Pharmacological properties of blister beetles (Coleoptera: Meloidae) promoted their integration into the cultural heritage of native rural Spain as inferred by vernacular names diversity, traditions, and mitochondrial DNA

Nohemí Percino-Daniel (1, 2), David Buckley (1) & Mario García-París (1,*)

(1) Museo Nacional de Ciencias Naturales, MNCN-CSIC. José Gutiérrez Abascal, 2. 28006 Madrid. Spain.

(2) Current address: Escuela Nacional de Antropología e Historia. Isidro Favela s/n, Delegación Tlalpan. DF. Mexico.

* Author for correspondence: mparis@mncn.csic.es. Phone: 0034914111328 ext 1124

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Abstract

Ethnopharmacological relevance

Beetles of the family Meloidae (blister beetles) are often reported in pharmacological literature because of their content of cantharidin. Cantharidin has a long history in human medicine and was commonly applied in the19th and early 20th centuries, although its use has been progressively abandoned since then. Contrary to most, even common, large species of coleoptera, blister beetles of the genera *Berberomeloe*, *Physomeloe* and to a lesser extent *Meloe*, are usually recognized and often incorporated into local folk taxonomy by inhabitants of rural areas in Spain.

Aim of the study

To demonstrate the role that pharmacological properties of blister beetles must have played in their integration in the culture of early Iberian human societies, but also in the preservation of their identity until today, a rare case for Spanish insects. To achieve this purpose we document the diversity of vernacular names applied in rural areas of Spain, and we determine, using molecular data, the antiquity of the presence of two species of the better-known blister beetle in rural Spain, *Berberomeloe majalis* and *B. insignis*.

Materials and methods

We try to document the extent of traditional knowledge of meloid beetles in rural areas by interviewing about 120 people from villages in central and southern Spain. We also use mitochondrial DNA sequences (Cytochrome Oxidase I and 16SrRNA) obtained from several populations of two species of the better known blister beetle in rural Spain, *Berberomeloe majalis* and *B. insignis*, to determine wether these beetles were already present in the Iberian Peninsula when earlier ancient cultures were developping.

Results

Our results show that, based on mitochondrial DNA, blister beetles of the genus *Berberomeloe* were present in the Iberian Peninsula long before humans arrived, so ancient Iberian cultures were in contact with the same beetle species occurring now in rural areas. On the other hand, people interviewed in rural communities provided us with more than 28 different vernacular names, a few short songs incorporated to local folklore, and some therapeutical uses.

Conclusion

Current knowledge of blister beetles of the family Meloidae in rural Spain was likely developped as a consequence of their pharmacological properties; we hypothesize this knowledge was inherited from ancient pre-Christian Iberian native cultures as part of their traditional therapeutical traditions. It is possible then, that current vernacular names and traditional songs are the only remanents of an ancient knowledge of pharmacological uses of meloid beetles, verbally transmitted from the ancestral cultures to modern day rural Spain. Our work suggests that this legacy, part of the European Cultural Heritage, is dissapearing fast, in parallel to the loss of traditional agricultural techniques.

1. Introduction

Vernacular or common names for insects, as for most other organisms, are known for species that share a close (economically based) relation with humans, either as pests, food resources, industrial activities and disease vectors, for instance, or less frequently for "cute" conspicuous harmless animals that are integrated in the "folk" traditions of local comunities (Conklin, 1961; Jain, 1963; references in Conklin, 1972). The number of species that receive enough popular attention to deserve a vernacular name or even to be properly identified is, thus, variable from country to country, and even from region to region (Bailenson et al., 2002: 26; Singh, 2008). In Spain, differences on the popular knowledge of marine and terrestrial invertebrates are notorious. While anyone in Spain is able to provide specific vernacular names for species of crabs or squids used mainly as food (the official list of common names for commercial seafood and marine fish takes 42 pages: MAPA, 2008; see also Löfling, 1753 for fishes), most people are unable to distinguish among all dark beetle-like terrestrial invertebrates which unvariably receive a general distasteful name like cockroaches ("cucarachas") or bugs ("bichos").

Blister beetles of the family Meloidae seem to escape from this rule. Not relevant as pests in Spain, not edible, poisonous but not harmful to people since they have no inoculation mechanisms, blister beetles of the genera *Berberomeloe*, *Physomeloe* and *Meloe* are known by different vernacular names in rural Spain (Fernández de Gata, 1900; Boscá Seytre, 1916; Bolívar Pieltain, 1926). This is surprising having into account that almost no other Coleoptera, except the ladybug [*Coccinella septempunctata* (Linnaeus, 1758)], the fireworm (*Lampyridae*), the stag beetle [*Lucanus cervus* (Linnaeus, 1758)], and a few pests, are clearly and generally identified as distinct by its own name in Spain (López de Guereñu, 1957; García Arias, 1975; Hernández & Molina Martos, 1999; Martín Piera & López-Colón, 2000; Zabala et al., 2003).

Why are meloid beetles known by rural people in Spain? Beetles of the family Meloidae are well known along historical pharmacological literature because of their high content of cantharidin (Dioscorides, 1636; Fischer, 1827; Tan et al., 1995; Fernández-Rubio et al., 2008). Cantharidin, a natural toxin (Carrel & Eisner, 1974; Karras et al., 1996; Dupuis & Berland, 2004) that, among other effects, inhibits the activity of serine-threonine protein phosphatases (Honkanen, 1993), has been used as an epispastic compound, an abortifacient, and even as an aphrodisiac, for quite a long period, particularly along late XIX and early XX centuries (Deyrolle, 1873; Amor Mayor, 1860; Górriz Muñoz, 1882; Bologna, 1991; Ferrer, 2012). Its use was progressively abandoned since then, except for dermatological treatments

(Moed et al., 2001) and recent studies in relation to antitumoral properties (Wang, 1989; Wang et al., 2012). Medical use of cantharidin in Europe was however concentrated in cities, and according to previous reports most of the specimens used for trade did not come originally from Spain (despite their widely used commercial name: “Spanish fly”) (Olivella Riu, 1930).

Our hypothesis is that current knowledge of blister beetles of the family Meloidae in rural Spain was developped as a consequence of their pharmacological properties; however, we believe this knowledge is not related to the relatively recent use of cantharidin in modern human medicine, but rather we hypothesize it was inherited from ancient Iberian native cultures. We discuss in this paper two lines of evidence: (1) We try to document the extent of knowledge of meloid beetles in rural Spain by interviewing about 120 people from villages in central and southern Spain. This survey was intended to evaluate wether villagers use a particular name for meloid beetles (particularly of the genus *Berberomeloe*), and also to inquire why they know about them. These field data were complemented with a bibliographic search on XIX and early XX century Spanish literature about the medical use and the trade of meloid beetles. And (2) we used mitochondrial DNA sequences obtained from different Spanish populations of two blister beetle species that are better known in rural Spain, *Berberomeloe majalis* (Linnaeus, 1758) and *B. insignis* (Charpentier, 1818), to determine wether the beetles were already present in the Iberian Peninsula when earlier cultures were developping (Taberlet et al., 1998; Hewitt, 1999, 2000; Recuero & García-París, 2011), or on the contrary, wether the species arrived recently following holocene climate changes or human mediated transport as some other well known Iberian species did (Carranza et al., 2004; Recuero et al., 2007).

Based on the results we propose that current knowledge of blister beetles of the family Meloidae in rural Spain developped as a consequence of their pharmacological properties; however, we believe this knowledge is not related to the relatively recent use of cantharidin in modern human medicine, but rather we hypothesize this knowledge was inherited from ancient pre-Christian Iberian native cultures as part of their traditional therapeutical traditions. It is possible then, that current vernacular names are the only remanents of an ancient knowledge of pharmacological uses of meloid beetles, verbally transmitted from the ancestral cultures to modern day rural Spain. Our work tried to provide an account of the vernacular names used in Spain for these beetles - an indication of their relevance in the past rural life -, as a contribution for the European Cultural Heritage, before they dissapear completely from the speaking tradition, in parallel to the loss of traditional agricultural techniques.

2. Materials: Blister beetles (Coleoptera: Meloidae)

Meloid beetles include about 2500 species (Bologna, 1991) distributed over most of the world, excluding New Zealand, Eastern Polynesian Islands, and the regions around glaciated areas. Most meloids have high concentrations of cantharidin, an organic compound with strong effects on both vertebrates and invertebrates. The eficiency of cantharidin as a deterrent for insect predators is well documented (Carrel & Eisner, 1974), while its action poisoning and killing horses and other livestock is widely reported in the veterinary scientific literature (Bahme, 1968; Ray et al., 1980a, b; Beasley et al., 1983).

