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## **Mice on the borders: genetic identification of rat and house mouse species in Lampedusa and Pantelleria islands (Southern Italy)**

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### **SUMMARY**

Biogeography and the occurrence of small mammals are usually hard to investigate due to the small size and secretive habits of these mammals. Available data are particularly insufficient on minor islands and at national borders, where research efforts are usually scarce. Here we briefly updated the knowledge on murid rodents on two remote Italian small islands (Lampedusa and Pantelleria) at the southernmost Italian borders. During summer 2019, house mice and rats were sampled in Lampedusa and Pantelleria and molecular markers were sequenced for species identification. The new sequences of *Mus domesticus* were also compared with samples from previous works collected in Lampedusa, Pantelleria, and several localities in the Mediterranean basin. Moreover, our analyses provided the first genetic evidence of the occurrence of *Rattus norvegicus* in Lampedusa. To conclude, *R. rattus* was confirmed to be present in Pantelleria. The newly collected haplotype of *M. domesticus* from

Pantelleria is similar to those currently known for Sicily, whereas the new haplotype from Lampedusa partially diverges from the ones previously described, and clusters with haplotypes from North Africa and the Middle East.

## INTRODUCTION

The most updated checklist of Italian Mammals reports the presence of 123 species and, amongst those, of 34 rodents (Loy et al., 2019). However, assessing the actual distribution of small mammal species may be challenging, as it requires detailed fieldwork including direct captures with traps and/or the analysis of pellets of raptor birds (Angelici et al., 2009; Amori et al., 2015). Assessing the small mammal assembly is particularly important on small islands, where the presence of alien rodents is reported to affect native ecosystems, e.g. native plants, insects and the breeding success of marine birds (Martin et al., 2000; Capizzi et al., 2010). In the Mediterranean islands, two species of rats affect native ecosystems and biodiversity (Duron et al., 2017): the black rat *Rattus rattus* (Linnaeus, 1758) and the Norway rat *R. norvegicus* (Berkenhout, 1769). Similar species e.g. the Polynesian rat *R. exulans* (Peale, 1848) and the Tanezumi rat *R. tanezumi* Temminck, 1844 are at the beginning of their invasion processes in several islands, and their identification mostly relies on genetic analyses (West et al., 2017; Guo et al., 2019; Camacho-Sanchez & Leonard, 2020). The invasion of the Mediterranean basin by synanthropic rodents is mostly due to the intensification of the trade with Asian countries, which are the native distribution areas of these species (Puckett et al., 2016). The Mediterranean islands (mostly the small ones) are currently mostly threatened by the black rat (Martin et al., 2000; Capizzi et al., 2010; Duron et al., 2017). The Norway rat, indeed, is usually quite rare on Mediterranean small islands (e.g. Angelici et al., 2009; Canale et al., 2019). Also, the house mouse *Mus domesticus* Linnaeus, 1758 (recently split from *Mus musculus* Linnaeus, 1758 as a valid species: see for example Loy et al., 2019) is present in Italy after ancient introduction events

at least since the Neolithic age (about 6000 BCE: Bona, 2020). Several chromosomal geographical variants (i.e. “races”) have been reported in Italy for this species (Loy et al., 2019), many of them settled on islands (Amori et al., 2008).

As to synanthropic murid rodents (*Rattus* spp. and *Mus* spp.) in Lampedusa and Pantelleria islands (Sicily, Southern Italy), previous works reported the presence of *R. rattus*, *R. norvegicus*, and of a subspecies of the house mouse, namely *M. musculus praetextus* Brants, 1827, which is now considered as a synonym of *M. domesticus* Schwarz & Scharz 1943 (Felten & Storch, 1970; Zava & Lo Valvo, 1990; Angelici et al., 2009; Cattaneo, 2015). House mice in Pantelleria were reported to show a coat pattern similar to the Algerian mouse *M. spretus* Lataste, 1883 (Felten & Storch, 1970), and further analyses are needed to check the presence of this species in Italy (Loy et al., 2019). Also, the Etruscan pigmy shrew *Suncus etruscus* Savi, 1822 is known to occur on both islands (Zava & Lo Valvo, 1990; AA.VV., 2008). Other small mammals recorded only in Pantelleria include the endemic subspecies of wood mouse *Apodemus sylvaticus hermanni* (Linnaeus, 1758), which needs to be genetically confirmed, the Mediterranean shrew *Crocidura pachyura cossyrensis* Contoli, in Contoli et al. (1989), genetically clustering with samples from Tunisia and Sardinia (Nicolas et al., 2014). However, no genetic identification for rats on these islands is available, despite this is an important requirement before any management action, including eradication, might be implemented (Capizzi, 2020; Mengoni et al., 2018).

For this reason, here we report the genetic identification of synanthropic murid rodents collected on Lampedusa and Pantelleria

islands, with the aim to provide reliable and geo-referenced baseline data to be used for future studies.

## MATERIALS AND METHODS

In summer 2019, we collected samples from Pantelleria (83 kmq, 0-836 m a.s.l.) and Lampedusa (20 kmq, 0-133 m a.s.l.). Pantelleria is located 100 km southwest of Sicily and 60 km east of the Tunisian coast and it is a volcanic satellite island of Sicily.

