:1920-1933.
- Poinar, H., Kuch, M., McDonald, G., Martin, P. & Pääbo, S. 2003. Nuclear gene sequences from a Late Pleistocene sloth coprolite. *Curr. Biol.*, 13: 1150-1152.
- Ramis, D. & Alcover, J.A. 2001. Revisiting de earliest human presence in Mallorca, Western Mediterranean. *Proc. Prehistoric Soc.*, 67: 261-269.
- Vrba, E.S. 1985. Africa Bovidae: evolutionary events since the Miocene. *S. Afr. J. Sci.*, 81 : 263-266.

Capricornis	ATG	ATT	AAC	ATT	CGA	AAA	ACT	CAC	CCA	CTA	ATA	AAA	ATT	GTA	AAT	AAT	GCA	TTT	ATT	GAT
NemorhaedusCCCCC
OreamnosCCCCCCC
RupicapraCC	..TCCC
AmmotragusCCCCCCC	..C
BudorcasCCCCC	...	C.C	...
OvibosCC	..T	.CCC	..GC
PseudoisCCCG	..CC	..CCC
HemitragusCCCCC	..CC
CapraCCCG	..C	T..C	..CC
OvisCCCC	..CC
PantholopsCCC	..CC	G..	..C
SaigaCCCTCCC
MYOTRAGUSCC	...	T.CC	A..C	..CCC
Capricornis	CTC	CCA	ACC	CCA	TCA	AAC	ATC	TCA	TCA	TGA	TGA	AAC	TTC	GGC	TCC	CTC	CTG	GGC	ATC	TGC
Nemorhaedus	C..TG	..GA
OreamnosTT	..TTT	...	T.A	..T
Rupicapra	G.T	..GTAA
AmmotragusT	..AA
BudorcasTT	..AACT
OvibosA	..T
Pseudois	G.TT	..AAA
Hemitragus	..TT	..AAT
CapraT	..AA	..A	..T	..G
Ovis	G.TT	..TT	..TTAT
Pantholops	G..TTAT
Saiga	G..A
MYOTRAGUS	G..A	...	G..
Capricornis	CTA	ATT	CTA	CAA	ATC	CTA	ACA	GGC	CTA	TTC	CTA	GCA	ATA	CAC	TAC	ACA	TCC	GAT	ACA	ACG
Nemorhaedus	T..	..CA	...
Oreamnos	T..	...	T..	..TCA	...
Rupicapra	T..	...	T..	..GG	G.A
Ammotragus	T..TTCTA	...
Budorcas	T..	GC.TA
OvibosTTCA
Pseudois	T..	..C.GTCTA
Hemitragus	..G	...	T..	..GGCTA
CapraC	T..GTCTA
Ovis	T..	...	T..	..G	..TT	...	C..	..CA
Pantholops	T.G	G..	T..TAA	...
SaigaCTG.T	..C	...	G.A
MYOTRAGUS	T..	..C	T..TCA
Capricornis	ACA	GCA	TGT	TCT	TCT	GTA	ACA	CAC	ATT	TGC	CGA	GAC	GTA	AAC	TAC	GGC	TGA	ATT	ATC	CGA
NemorhaedusCT	..T
OreamnosC	..CC	..TT
RupicapraC	..CCTC
AmmotragusTC	..C	...	G.CC
Budorcas	..T.C	..C	...	G.CTTC
OvibosTCG	..TC	..T
PseudoisTC	..CG	..T	..CG	..T	..CT
HemitragusTCCTTTT	..TC
CapraT.	..CTT	..TT	..TT	..T	..C
OvisTC	..CCGTGT
PantholopsTCCT	..TTTT
SaigaTC	CACTCCTT
MYOTRAGUSC	..CC	G.C	..TT
Capricornis	TAT	ATA	CAC	GCA	AAC	GGA	GCA	TCA	ATA	TTC	TTT	ATC	TGC	CTA	TTC	ATA	CAC	GTA	GGA	CGA
Nemorhaedus	..CT	..C
OreamnosT	..CT	..CT
Rupicapra	..CTTT	..CTT
AmmotragusGTT
BudorcasGGTT	..G	..T
OvibosCGG
Pseudois	..CCCTG
Hemitragus	..CGGT	A.C
Capra	..CTT	..G	..T	A.C
OvisGTT	..G	..T
Pantholops	..CTT	..G	..T
SaigaTC
MYOTRAGUSTCT	..C	G..T	..GGG