Figure 1

Besides its general toxic action, high concentrations of cantharidin in the blister beetle tissues favored their use in pharmacological practices including poisoning, abortion, skin blistering (Fig. 1), and even as a powerful aphrodisiac (Beauregard, 1890). As documented in classic texts, beetles of the family Meloidae were well known by ancient European cultures well before Christ. According to Tan et al. (1995), for instance, references to the use of Meloid beetles for medicinal purposes in China date back to one or two centuries before Christ. Similarly, Dioscorides published *De materia medica* original text, compilating all previous pharmacological knowledge, around the year 77 AD (Encyclopaedia Britannica, 2012). Blister beetles were also recorded in the Compendium of Materia Medica published by Li Shi-zhen of Ming Dynasty in 1596. Ancient Greeks used for these beetles the names "*buprestis*" and particularly "*cantharis*", names which unfortunately were applied by Linnaeus (1758) to other coleopteran families that are neither poisonous, nor contain cantharidin (Buprestidae and Cantharidae). Geoffroy (1762) tried to solve the problem renaming some of these groups, but rules of Zoological Nomenclature do not allow for such changes (ICZN, 1999). Cantharidin was well known for causing blisters in human skin and for strong irritation of nephritic and urinary tracts following ingestion (Craven & Polak, 1954; Nickolls & Teare, 1954; Wertelecki et al., 1967). This latter property generated its "fame" as an aphrodisiac in the High Middle Age (Covarrubias, 1611), while skin blistering attracted the attention of many physicians and pharmacy dispatchers along most of the XIX and early XX centuries (Amor Mayor, 1860; Górriz Muñoz, 1882). References to the use of blister beetles because of their pharmacological properties are extensive in Chinese scientific literature (see references in Wang, 1989, and Tan et al., 1995), including references to antitumoral properties of cantharidin or fatal intoxication cases (references in Wang, 1989). A drug containing cantharidin is currently in use to treat human skin warts and other dermatological affections (Moed et al., 2001).

Trading of blister beetles as the only source for cantharidin in Europe reached a considerable level (Olivella Riu, 1930) and, as a consequence, the Greek names were replaced by a more sounded one, "*Spanish fly*", which was used in pharmacy until recent times. This name was specifically applied for one of the commonest meloid beetles in Central and Western Europe (*Lytta vesicatoria*) although, in general, specimens from different meloid species were pooled and mixed for the commercial trade and compounds (Olivella Riu, 1930). Despite its name, *L. vesicatoria* has a restricted distribution in Spain, limited to hilly or montane areas in the northern regions and along the central mountains (García-París *et al*., 1993)

Popular pharmacological applications of the "*Spanish fly*" ranged from the absurd to the medically founded (Moed et al., 2001), but most of the information available came from commercial uses and their publicited properties. We lack, however, of information about the local pharmacological use of the cantharidin in rural areas, areas in which, as a matter of fact, the beetles live and are found (Amor Mayor, 1860; Górriz Muñoz, 1882; Lückmann &

Niehuis, 2009). It is also not known when these pharmacological uses were initiated, whether they were recently imported or have been traditionally used. We here explore these issues, through the study of the popular knowledge, folklore, and traditional uses of meloid beetles in rural areas of Spain. We conducted a field ethonographic survey to determine the extent to which the species and their pharmacological uses are known. We also conducted a molecular study to infer the history (time ranges) of the presence of blister beetles in the Iberian Peninsula.

Figure 2

3. Methods

Field data on vernacular names were gathered through a field ethnographic survey directed to local people in rural areas, mostly in central and southern Spain. One or two live adult specimens of *Berberomeloe majalis* or *B. insignis* (in the southeastern areas), or alternatively *Physomeloe corallifer* (Germar, 1818) or *Meloe proscarabaeus* Linnaeus, 1758 (particularly in the northern areas) (Fig. 2a, b, c, d) were presented in plastic containers to the selected subjects. One of the main objectives of the survey was to assess whether people from rural areas knew the name of the insect presented, and second, how much they knew about the local uses of the insect shown. Subject selection was initially close to random, but always in rural environments, outside the summer vacation periods. People selected, male or female indistinctly, were working or walking in the fields near villages, or sitting, isolated or in groups, within the villages. Once the specimen was presented, we lead a casual conversation with the interviewed person not asking directly for uses of the animals, allowing them to express freely whatever observation they considered pertinent. After a series of negative responses by young people (see results), selection was centered on aged persons well above 40 years old. Villages were selected in order to maximize the geographic area where *Berberomeloe* beetles occur. The list of villages where interviews were carried out is indicated in Table 1 (Fig. 3). A second set of data came from colleagues and entomologists that knew about local names or traditions related to these animals from their regions of origin. Finally, a third source of data was obtained from Spanish pharmaceutical or pharmacological reports (mostly dissertations) published during the second half of the XIX century or the first half of the XX century. Vernacular names recorded in Extremadura (southwestern Spain) were added from a web resource (Vozdemitierra, 2008) (Table 1, Fig. 3).

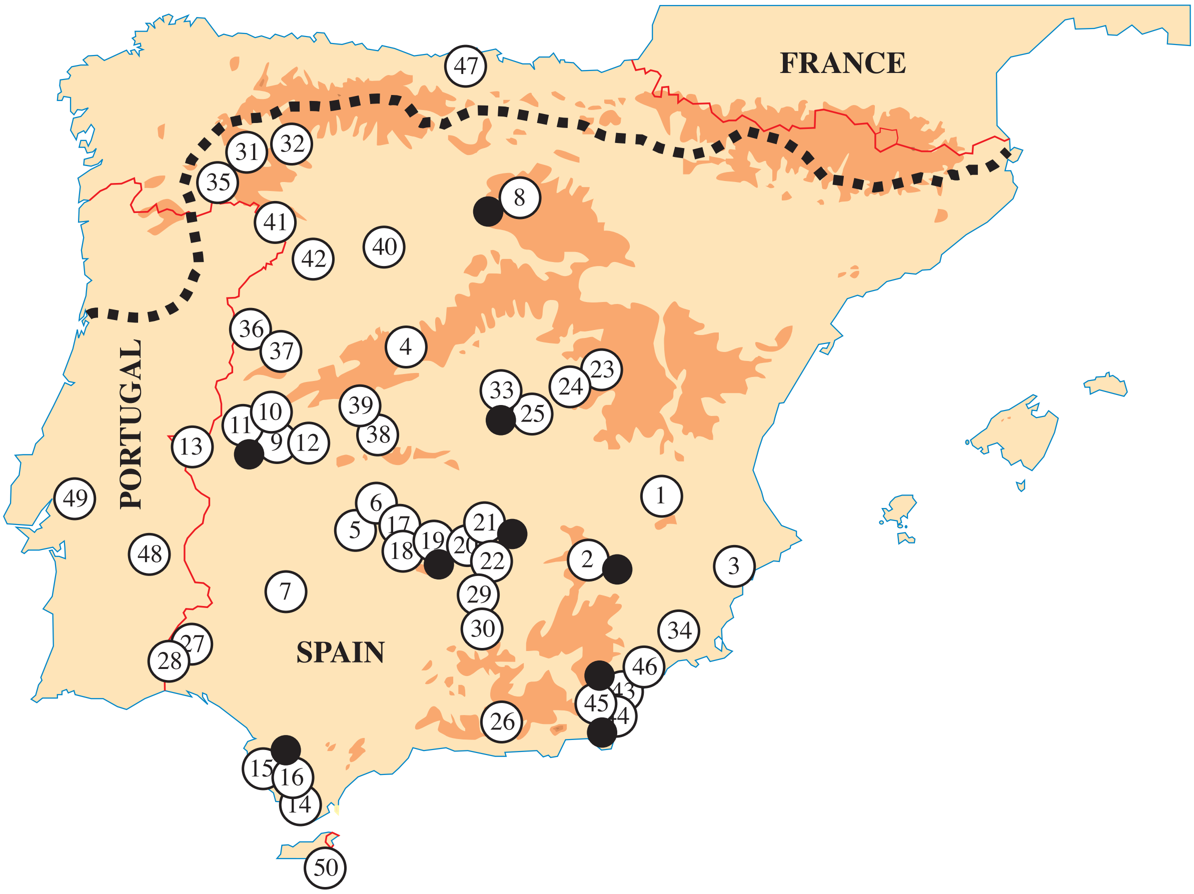

Figure 3

A detailed search on blister beetle related scientific literature published before 1933 (García-París & Ruiz, 2005) rendered 105 publications available for study. We added European and American literature for the same period, focussing in Spanish language, and publications from 1994 to present, centered in the study of Spanish species. We performed additional searches using non-zoological bibliographical sources, trying to find supplementary information for the period included along the late XIX century and the early XX century, a period of intense commerce of dehydrated blister beetles (Olivella Riu, 1930).

For the molecular part of the study, we sampled in the field for specimens of four species of Iberian meloid beetles, *Berberomeloe majalis*, *B. insignis*, *Oenas fusicornis* Abeille de Perrin, 1880 (all three belonging to the tribe Lyttini) and *Physomeloe corallifer* (tribe Meloini). We focused on the two *Berberomeloe* species, which are the two better-known blister beetles in rural Spain. We wanted to analyze whether these two species were present in the Iberian Peninsula well before the first human settlements, or if the species could have arrived following Holocenic climatic changes or through more recent human mediated transport and introduction. Therefore, we designed the sampling of specimens of *Berberomeloe majalis* and *B. insignis* to cover a large portion of the species' known distribution range in the Iberian Peninsula (Table 2; Fig. 3: black dots), in order to capture the genetic variability of the species in the area and to estimate the time to the most recent common ancestors (TMRCAs) of the different populations, lineages, and species. Twenty-one beetles from 13 localities distributed along the Iberian Peninsula were collected in the field (Table 2, Fig. 3), and preserved in ethanol as vouchers at the Museo Nacional de Ciencias Naturales (MNCN-CSIC). We included the species *O. fusicornis* and *P. corallifer* as outgroups for the phylogenetic analyses.

Tissue was extracted from the coxae or femora of specimens preserved in ethanol (96º to absolute) stored at -20ºC. Total genomic DNA was extracted using protocols described by Alcobendas et al. (2008). Polymerase chain reaction (PCR) was used to amplify 630 base pairs (bp) of the mitochondrial Cytochrome Oxidase I gene (cox1), using the primers LCO1490 (Folmer et al., 1994) and COI-H (Machordom et al., 2003), as well as another mitochondrial fragment of 760 bp including a small portion of the ND1 gene, the tRNA Leucine and part of the 16S ribosomal RNA using the primers 16Sbr and 16Sar (Palumbi et al., 1996). PCR reactions were performed in a total volume of 25 ml, including one unit of Taq polymerase (Biotools, 5 U/ml), 2.5 mM of each primer, 0.4 mM of dNTPs, 1.5 mM of $MgCl_2$ and 67 mM of a reaction buffer (Tris-HCl, pH=8.3, Biotools). PCR reactions typically consisted of 35 cycles with a denaturing temperature of 94 ºC (30 sec), annealing at 42 ºC (45 sec), and extension at 72 ºC (90 sec). Double strand templates were cleaned using Sodium acetate and ethanol to precipitate the PCR products and then re-suspended in 22 ml of ddH_2O. Sequencing reactions were performed for both strands and sequenced on an ABI PRISM 3730 DNA sequencer as explained in Martínez-Solano et al. (2006).

Sequences were compiled using Sequence Navigator™ version 1.0.1 (Applied Biosystems) and aligned manually. We determined the partition scheme and substitution models that best fit the character matrix by means of the software PartitionFinder (Lanfear et al., 2012) under the Akaike Information Criterion (AIC). Phylogenetic analyses were performed using Maximum Likelihood and Bayesian Inference, using the software packages raxmlGUI (Stamatakis, 2006; Silvestro & Michalak, 2011) and Mr. Bayes 3.2 (Ronquist et al., 2012), respectively. In both cases, we partitioned the data matrix by genes and by codon positions (cox1), following the partition scheme suggested in PartitionFinder. Maximum Likelihood analyses were run under the GTRGAMMAI model, with 1000 bootstrap replicates to assess support for the nodes in the resulting phylogenetic tree, which was visualized and edited in FigTree v1.3.1 (http://tree.bio.ed.ac.uk/software/figtree/). For the Bayesian analysis, instead of specifying as priors for the analysis the substitution model defined in PartitionFinder for each partition, we let the analysis to explore the substitution model space (option ‘lset nst=mixed rates=invgamma’). Setting it up this way, the analysis returns the probabilities of each substitution model (above a threshold of 0.05) together with the uncertainty associated to the models. Furthermore, all the parameter values resulting from the analysis, including the topology and branch lengths, are averaged across the estimated models and weighted according to their posterior probabilities. To estimate the posterior probabilities of all the parameters, we ran four Markov Chains Monte Carlo (MCMC) for $100x10^6$ generations sampling every 10.000 generations, in two independent runs. We repeated this procedure twice, and check for convergence and stationarity of the MCMC runs assessing the Estimated Sample Size (ESS) values, the Potential Scale Reduction Factor (PSRF), and the trace of each parameter and runs in Tracer v1.5 (Rambaut & Drummond, 2007). To generate the final phylogenetic hypothesis, we discarded the 25% of the obtained trees as burnin, and generated an annotated 50% Majority Rule consensus tree in Mr. Bayes, which was visualized and edited in FigTree v1.3.1 (http://tree.bio.ed.ac.uk/software/figtree/). A general overview of the phylogenetic methods employed can be found in Yang & Rannala (2012).

To estimate the age of the two *Berberomeloe* species and, therefore, to estimate the temporal range of the presence of the species in the Iberian Peninsula, we followed a dating approach based on the use of molecular clocks. Basically, molecular clocks relate the amount of genetic differentiation between pairs of taxa to temporal estimates. Through the use of a molecular clock, we can potentially transform the branch lengths of a phylogenetic tree (i.e., a measure of the genetic differentiation among taxa) on temporal estimates, provided we know this relationship between ‘the amount of evolution’ expressed in the branch lengths occurring per unit of time. Specifying this relationship is known as ‘calibrating’ the molecular clock. Ideally, molecular clocks should be calibrated *ad hoc* for each specific study, normally incorporating information form the fossil record or paleobiogeographic events, bringing an independent source of temporal information for some nodes of the phylogenetic tree. From this independent information, the relationship between substitution rates, branch lengths, and time estimates can be established, and the divergence times calculated for every node in the tree. Since there are no explicit fossil records with which we could calibrate the molecular clock for the present data set, we checked on the literature for reported substitution rates for cox1 and 16S in beetles from related and unrelated families [Tenebrionidae (Papadopoulou et al., 2010), Carabidae (Andújar et al., 2012), Zopheridae (Baselga et al., 2011)]. As a conservative approach, we specify broad substitution rates intervals per unit of time that would include known substitution rates for beetles, as well as potential outliers with fairly low or high substation rates (Brower, 1994; Papadoloulou et al., 2010). We used this substitution rates (substitutions/site/my) to calibrate the molecular clocks in the analysis. Although this approach is not exempt from error, we were not interested in obtaining close estimates of divergence times between species, but realistic time ranges for which the presence of the species in the Iberian Peninsula could be determined.

Until recently, most of dating studies relied on the use of 'strict molecular clocks' to estimate divergence times. Basically, a strict molecular clock assumes that the rate of evolution is constant and uniform among all the lineages in a tree. Once a calibration for the clock is provided, it is then rather straightforward to transform all the branch lengths of the tree in times estimates. This basic biological assumption of constancy, however, does not hold true in many real natural systems. Therefore, a new suit of molecular clocks has been developed to account for heterogeneity and variation in rates of evolution among lineages. These clocks are known as 'relaxed molecular cloks' (e.g., Drummond et al., 2006). We here take advantage of these new methods to explore the temporal ranges for the presence of the studied species in the Iberian Peninsula.

Dating analyses were run under a Bayesian-coalescence framework as implemented in BEAST v.1.7.4 (Drummond & Rambaut, 2007; Drummond et al., 2012). Basically, BEAST is a Bayesian framework that uses a population/demographic model (the coalescent) to infer the genealogical history of a set of molecular sequences, together with all the possible parameters that have shaped this genealogical history (e.g., population size, substitution rates, divergence times, etc.). Interestingly, BEAST takes advantage of the Bayesian statistical inference to jointly estimate all the parameters involved (including the topology of the tree, branch lengths, and divergence times, for instance). For each parameter, then, we obtained its posterior probability, which is inferred from a Markov Chain Monte Carlo (MCMC) sampling method. A full account of these methods can be found in Beaumont & Rannala (2004), Drummond & Rambaut (2007), Drummond et al. (2012) and Yang & Rannala (2012). We first run a series of analyses following the partition scheme suggested in PartitionFinder and a relative substitution rate of 1 for the 16S, to analyze the relative substitution rates of the two gene fragments, and to check for the adequacy of the model priors for the convergence and stationarity of the MCMC runs. These analyses were run for 100×10^6 generation sampling every 10.000 generations, using different combinations of models and parameters. The best results were obtained through the use of a lognormal relaxed molecular clock, a substitution model of HKY+I for 16S and HKY+G for cox1, a gamma prior for the relative rate parameters for each codon position (gamma prior: initial value=1; shape=1; offset=0; range: 0-1000), and a prior for the topology of the tree of 'birthand-death with incomplete sampling’. We then ran a new analysis, specifying this time as a prior the substitution rates for 16S and cox1 retrieved from the literature [16S ucld.mean (subst/site/my): lognormal distribution in real space, with initial value=0.006; Log(Mean)=0.006; Log (Stdev)=0.23][cox1 ucld.mean(subst/site/my): lognormal distribution in real space, with initial value=0.015; Log(Mean)=0.015; Log (Stdev)=0.2]. These distributions encompass pairwise differentiation ranges of 0.8-1.6% for the 16S and 2-4% for cox1. For every node of the phylogenetic tree, we recorded the mean temporal estimate (TMRCA) and the associated Bayesian 95% High Probability Density interval (95% HPD, i.e., the shortest interval in the parameter space that contains 95% of the posterior distribution). Finally, we ran the same analysis but with no data, sampling only from the prior distribution, to check for the influence of the priors used on the final the posterior probability of each parameter, including the time estimates.