Table 2

Capricornis	GGC	CTA	TAC	TAC	GGA	TCG	TAC	ACT	TTC	TTA	GAA	ACA	TGG	AAC	ATC	GGG	GTA	ATT	CTC	CTA
NemorhaedusATA	...	G.C
OreamnosT	..T	..A	..T	..C	..TAT	..ACT	..
RupicapraTAT	C..AAC
AmmotragusTAT	C..A	..T	G.A	..A	..G	..C
BudorcasG	..TACAA
OvibosAAAT	...
PseudoisTA	..T	..C	..T	C..	..GAA	A.C
HemitragusTAC	...	C..AT	..AC
Capra	..TT	..T	..A	..T	..C	..T	C..AT	..ACG	...
OvisT	..T	..A	..T	..C	...	C..AAC
Pantholops	..TATCGAC
SaigaT	..TC	..T	..A	...	C..AT	..A	..TT
MYOTRAGUSA	C..AA	A..	..C
Capricornis	CTC	ACA	GCG	ATA	GCC	ACA	GCA	TTC	ATA	GGC	TAT	GTC	CTA	CCA	TGA	GGA	CAA	ATA	TCA	TTC
Nemorhaedus	T.T	G..	A.AG
Oreamnos	..T	...	A.AG
Rupicapra	A.AG	..T	..GCG
Ammotragus	T..	G..	A.AAGG
Budorcas	T.T	G..	A.A	T..T	...
OvibosT	A.AT
Pseudois	T..	G..	A.AC	...	T.GT
Hemitragus	A.A	T..G
Capra	...	G.G	A.A	..GT	T..
Ovis	T.T	G.G	A.A	T..
Pantholops	T.T	G..	A.TT	T..
Saiga	T..	G..	A.AA
MYOTRAGUS	T..	...	A.ATT	..C	..T	T..T	..T	...
Capricornis	TGA	GGG	GCT	ACA	GTT	ATT	ACC	AAC	CTC	CTC	TCA	GCA	ATC	CCA	TAC	ATT	GGC	ACA	AAC	CTA
NemorhaedusAT	..TT	..TC	...
OreamnosAC	..C	..TC
RupicapraCC	..CTC
AmmotragusAC	..CT	G..	..G	...
BudorcasA	..AC
OvibosAC	..C	..TC
PseudoisAC	..CTC	..TT
HemitragusAC	..CTTT	..C
CapraAC	..C	..T	..T	..TT
OvisA	..ATTTT
PantholopsA	..AAT	G..
SaigaA	..AC	..CTTT	..C	G..
MYOTRAGUSA	..A	..CCTA	..C	..T	..T
Capricornis	GTA	GAA	TGA	ATC	TGA	GGA	GGA	TTC	TCC	GTA	GAC	AAA	GCC	ACC	CTC	ACC	CGA	TTC	TTT	GCC
Nemorhaedus	..CGAT	..TC
Oreamnos	..CG	..G	..ATC
Rupicapra	..C	..GG	..C	..GTT
Ammotragus	..CGAT	..TT	..TC
Budorcas	..T	..GAA	T..A	T..
Ovibos	..CT	..T
Pseudois	..CGGAGTCC	...
Hemitragus	..CATAT	..AC	..T
Capra	..CATTTCC	...
Ovis	..CGATTT	..C
Pantholops	..CGATTT
SaigaG	..T	..T	..AT	..AC
MYOTRAGUSGAGAAC	..T
Capricornis	TTC	CAT	TTC	ATT	CTC	CCA	TTC	ATC	ATC	ACA	GCC	CTC	GCC	ATA	GTG	CAC	CTA	CTT	TTC	CTC
NemorhaedusCCTTT	ACT	..TC
OreamnosCTGGCC	...	T..
Rupicapra	..T	..CCTT	G..	...	T.ACCC
AmmotragusCCT	G.A	...	G..AC	...	T..
Budorcas	..T	..CCT	G..C	..T	T..
OvibosC	..T	..CTGTTA	..T	T.G	..C
PseudoisCCT	..T	..T	..TC
HemitragusCTT	G..CG	..C
CapraC	..T	..CT	G..TCG	..C
Ovis	..T	..C	..T	...	T..	G..TCC
Pantholops	G..CCC
SaigaCCT	..T	...	G..	..T	..TCT	..T
MYOTRAGUS	..T	..CCT	..TCA