4. Results

4.1. Blister beetles rural knowledge

Twenty-five persons out of 119 interviewed replied that they had no idea of any name or usage for the blister beetle shown. Eighteen out of the 25 were younger than 40 years old, the remainig 7, older, were not interested in our query. Only 3 persons younger than 40 years old knew something about the beetle, but they were in company of older people, and since older people answer first, we did not take into account their answers. Repeated and consistent negative responses by young people, prompted us to stop asking the population group below 40 years old. Among the remaining 94 interviewed people, 6 were unable to remember the name of the insect, although they were sure the beetle had a name. In some cases frustration was so hard amongst these people, that they grabbed us by the arm and kindly walked us to ask other people from the village, so we could get the information we asked for. The other 88 subjects interviewed provided us with a name, but only 80 were confirmed for blister beetles (Table 1).

People interviewed did not show any annoyance or felt appareantly too uncomfortable to take a closer look to the insect. On the contrary many people, particularly well above 60 years old (indistinctly male or female), show real interest or even smiled openly when they saw an animal that, according to some of them, brought memories from their childhood.

Figure 4

Some of the oldest people interviewed said that the beetles were known to them because they were familiar with their “disgusting oily red or bright orange secretions” and because of their “wandering behaviour, often in tandem” (*Berberomeloe* males follow females for quite a long period during courtship) (Fig. 4), but many were unsure about their toxicity or did not know about any specific use. Only 37 people responded with specific answers about the insects "oil" properties. Twenty-six indicated that the "oil" was toxic, but actually, most of them added that it was “not dangerous”. Six of them knew or have heard that the "oil" was very effective eliminating skin-warts, but only two have tried the oily secretion on themselves. Both complained about the consequences (“disgusting blistering and nothing else” in one case, and “deep, long lasting, ulceration of flesh around the area treated” for the other). This second person described the situation quite graphically: “*I tore apart the head of the beetle and applied the bleeding body against the wart in my neck; after a few hours I´ve got such a deep ulceration that anyone looking through the hole in my throat could almost see my Adam´s apple*”.

Seven persons commented that blister beetle oil was used for healing livestock; of those, four (from four different villages) indicated that it was used to heal infected wounds (probably with myasis), particularly in the legs of sheep, goats and horses. One commented that it was used to interrupt lactancy of calves. The other two did not explain how it was used. Three others indicated that they were useful for skin diseases but without further specifying, while four other people provided us with sarcastic (or very naïve) answers including that the "oil makes head hair to grow again". No one signaled any possible usage as aphrodisiac or related them with either urinary of genital tracts. A person commented that meloid beetles (although probably not the ones we were showing) killed three of a neighbour's mares.

Besides these reports gathered in the field, we obtained names and traditions referring to meloid beetles from colleagues and friends that expent part of their lives in rural areas. Their information came from either personal rememberings from childhood, or from asking directly their family members. Other colleagues, particularly entomologists, remembered what other people have said when they saw them collecting blister beetles; these addtional sources of information were included also in the following paragraphs (Table 1).

4.2. Vernacular names and traditions

Amongst the names applied exclusively to blister beetles, many are related to the rather evident oily articular secretion produced by *Berberomeloe* species (Fig. 5). In this category, we included names as: "*aceitera*" (oil bottle) (the most widespread name), "*aceitero*" (oil merchant), "*alcucilla*" (small oil can), "*tunos*" (derived from "*aceituna*" – olive), "*carraleja*" (possibly derived from the arab word used for olive, see below), "*vinagrera*" (term used for small vinegar and oil bottles used to dress salads); "*aceiteira*" and "*carralega*" are used in Galician language, specially in the Galician speaking areas of Castilla–León. Other set of names refers to the color of the haemolymph, like "*sangraor*" (bleeder) (Vozdemietierra, 2008).

Figure 5

Other widespread set includes names related with either "healing" or "priest" (both terms can be referred with the same word in Spanish) as follows: “*cura*” (cure or treatment, but also priest – since, according to its ethymology, he is a cure for souls, in charge of the care and spiritual instruction of a religious community), “*curandero*” (traditional healer), “*curato*”, “*curacurato*”, “*cureso*” (all three derived from cure), “*curavache*” (derived from cure or priest), “*curilla*”, “*curita*”, “*curica*” (all three derived from cure, but also affectively referring to a priest), “*currito*” (probably a corruption of “*curita*”), “*fraile*” (friar), and “*frailecillo*” (little friar).

Some names refer to the extreme pharmacological properties of the beetles, often killing when taken in excess: “*boticaria*” or “*boticario*” (chemist or druggist), “*matahombres*” (men killer), “*matasiete*” (this name refers to the story of seven farm-workers that died when drinking water from a “*botijo*” –typical Spanish vessel using during agricultural field work– in which a specimen of *Berberomeloe* had drowned).

Another set of names refer to the beetle shape: “*reventón*” (bursting), “*reventín*” (small bursting), "*cisterna*" (tank to carry liquids), “*tabernera*” (woman who keeps a tavern; usually viewed as plump strong women). This last one, together with “*vinatera*” (woman wine merchant) might also account for the red articular oily secretion, which looks like red wine in some geographic areas (Fig. 5). In this category could be also included some of the names related to "olive" previously reported.

Other names are of uncertain origin. Some of them are also used for other large black beetles (particularly the genus *Blaps* of the family Tenebrionidae), but also for species of Meloidae: "*demoniones*" (ugly demons; used for *Meloe* and other beetles); "*comehuevos*" (egg-eater; or also a childish "ball-eater"). The names "*vaquita*" (small cow) and "*vaca lora*" are used in areas close to the southern Portuguese border, where the species is called "*vaca loira*" (blond cow). The traditional name "*abadejo*" one of the common names used for these beetles according to the Royal Spanish Academy (RAE, 2001) was not recovered among the interviewed people, but in one village they used the derived name "*abaejo*". The name "*sietecueros*" (seven leathers), registered for *B. majalis* in Extremadura (Vodzdemitierra, 2008), is used in some American countries for a tumour in the foot (RAE, 2001). It is also used as a vernacular name for slugs (Gastropoda) in Venezuela, and for a tree in Colombia (RAE, 2001).

People of Moroccan origin in the Spanish North African city of Ceuta, called *Berberomeloe majalis* with the name "*Hmar jed'di*" (grand father´s small donkey), referring to the shape and the way the insect moves along the wheat fields. They did not mention any use for it, and they considered the animal harmless. This name is likely used in regions of the Rif Mountains where *B. majalis* is commonly present, since we do not have evidence of its presence in Ceuta (J.L. Ruiz pers.com.).

Most sets of names were not restricted to any particular region, and many names were used in distant villages from different geographical and administrative regions (Autonomous Communities), for example "*tabernera*" was used in isolated villages from Burgos and also in Salamanca, both in Castilla-León, but also in villages of Guadalajara in Castilla-La Mancha. Names varied among villages only distant a few kilometers apart, while the intra-village variability was apparently low (not statistically tested); this was especially true along the contiguous provinces of Cáceres (Extremadura), Ciudad Real, and Albacete (both in Castilla-La Mancha), where more than nine names were consistently used, but not shared across contiguous villages.

Some local folklore is also associated with meloid beetles. We found four small songs involving blister beetles of the genus *Berberomeloe*. Two were chanted apparently in order to obtain a religious "justification" to kill the animal. This need of a justification to kill an insect is probably a reflection of the deep relevance of the beetle, which according to people's beliefs, should not be killed without a purpose. One of these, is a song from villages in the province of Ciudad Real: "*Reventón, reventón, si no revientas tú te reviento yo*" ("Blister beetle, blister beetle, if you do not burst yourself, I will burst you") which is chanted while crossing oneself, following which, the meloid is crushed under the foot. Another was registered in the province of Badajoz: "*Cura, curato, si no me das misa te mato*" ("Priest, blister beetle, if you do not say mass to me I will kill you"), which obviously ends with the beetle crushed again. Another similar, also from Badajoz, but without religious implications: "*Cura, curato con el dedo corazón te mato*" ("Priest, blister beetle, I kill you with my middle finger") (Vozdemitierra, 2008). The fourth song is more a marching song related to village festivals registered in the province of León: "*Vinatera dame vino, vinatera dame pan. Unos vienen y otros van a las bodas de San Juan, los de adelante corren mucho y los de atrás se quedarán. Vinatera dame vino y dame pan.*" ("Wine merchant (blister beetle figurately) give me wine, wine merchant give me bread. Some people come, some people go, to the weddings for San Juan, the ones in front run fast, the ones behind will not arrive. Wine merchant give me wine and give me bread").

4.3. Bibliographic search

Some of the first accounts of Iberian invertebrates (Asso del Río, 1784) already mention vernacular names for blister beetles, but most of these names were registered previously in classic zoological texts. Aldrovandi (1638) mentioned that in "Hispania" blister beetles known as "*cantharis*" were named "*cubillo abadeio*", while those referred classsically as "*buprestis*" were named as "*archentabuei*". Lusitano (1558: 285), Mattioli (1578: 221), and Aldrovandi (1638: 470) also indicated that in the Arab speaking regions the name used for "*cantharis*" was "*carariha*", a word fonetically similar to the name "*carraleja*" currently used in many areas of Castilla. The name "*vinatera*" (woman selling or transporting wine) was reported for species of the genus *Meloe* in Aragón (Asso del Río, 1784).

During the XIXth century in Spain, only a few group of invertebrates received as much attention as Meloidae did. Most works published during the last decades of the century included also vernacular names for some species, particularly of the genera *Meloe*, *Physomeloe* and *Berberomeloe* (Amor Mayor, 1860; Díaz Lizana, 1864; Dieck, 1870; Cardona Orfila, 1872; Górriz Muñoz, 1882). Among those were included the already mentioned "*carraleja*", "*aceitero*" and "*abadejo*" for *Berberomeloe majalis,* the name "*matahombres*" for *Physomeloe*, and the name "*papachu*" used in Catalan (Mallorquí) for species of *Meloe* in the Island of Menorca (Amor Mayor, 1860; Cardona Orfila, 1872; Górriz Muñoz, 1882). Early XXth century continued with the tradition of including vernacular names as “*carraleja*” and “*tabernera*” in western Castilla (Fernández de Gata, 1900), and “*cuca papera*” used in Catalan (Valencià) for Meloe in Valencia (Boscá Seytre, 1916), but with the exception of those cited, most of them were simply transcribed from previous works. From the mid XXth century to now, vernacular names for meloids were almost ignored in the scientific literature, although the name “*naipa*” was registered for *Meloe tuccia* in El Hierro (Canary Islands) (Pardo Alcaide, 1951). During this period, common names were proposed and used instead or together with vernacular names (Bolívar Pieltain, 1926; García-París, 2001), although these common names have no cultural intrinsic value since they are simply used to replace the scientific name. The Dictionnary of the Royal Spanish Academy (RAE, 2001) include the names “*carraleja*”, “*aceitero*” y “*abadejo*” for *Berberomeloe majalis*, precisely those registered by Amor Mayor (1860), and “*cantárida*” for *Lytta vesicatoria*. Blas et al. (1987) recorded the Catalan names “*cantàrida*” and “*encantari*” for the latter. Recently, the Diccionario Virtual Extremeño (Vozdemitierra, 2008) recovered a detailed list of common names for *Berberomeloe majalis* in the province of Badajoz, and Benítez (2011) reported the names “*curica*” and “*aceitera*” in the province of Granada also for *B. majalis*.

Names applied in entomological works in Spanish to blister beetles as a group (family Meloidae) are translated from classic texts, and relate to their pharmacological properties: “*coleópteros epispásticos*” (Amor Mayor, 1860), “*coleópteros vesicantes*” (Valladares Díez, 1984), or “*cantáridas*” (Boscá Seytre, 1918). For commecial purposes, however, they were always referred as “*mosca de España*”, the traslation of “*Spanish fly*”, the commonly used name through most of Europe (Blas et al., 1987; Lückmann & Niehuis, 2009).

4.4. Mitochondrial DNA study

For all these specimens collected, we amplified and sequenced two mitochondrial markers (Cytochrome Oxidase I gene –cox1– and 16S ribosomal RNA). The cleaning and alignment of the sequences of these two genes resulted in a combined character matrix of 1165 bp [partial alignments: 508 bp (16S) and 657 bp (cox1)] for the 21 specimens. Sequences were deposited in GenBank, and accession numbers are given in Table 2 [*to be added upon manuscript acceptance*]. Running the data matrix in PartitionFinder to determine the best partition scheme and substitution model yielded a scheme of four partitions including the 16S and the three codon positions of cox1, with the following substitution models: 16S: HKY+G; cox1-pos.1: TrNef; cox1-pos.2: HKY+I; cox1-pos.3:TIM+G.

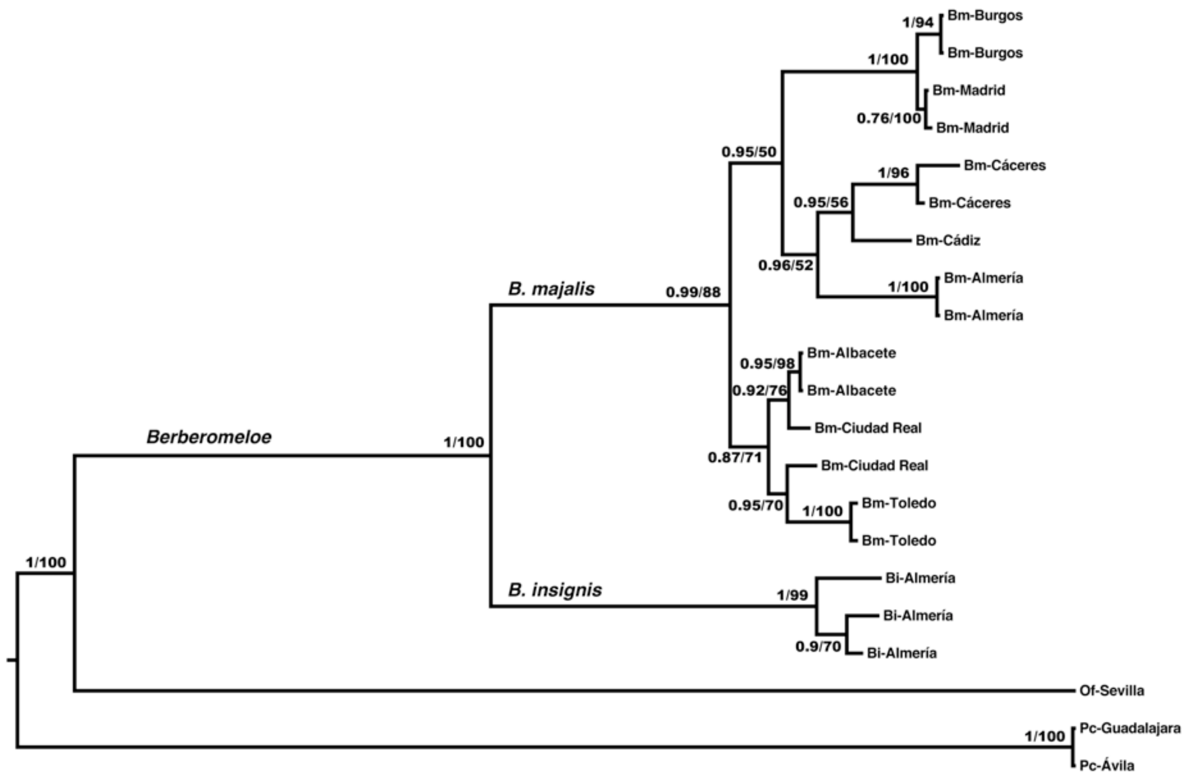

Figure 6

The phylogenetic analysis of the partitioned data set either under Maximum Likelihood or Bayesian Inference yielded the same topology (Fig. 6), which was also recovered in the coalescence-based analysis of divergence time estimates (Fig. 7). The Bayesian tree showed strong support (posterior probabilities) for most of the nodes; the same pattern was found for bootstrap values in the Maximum Likelihood analysis (ML tree, -ln= 3603.4115). In these two analyses, the specimens of *P. corallifer* were specified as outgroup, and the three other species (*Oenus fusicornis*, *Berberomeloe majalis* and *B. insignis*) were consistently recovered as monophyletic groups with very strong support (Fig. 6). Two major clades are recovered within *B. majalis*. The first one includes the populations from the central-southern Iberian plateau (Castilla-La Mancha) (population from Albacete, Ciudad Real and Toledo, Fig. 3). The second clade contains two well-supported lineages, grouping the populations from the center and North (Burgos, Madrid), and the western and southern populations (Almería, Cádiz, Cáceres), respectively.