Table 2

Capricornis	CAC	GAA	ACA	GGA	TCC	AAC	AAC	CCC	ACA	GGA	ATC	TCA	TCA	GAC	ACA	GAC	AAA	ATC	CCA	TTC
Nemorhaedus	..T	..G	.T.T	...	C..T.T
OreamnosT	..TAT	.T.	G..TA
Rupicapra	..TA	C..T	G.G	..TT
Ammotragus	..TGT	G..
BudorcasT	C.G	..T	G..	..TTTT
OvibosT	C..G
PseudoisT	C..
HemitragusGG	.T	C..TT
CapraT	C..T
OvisT	C..	..GTT	..C
PantholopsT	C..T	G..T
SaigaT	C..T	T..
MYOTRAGUS	T..T	C..	G..T
Capricornis	CAC	CCC	TAC	TAC	ACA	ATC	AAA	GAT	ATC	CTA	GGC	ATC	GTG	CTA	CTA	ATC	CTC	ACC	CTC	ATA
NemorhaedusT	..T	..TT	GCT	A.AT	..T
OreamnosT	..T	..T	GC.	A.ACT	...
RupicapraT	..C	..TC	..T	GC.	.T.T	...
AmmotragusTCT	GC.	A..C	...
BudorcasT	..TC	..TA	G..	A.A	GT.G	...
OvibosTC	..T	GC.	A.ATT	...
PseudoisTC	..TT	GCT	.CA	..G	G..	..G
HemitragusTC	..TT	T..	GC.	A.AT	..T	GT.	..A	...
CapraTC	..T	T..	A..T	..T	GT.	..A
OvisT	..TC	..TCT	GCT	A.CTG	...
PantholopsT	..C	..TC	GCT	A.AA	..T
SaigaC	..TC	..T	GC.	C.AT	..T	..A	..T
MYOTRAGUSTC	..TCG	A.AT	T.A	..TG
Capricornis	CTA	CTA	GTA	CTG	TTC	ACA	CCC	GAC	CTA	CTC	GGA	GAC	CCA	GAC	AAC	TAC	ACT	CCA	GCA	AAC
Nemorhaedus	T..	..G	...	T.AT	...	T..	..TTT	..C
Oreamnos	T.A	...	T..	T..
RupicapraGA	..TTTT	..CG
AmmotragusA	..TTGT	..CT
Budorcas	T.GA	..T	.T.	..TT	...	G..	..TT	..T	..CTT
Ovibos	T.ATT	..C
Pseudois	T..	T.A	..TC
Hemitragus	T..A	..T	.T.TT	..CTT
Capra	T..AG	..TTC
OvisAG	..T	...	T..C
Pantholops	T..A	..T	T..T	..T	..C
Saiga	..TC	..A	..T	T..	..AG	..TR
MYOTRAGUSAAT	..T	..T	..AC
Capricornis	CCA	CTC	AAC	ACA	CCC	CCT	CAC	ATC	AAG	CCC	GAG	TGA	TAC	TTC	CTA	TTT	GCA	TAC	GCA	ATC
NemorhaedusGT	..A	..T	..ATT
Oreamnos	..G	..A	..TT	..C	..TA
RupicapraAAT	...	T.GT	..G
AmmotragusT	..T	..A	..T	..A
BudorcasA	..T	..AT
OvibosT	..A	..A
PseudoisT	..A
HemitragusT	..A	..T	..AT	..TG
CapraT	..A	..T	..G	..G	..T
OvisTTA	..T	..AG
Pantholops	..CA	..CT	..A	..T	..A	..GT
SaigaTA	..T	..T	..A	..AC
MYOTRAGUSAAACT
Capricornis	CTA	CGA	TCA	ATC	CCC	AAC	AAA	CTA	GGC	GGA	GTT	TTA	GCC	CTA	GTC	CTC	TCA	ATT	CTA	ATC
Nemorhaedus	T..TC	C..GTT	...
Oreamnos	T..TAC	C..CCCG	A
RupicapraTT	..AC	C..ACC
AmmotragusT	..TG	..A	..C	..C	C..	..AC	T..
Budorcas	T..AC	..CACCG
OvibosT	..TC	..CCCCT	...
PseudoisTGAC	C..ACC
Hemitragus	T..TAC	C..CC
CapraTAC	C..CC
Ovis	T..T	..TAC	C.CACGG
PantholopsAC	C.GGCTT
Saiga	..CT	..T	..TAC	C..AC
MYOTRAGUSTAC	C..G