In order to estimate divergence times between clades in the phylogenetic tree, we followed a conservative approach to calibrate a molecular clock, using broad substitution rate intervals including unusually fast and slow evolving rates. This approach tends to result in very broad 95% HPD intervals. Nevertheless, the temporal estimates (95% HPDs) recovered in the analyses are consistently found to be older than the first record of human settlements in the Iberian Peninsula. Times to the most recent common ancestors (TMRCAs) for *Berberomeloe* species fall well within the Miocene-Pliocene, and differentiation of main lineages within species occurred during the Pliocene-Pleistocene range (Fig. 7, Table 3). For instance, the TMRCA of the two *Berberomeloe* species can be traced back to ca. 9 million years ago (mya) (5.6-13.4mya), and the main lineages within *B. majalis* diverged between 1 and 3mya. The TMRCA of the lineages included in *B. insignis* date to ca. 1mya (0.55-1.7mya).

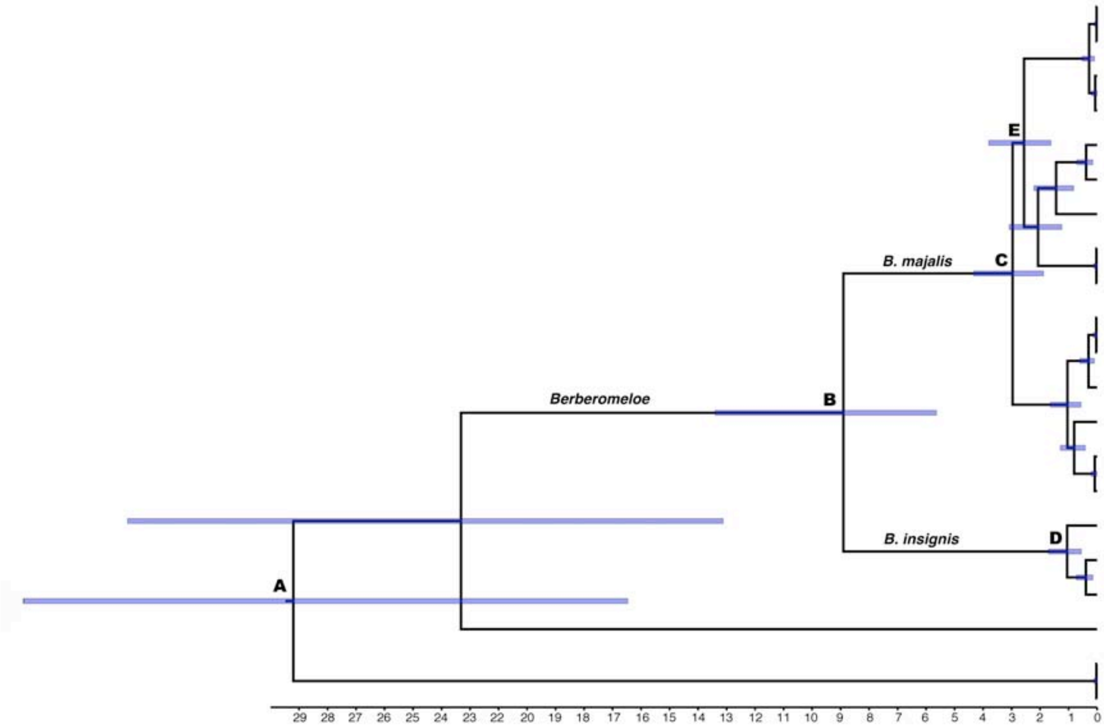

Figure 7

5. Discussion

5.1. Vernacular names

During the field survey, we found at least 28 different vernacular names applied unequivocally to the species of genus *Berberomeloe* and *Physomeloe*, plus other six reported from literature (Vozdemitierra, 2008). Fieldwork was concentrated in the Spanish speaking regions of the Iberian Peninsula, where meloid beetles are common (Andalucía, Castilla – La Mancha, Castilla – León, Extremadura and Murcia). Blister beetles, however, are also present in other regions that were not sampled (particularly Aragón), so it is likely that the number of names applied to these insects is much larger.

It is generally assumed that referring to an animal by its own name reflects explicitly some degree of knowledge. Among the answers we got in the interviews, very few names were obvious misuses of other animal names. Among those, two persons (together) from the province of Zamora gave us the name: “*sapa de san Antonio*”, a name commonly used for the frog *Hyla arborea* (García-París, 1985), and, as a combination ("sapa" or "vaquita de San Antonio"), occasionally for *Coccinella septempunctata* (Zabala et al., 2003); other three people from two different villages in northern Toledo provided us with the name “*sapos*” (toads), but in relation to this case M. París (Entomology curator at MNCN) indicated that in certain areas of Castilla – La Mancha region (as it was the case) the name “*sapos*” is used instead of “*bichos*” (bugs) referring to any small uninteresting creature. Three people refer to them as “*escarabajos*” (beetles) and one as “*insectos*” (insects), but not a single person referred to them as cockroaches. It would be obvious, therefore, that rural people from Spain know about blister beetles. If a correlation between names and knowledge could be made, it can be said with certainity that people in Spain know more about blister beetles than they do for any other Coleoptera [except maybe the lady bug (Zabala et al., 2003)], and probably with respect to any other terrestrial invertebrate non edible or pest.

During the interviews, we also showed in a few occasions (12 persons) large beetles of the family Tenebrionidae (*Scaurus punctatus* and *Blaps hispanica* or *B. lusitanica*). These are all year round common insects in fields and villages in the study area. Beetles of the genus *Blaps* are able to project a highly irritant secretion with a strong disgusting odor over large distances (quite dangerous if it enters in contact with the eye). However, not a single person (except in the northern Cantabrian region, were they called them - as well as to species of the genus *Meloe* - "*demoniones*" - ugly demons) provided us with a specific name to them, even though they knew they were somehow toxic or dangerous.

Based on the lack of vernacular names for tenebrionids, and also on previous personal data about Carabidae (García-París unpublished), it seems that rural Spaniards are not interested in beetles. Toxicity of blister beetles of the genus *Berberomeloe* is not strong enough (unless swallowed) to represent a serious threat for humans and they do not bite nor sting. They are large obvious animals that can be seen wandering around trails and cultivated fields, but their period of activity is restricted to a few months per year, in spring and early summer (García-París et al., 2003).

5.2. DNA, pharmacology and spoken tradition

So, why do Spanish people actually know about blister beetles? Our hypothesis is that meloid beetles were incorporated to the ancestral medicinal traditions of ancient pre-Christian cultures in Spain. Although these traditions have become progressively lost as veterinary and pharmacy practices found modern substitutes for treatment of wounds and infections, these ancient traditions still persist within the collective memories of rural people transmited from parents to children, reinforced by the obvious presence of the beetles around kids playground areas near villages. This hypothesis was difficult to test. A first problem was derived from the fact that *Berberomeloe* species past geographical range might not be coincident with their current geographical distribution, and therefore these blister beetles might not have been present in Spain until recent times as documented for other small animals (Carranza et al., 2004; Recuero et al., 2007). A second problem was posed by the fact that we do not know if ancient Spaniards used the pharmacological properties of blister beetles for medical purposes at all.

To test the first question we sequenced two different mitochondrial DNA gene regions (Cytochrome Oxidase I and 16S) with different evolutionay rates, in geographycally widespread samples of the two species of *Berberomeloe* (the largest and most frequently recognized blister beetles). Results indicate that the basal split within *Berberomeloe* took place about 9 mya, separating *B. insignis* and *B. majalis*. Mitochondrial lineages within the widespread *B. majalis* have been evolving independently since the Pliocene (Fig. 7, Table 3). Lineages within *B. insignis* moreover, share a common ancestor ca. 1mya. This is an interesting result, since the species is an Iberian endemic with a rather restricted distribution area (southeastern corner of the Iberian Peninsula) and, as such, it may be hypothesized to have originated and remained in the area since its origin. Time estimates show a relatively high standard error (95% HPD), but in any case, splitting within *Berberomeloe* and even within Iberian clades of *B. majalis* and *B. insignis* are much older than the earliest human cultural expressions in the Iberian Peninsula (paintings and carvings in Altamira Cave mainly date back to the Magdaleniense and Solutrense periods, or at its earliest to the Aurignaciense, about 35,600 years ago – Pike et al., 2012). Our analyses cast no doubts about the geographic location of the phylogenetic splitting between and within *Berberomeloe* species, which took place in the Iberian Peninsula during the Pliocene and Pleistocene. Therefore the same species of blister beetles that we found today in the Spanish fields were present along the development of the early pre-Christian native groups in Spain.

Answering the second query is more diffcult. We have not been able to find either zooarchaeologic references or literature about the use of blister beetles by ancient cultures in Spain. Information on this respect dates back to the High Middle Age, since one of the vernacular names for the species in Castilla-León, "*carraleja*", was likely derived from the arab word "*carariha*" (Lusitano, 1558). Also, Covarrubias Horozco (1611) reports the use of blister beetles as a poison and as an aphrodisiac.

Ancient cultural groups in different continents seem to have used blister beetles for medical purposes. Extensive documentation for the ancient use of pharmacological properties of cantharidin, mostly obtained from blister beetles of the genus *Mylabris*, are available from China (Wang, 1989; Tan et al., 1995) and for the South African Tswanas (Moed, 2001). Diverse prehispanic American cultural groups had specific names for blister beetles (Navajo, Mexica, Otomi, Mapuche) suggesting that, at least, some large species of the genera *Megetra, Meloe, Lytta* and *Pseudomeloe*, were well known, and probably used for medical purposes (Herrera & Mendoza, 1866; Peñafiel Barranco, 1866; Cepeda Pizarro, 1997; García-París et al., 2007; Percino-Daniel, unpublished data). We have no reason to reject a similar degree of knowledge for local ancient cultures in temperate Western Europe, where contact with the conspicuous *Berberomeloe* was unavoidable.

A further argument came from the religious folk songs chanted by farmers before killing a blister beetle. Why people needed an almost religious justification to kill blister beetles, in a traditionally merciless continent – Europe – with respect to insect life? The need for religious forgiveness can only be developed if the actual action is perceived as a negative social behaviour. Reasons for that perception could come from the fact that killing the beetle eliminates an important resource for the community (Campbell, 1991), or because the beetle in itself is considered a relevant "social icon" which had been considered sacred or totemic in past times (Alinei, 1981). First case is unlikely, since population densities of *B. majalis* are generally very high (García-París et al., 2003), and even the endangered *B. insignis* may present locally dense populations (García-París & Ruiz, 2011). Treating *Berberomeloe* with any kind of respect is surprising for Christian societies (or Muslim communities) in the Spanish Middle Ages, because insects are generally considered impure creatures (*Leviticus* 11; Holman Christian Standard Bible). Social respect for blister beetles despite possible disaproval from the Church, favors the hypothesis of considering their knowledge as a surviving portion of the pre-Christian pharmacological traditions that managed to persist until today.

In conclusion, by using molecular DNA date we have demonstrated that blister beetles of the genus *Berberomeloe* were part of the natural landscape of Spain well before the settlement of modern humans. The ability of *Berberomeloe* to adapt to rural environments and thrive there is easily determined by their large population sizes in agricultural fields all over temperate Iberia. *Berberomeloe* are amongst the largest beetles of Europe, with a maximum length up to 75 mm (Bologna, 1991), only matched by the stag beetle, *Lucanus cervus*. They are diurnal and wander from place to place, showing an aposematic coloration and an evident bleeding defensive reaction, which makes them easy to spot, and uniquely attractive for children to play with. *Berberomeloe* haemolymph contact with senstive human skin causes a difficult to forget blistering, but not harmful enough to become afraid of. At the same time, it is not difficult to imagine that early symptoms will follow testing by adults in animal tissues and wounds, with an immediate effect eliminating myasis and cleaning damaged tissues, in what we could probably considered one of the earliest pharmacological uses derived from animals. At that time, names for *Berberomeloe* must have spread quickly all over the range of the species. Since then, the process of becoming sacred, totemic, and tabu along the succesive cultural stages in European societies is easly conceivable (Alinei, 1997; Viereck 2000). The replacement of erliest names would have led to another set of names and traditions, with such strenght, that subsequent sucessive establishment of Muslim and Christian societies were unable to erase or even to transform, as happened with many of the names applied to other insects as *Coccinella septempunctata*, which became in large part Christianized (see Zabala et al., 2003). Pharmacological properties of *Berberomeloe* must have played an undisputable role in its integration in the culture of early Iberian human societies, but also in the preservation of their identity until today, a rare case for Spanish insects.

Nowadays, the concentration of population in large cities precludes regular contact with nature, and vernacular names as well as rural traditions are rapidly disapearing (Zabala et al., 2003; Singh, 2008). Even naturalistically oriented people are using the "official common names" consensued by Zoological Societies, instead of local vernacular names (Parkes, 1978; Stiles & Skutch, 1990; de Juana et al., 2005), contributing to their dissapearance. Neither the current pharmacological relevance of meloid bettles nor their ancient pharmacological applications are appropriately documented (Moed, 2001). At the same time, recent migration into rural areas by populations that have not been in previous contact with these large beetles, might be starting to generate an overlaping new folk taxonomy, completely unrelated with ancient traditions. This seems to be the case in northern Mexico, where the English word "trailer", which is frequently used by native Spanish speakers for large articulated trucks, is now used to refer to large meloid beetles of the genera *Meloe* and *Megetra*. We hope the data presented here contribute to call attention for this ancient knowledge that has to be considered part of the disappearing ancestral European Cultural Heritage.

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Figure legends

Figure 1. Minor ulceration following blistering in human skin (neck), as a consequence of direct contact with haemolymph of a single alive blister beetle (*Cerocoma schreiberi*). The photograph was taken 36 hours after accidental contact during field research in Central Spain on MG-P skin (Photograph by I. Martínez-Solano, modified from Fernández-Rubio et al., 2008).

Figure 2. Representative specimens of blister beetles (family Meloidae) shown during the ethnograpic study in Spain. A.- *Berberomeloe majalis* (specimen photographed from Pinilla de los Moros, province of Burgos, Spain). B.- *Berberomeloe insignis* (specimen from Tabernas, province of Almería, Spain). C.- *Physomeloe corallifer* (specimen from Serranillos, province of Avila, Spain). D.- *Meloe proscarabaeus* (specimen from Jerte, province of Cáceres) (Photographs M. García-París).

Figure 3. Map of the Iberian peninsula depicting localities where interviews rendered positive answers identifying blister beetles with a vernacular specific name. Dotted line indicates the northernmost limit of current geographic distribution for the genus *Berberomeloe*, the better known meloid in Spain an Portugal. Black dots mark the localities for mtDNA study, most of them coincident with interview localities (see Table 1 and 2). Villages with information about vernacular names provided by Vozdemitierra (2008) are located between locality numbers 5, 7 and 13.

Figure 4. Initial stages of the courtship in *Berberomeloe majalis*; the male follows the female for a long time before iniciating the remaining phases of the courtship (specimens form Alhama de Granada, province of Granada) (Photograph M. García-París).

Figure 5. Defensive behaviour of *Berberomeloe majalis*; droplets of blood-red haemolymph containing cantharidin are expelled through articulations in the legs and thorax (specimen form Malcocinado, province of Cádiz) (Photograph M. García-París).

Figure 6. Phylogenetic relationships among the Iberian blister beetles of the genus *Berberomeloe* included in this study. The phylogenetic tree has been generated

through a Bayesian analysis. The same topology was recovered under a Maximum Likelihood approach. Nodal support for the resulting clades is given by the Bayesian posterior probabilities (pp), and the bootstrap values in the Maximum Likelihood approach (bb); they are shown for each node of the tree (pp/bb).

Figure 7. Temporal Estimates: Temporal estimates (95% High Probability Intervals) are plotted in the phylogenetic tree generated through the relaxed molecular clock approach in a Bayesian-coalescence framework. The same topology and phylogenetic relationships were obtained in this analysis and through the Maximum Likelihood and Bayesian Inference approaches (Fig. 6). The exact values for these intervals are shown in Table 3. (units of the temporal axis in millions of years).

Table 1. List of villages where interviews rendered positive answers identifying blister beetles with a vernacular specific name in Spain. We indicate the name given, the species shown, the number of people who identified the animal with the name and the pharmacological uses mentioned during interviews. Numbers in the first column correspond to localities shown in Fig. 3. Localities close to each other are included with the same number. Village codes 48-60 correspond to Vozdemitierra (2008).

Map code	Locality	n	Name applied	Species	Pharmacological use
01	Albacete: Casas Ibáñez	4	**cureso**	*B. majalis*	
02	Albacete: El Bonillo	2	**aceitera**	*B. majalis*	
03	Alicante: Alicante	-	**aceitera**	*B. majalis*	
04	Ávila: Ávila	-	**carraleja**	*P. corallifer*	
04	Ávila: Ávila	-	**comehuevos**	*B. majalis*	
06	Badajoz: Castuera	2	**curita**	*B. majalis*	
06	Badajoz: La Siberia	-	**curato**	*B. majalis*	
07	Badajoz: Pallares	1	**curita**	*B. majalis*	skin treatment (h)
07	Badajoz: Pallares	1	**curita**	*P. corallifer*	
08	Burgos: Mecerreyes	2	**tabernera**	*B. majalis*	
09	Cáceres: Cáceres	-	**cura**	*B. majalis*	
10	Cáceres: Garrovillas	2	**curita**	*B. majalis*	healing livestock (v) healing infected wounds (v)
11	Cáceres: Navas del Madroño	2	**curita**	*B. majalis*	healing infected wounds (v) against warts (h)
12	Cáceres: Sierra de Fuentes	1	**currito**	*B. majalis*	
13	Cáceres: Valencia de Alcántara	-	**tuno**	*B. majalis*	
14	Cádiz: Benalup de Sidonia	2	**curita**	*B. majalis*	interrupting lactancy (v)
15	Cádiz: Chiclana de la Frontera	1	**cura**	*B. majalis*	skin treatment (h)
15	Cádiz: Chiclana de la Frontera	1	**curilla**	*B. majalis*	against warts (h)
15	Cádiz: Chiclana de la Frontera	2	**abaejo**	*B. majalis*	against warts (h)
16	Cádiz: La Muela	3	**curita**	*B. majalis*	healing livestock (v) healing infected wounds (v)
17	Ciudad Real: Alcoba	-	**curavache**	*B. majalis*	
18	Ciudad Real: Arroba de los Montes	2	**curato**	*B. majalis*	
17	Ciudad Real: Horcajo los Montes	-	**curavache**	*B. majalis*	
19	Ciudad Real: Piedrabuena	1	**curato**	*B. majalis*	
20	Ciudad Real: Pozuelo de Calatrava	1	**reventón**	*B. majalis*	
21	Ciudad Real: Villarubia de los Ojos	-	**reventón**	*B. majalis*	
22	Ciudad Real: Viso del Marqués	2	**alcucilla**	*B. majalis*	
23	Cuenca: Beteta	2	**tabernera**	*B. majalis*	
24	Cuenca: Canalejas del Arroyo	4	**boticaria**	*B. majalis*	