Table 2

Capricornis	CTA	GCA	CTC	GTA	CCC	TTC	CTC	CAC	ACA	TCC	AAA	CAA	CGA	AGC	ATA	ATA	TTC	CGA	CCA	ATC	
Nemorhaedus	A.TT	C..G	
Oreamnos	...	AT.	T.TTT	..T	..TTGT	
Rupicapra	..G	..T	C..	..TG	
Ammotragus	...	AT.TGG	
BudorcasT	A..	A.G	...	C..A	..G	
OvibosT	
PseudoisT	..T	A..TG	
Hemitragus	T..	A..	..TT	
Capra	T..	..T	..TTTC	...	
OvisT	A.T	A..	...	C..TA	..GG	
PantholopsTTT	..TGTT	..T	..T	
SaigaTC	...	A..GTGT	..T	
MYOTRAGUSTG	..T	A..	...	C..ATAGT	..T	
Capricornis	AGC	CAA	TGT	ATA	TTC	TGA	ATC	CTA	GTA	GCA	GAT	TTA	CTT	ACA	CTC	ACA	TGA	ATT	GGA	GGA	
NemorhaedusC	T..CTA	G..	
Oreamnos	..TC	G..CC	C..	..A	G..C	
RupicapraCTC	C..	T.A	
AmmotragusCGCA	
BudorcasCTC	..C	C..	..AC	...	
OvibosACG	
PseudoisCC	C..	..AC	
HemitragusCC	..G	..C	..GG	
CapraC	G..C	..T.A	
Ovis	..TC	..C	C..	T.A	
Pantholops	..TCC	C..	T.ATC	
SaigaC	C..	...	G.TC	C..	..A	
MYOTRAGUS	..T	C..	G..T	T..C	C..	..AC	...	
Capricornis	CAG	CCA	GTC	GAA	CAC	CCC	TAC	ATT	ATT	ATT	GGA	CAA	CTA	GCA	TCC	ATC	ATA	TAC	TTC	CTA	
Nemorhaedus	..A	T.TTCG	..TTT	...	T.C	
Oreamnos	..ATA	G.CCGTCC	
RupicapraTCTTCC	
Ammotragus	..A	A..T	..A	..CTTCC	..C	
BudorcasTTG	..TTTCC	
OvibosGT	..T	..CC	...	
Pseudois	..AT	..CTTTTT	
Hemitragus	..AT	..T	..TCGTTTTC	
CapraT	..T	..TT	..TT	..TTCC	
OvisTCT	..TT	..TTTT	
Pantholops	..AT	..T	..CTTT	
Saiga	..AT	...	T.T	..A	..TCA	..CATT	..T	..T	
MYOTRAGUSTTGT	..TTTT	..T	
Capricornis	ATC	ATC	CTA	GTA	CTG	ATG	CCA	GTA	GCT	AGT	ACC	ATC	GAA	AAC	AAC	CTC	CTA	AAA	TGA	AGA	AAA
Nemorhaedus	..TA	..AG.CT	
OreamnosTA	..AC	..T	
Rupicapra	..TA	..ACA	..T	
AmmotragusTA	..ACG	..TTT	
BudorcasTT	..A	..ACTT	
OvibosG	..G	..A	...	A.GC	..T	
PseudoisT	T..	..A	..AC	..G	..TNC	...	
Hemitragus	..T	..TA	..AC	..C	..C	..TG	C..	...	
Capra	..T	..TA	..AC	..C	..C	..TTCC	
OvisTC	..A	..AC	..TCC	
Pantholops	..TT	..A	..AC	..C	..TCC	
Saiga	C.T	..TA	..AC	..C	..T	..TTGA	..TT	..T	
MYOTRAGUSTC	..A	..AG	..G	..CCC	

Table 2 [pages 0-0]. *Myotragus* sequence of the complete mtDNA cyt b, aligned with the sequence of all Caprinae species. Dots indicate sequence identity.

Taula 2 [pàgines 0-0]. Seqüència completa del mtDNA cyt b de *Myotragus*, alineat amb la seqüència de totes les espècies de Caprinae. Els punts indiquen identitat a la seqüència.

BOS	CATAAACCGTGGGGGTCGCTATCCAATGAATTTACCAGGCATCTGGTTCCTTCTTCAGGGCCATCTCACC
CAPRA	...T.....A.A..TG.....CC..TA..AG.....T
MYOTRAGUS	..T...T.....A...AAG.G...CCG..TA..A.....G..
OVIS	..T..T.....A.A..TG.....C..TA..AG.....T
CAPRICORNIS	..T..TT.....A...TT.....T..A.....
BUDORCAS	...T..T.....A...TG.....T.....
BOS	TAAACAGTCCATCTTCTCTTAAATAA
CAPRAC.C.....
MYOTRAGUSC.....C.C.....
OVISC.C.....C.....
CAPRICORNISTC.C...CA.....C.....
BUDORCASC.C.....C.....

Table 3. *Myotragus* sequence of a mtDNA HVRI fragment, aligned with sequences found in GenBank of other Caprinae (including *Bos* for comparison).

Taula 3. Seqüència d'un fragment del gen mtDNA HVRI de *Myotragus*, alineada amb les seqüències d'altres Caprinae trobades al Banc de Genes (incloent-hi *Bos* per comparació).