25	Cuenca: Tarancón	1	**cura**	*B. majalis*	
25	Cuenca: Belinchón	2	**cura**	*B. majalis*	
25	Cuenca: Barajas de Melo	-	**cura**	*B. majalis*	
26	Granada: Granada	-	**vinagrera**	*B. majalis*	
27	Huelva: Cabezas Rubias	3	**curita**	*B. majalis*	skin treatment (h)
28	Huelva: Sanlúcar del Guadiana	1	**vaquita**	*B. majalis*	healing goat wounds (v)
29	Jaén: Baños de la Encina	-	**curica**	*B. majalis*	
30	Jaén: Jaén	-	**curica**	*B. majalis*	
31	León: Ponferrada	-	**carralega**	*B. majalis*	
32	León: Quintanilla del Monte	-	**vinatera**	*B. majalis*	
33	Madrid: Perales de Tajuña	3	**aceitera**	*B. majalis*	
33	Madrid: Colmenar de Oreja	2	**aceitera**	*B. majalis*	
33	Madrid: Aranjuez	2	**sapo**	*B. majalis*	
33	Madrid: Rivas de Jarama	1	**aceitera**	*B. majalis*	
33	Madrid: Rivas de Jarama	1	**cura**	*B. majalis*	
33	Madrid: Madrid	-	**cura**	*B. majalis*	
34	Murcia: Murcia	1	**matahombres**	*B. majalis*	
34	Murcia: Murcia	-	**matasiete**	*B. majalis*	
35	Ourense: Verín	-	**aceiteira**	*B. majalis*	
36	Salamanca: El Tejado de Béjar	1	**carraleja**	*B. majalis*	
37	Salamanca: Bocacara	1	**fraile**	*B. majalis*	
37	Salamanca: Santibáñez de la Sierra	-	**frailecillo**	*B. majalis*	
33	Toledo: Zarza de Tajo	1	**sapo**	*B. majalis*	
38	Toledo: Fuentes	2	**curilla**	*B. majalis*	
39	Toledo: Navalcán	2	**aceitero**	*P. corallifer*	
40	Burgos: Fuentespina	3	**aceitunero**	*B. majalis*	
41	Zamora: Aliste	2	**sapa de San Antonio**	*B. majalis*	
42	Zamora: Fuentesaúco	3	**aceitera**	*B. majalis*	
43	Almería: Pulpí	1	**fraile**	*B. insignis*	
44	Almería: San Juan de Terreros	1	**matahombres**	*B. insignis*	
45	Almería: Zúrgena	2	**curica**	*B. insignis*	
46	Murcia: Tebar	3	**matahombres**	*B. insignis*	
46	Murcia: Águilas	2	**matahombres**	*B. insignis*	
47	Cantabria: Cóbreces	2	**demoniones**	*M. proscarabaeus*	
48	Badajoz: Cabeza La Vaca	-	**boticario**	*B. majalis*	
49	Badajoz: Campanario	-	**cura**	*B. majalis*	
50	Badajoz: Llerena	-	**cura**	*B. majalis*	
51	Badajoz: Burguillos del Cerro	-	**curandero**	*B. majalis*	
52	Badajoz: Nogales	-	**curita**	*B. majalis*	
53	Badajoz: Barcarrota	-	**curato**	*B. majalis*	
54	Badajoz: La Roca de la Sierra	-	**curita reventón**	*B. majalis*	
55	Badajoz: Villarta de los Montes	-	**reventín**	*B. majalis*	

56	Badajoz: Cheles	-	**vaca lora**	*B. majalis*
57	Badajoz: Alburquerque	-	**siete cueros**	*B. majalis*
58	Badajoz: Oliva de la Frontera	-	**sangraor**	*B. majalis*
59	Badajoz: Torrequemada	-	**curato**	*B. majalis*
60	Cáceres: Madroñera	-	**curacurato**	*B. majalis*

Table 2. List of localities from where blister beetles of the family Meloidae were collected for the mtDNA study (see also Fig. 3). Voucher number and GenBank accessions are included.

Taxon	**Locality**	**Voucher field number**	**GenBank accession *cox 1 16S***
Berberomeloe insignis	Spain: Almería: 4.5 km S Zúrgena	MAB 182	------------------
Berberomeloe insignis	Spain: Almería: El Puntal, Uleila	MAB 181	
Berberomeloe insignis	Spain: Almería: El Puntal, Uleila	MAB 184	
Berberomeloe majalis	Spain: Albacete: El Bonillo	MAB 169	
Berberomeloe majalis	Spain: Albacete: El Bonillo	MAB 140	
Berberomeloe majalis	Spain: Almería: El Puntal, Uleila	MAB 185	
Berberomeloe majalis	Spain: Almería: El Puntal, Uleila	MAB 197	
Berberomeloe majalis	Spain: Burgos: Monasterio de Rodilla	MAB 123	
Berberomeloe majalis	Spain: Burgos: Monasterio de Rodilla	MAB 116	
Berberomeloe majalis	Spain: Cáceres: 3 km S Navas del Madroño	MAB 174	
Berberomeloe majalis	Spain: Cáceres: 3 km S Navas del Madroño	MAB 175	
Berberomeloe majalis	Spain: Cádiz: 3 km S Alcalá de los Gazules	MAB 107	
Berberomeloe majalis	Spain: Ciudad Real: Piedrabuena	MAB 500	
Berberomeloe majalis	Spain: Ciudad Real: Piedrabuena	MAB 501	
Berberomeloe majalis	Spain: Madrid: 4 km S Colmenar de Oreja	MAB 146	
Berberomeloe majalis	Spain: Madrid: 4 km S Colmenar de Oreja	MAB 159	
Berberomeloe majalis	Spain: Toledo: 2 km NE El Emperador	MAB 122	
Berberomeloe majalis	Spain: Toledo: 2 km NE El Emperador	MAB 173	
Oenas fusicornis	Spain: Sevilla: Gerena	MAB 205	
Physomeloe corallifer	Spain: Avila: Peguerinos	MAB 098	
Physomeloe corallifer	Spain: Guadalajara: Uceda	MAB 099	

Table 3. Time estimates for the nodes of the phylogenetic tree represented in Figure 7 (A-E). For each node, we indicate the mean and median values estimated through the relaxed molecular clock approach, together with the lower and upper bounds of the 95% High Probability Density intervals (95% HPD), which show the ranges of possible values of the temporal estimates and, therefore, the error associated to the analysis [dates in millions of years (mya)].

Summary Statistic/Node	***A***	***B***	***C***	***D***	***E***
mean	29.12	9.14	3.04	1.09	2.77
median	28.20	8.90	2.97	1.05	2.70
95% HPD lower	16.46	5.64	1.88	0.55	1.62
95% HPD upper	43.92	13.40	4.34	1.70	3.96

CÁDIZ: Manzanete, 36º13'59.3"N-5º53'26.6"O, 54 m

Berberomeloe majalis

4+3 ejemplares cruzando la carretera en zona cultivos.

n.v. "cura", "curilla", "abaejo" (2 personas, se usaba para curar heridas del ganado, produce ampollas en la piel).

n.v. "vaquitas"

llaman en Sanlúcar de Guadiana (H) a las *B. majalis* negras y usan su sangre para curar las inflamaciones de las patas de los cabritos. 1 persona de >65 años.

n.v. "curitas"

llaman en Cabezas Rubias (H) a las *B. majalis* negras y usan su sangre para curar verrugas, aunque uno que lo ha probado dice que lo único que hace es provocar una inflamación dolorosa. 3 personas de >65 años.

n.v. "frailes"

llaman en Bocacara (SA) a las *B. majalis*. 1 persona de >60 años.