Lampedusa is located 113 km away from Tunisia and 205 km southwest of Sicily and represents an almost flat limestone island belonging to the African Plateau.

Samples of rats and house mice were collected in summer-autumn 2019, from individuals found dead in the case of rats (i.e. road-killed or killed by domestic cats) and from individuals found within human settlements, in the case of the house mouse in Pantelleria (Table 1; Figure 1).

Table 1. Locations of sample collection (WGS84 geographical coordinates).

Taxon	Island	Latitude	Longitude	Habitat type
<i>Rattus</i> sp.	Lampedusa	35.52426° N	12.53574° E	Road between fallows
<i>Rattus</i> sp.	Lampedusa	35.52426° N	12.53574° E	Road between fallows
<i>Rattus</i> sp.	Pantelleria	36.82858° N	11.96051° E	Road between fallows
<i>Mus domesticus</i>	Lampedusa	35.50876° N	12.59062° E	Human settlements
<i>Mus domesticus</i>	Pantelleria	36.83123° N	11.96207° E	Human settlements



Figure 1. The individual of house mouse sampled in Pantelleria.

In the last case, the individual was handled, and a fragment of 1 mm<sup>2</sup> of ear tissue was obtained through a biopsy punch; afterward, the individual was set free outside the building.

For the molecular analyses, genomic DNA from 2 tissue samples of *M. domesticus* (one from Pantelleria and one from Lampedusa, the latter killed by a domestic cat) and 3 tissue

samples of *Rattus* spp. (one from Pantelleria and two from Lampedusa) have been extracted from body muscle preserved in 96% ethanol by salting out procedure (Aljanabi & Martinez, 1997). Two mitochondrial fragments were amplified for the two genera in order to produce sequences comparable to the ones available in GenBank and previous works (Solano et al., 2013). Obtained sequences were checked,

edited, and aligned with Geneious v4.8.3 (Biomatter).

#### *Genetic analyses of house mouse samples*

The mtDNA Control Region (CR) and part of the flanking pro-tRNA were amplified in *M. domesticus* following Prager et al., (1993) under standard PCR conditions. The 822 bp long fragments obtained are made available in GenBank (Accession numbers will be provided upon acceptance: XXX). The resulting haplotypes were combined with the dataset used by Solano et al. (2013) which included 183 haplotypes from all the Mediterranean basin and northern Europe including Africa, and previous haplotypes from Pantelleria (N = 1) and Lampedusa (N = 2). Moreover, further genetic sequences available on GenBank and belonging to haplotypes from other small central Mediterranean islands (Linosa, Malta, and the seven Aeolian islands) and Sicily have also been included. A Neighbor-net network connecting all retrieved haplotypes was obtained with SplitsTree v. 4.10 (Huson and Bryant 2006) to visualize the relationship among the haplotypes observed in the frame of this work and those from previous phylogenetic/phylogeographic analyses of mouse population from Mediterranean area, and to assign the new samples to the haplogroups (Hg) identified in previous works (Bonhomme et al., 2011; Jones et al., 2011; Solano et al., 2013).

#### *Genetic analyses of rat samples*

For *Rattus* spp., the cytochrome b gene was amplified using the primers L14841 and H15149 already used for rats under standard PCR conditions (Kocher et al., 1989). The obtained sequences span from 1024bp to 1057bp. Molecular identification of each amplified sequence was performed with the BLAST algorithm (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) using the newly obtained sequences and searching for highly similar sequences (Mega BLAST) on the entire nucleotide collection database.

For *R. norvegicus* there is not a detailed work dealing with its phylogeography in Europe and Africa based on mitochondrial markers, so that the present comparative analysis could not go beyond the species identification. Concerning *R. rattus*, conversely, we aligned the new sequences with those retrieved from GenBank from Europe, Africa and other localities world-wide (Aplin et al., 2011; Colangelo et al., 2015; Lack et al., 2012). In this case, a TCS parsimony Network (Clement et al., 2002) connecting haplotypes was obtained with popART (<http://popart.otago.ac.nz>) with the aim to visualize the relationship among the new and previously described haplotypes.

## RESULTS

### *Mus domesticus*

The new sequence from Pantelleria represents a new haplotype, which differs from the one previously observed in in the same island (cf. Solano et al., 2013) for two mutations (one substitution and one indel) over the 822 bp considered, showing therefore a divergence of 0.24%. The Neighbor-net network evidenced that the new haplotype clusters with the other one from Pantelleria, within the haplogroup named “Hg9” (Bonhomme et al., 2011). Hg9 includes samples from the Middle East (EU194629 and EU194640; Rajabi-Maham *et al.*, 2008), Central-Northern Italy (Accession Numbers: AY560815, EU194669, EU194668, and EU194661; Castiglia et al., 2005; Rajabi-Maham et al., 2008), Sicilian mainland (SIC haplotype: JQ646052), and Aeolian Islands (AEO11,9,10,2; Solano et al., 2013).

The sequence from Lampedusa is a new haplotype differing from those previously reported from the island by 13 substitutions/mutations over the 822 bp considered, showing, therefore, a divergence of 1.5% and being similar to North African sequences. The Neighbor-net network evidenced that the new haplotype clusters

within the Hg7 haplogroup, which is widely distributed in North Africa and the Middle East and less common in Italy; the observed haplotype is closely related to haplotypes from Morocco (U47478; Prager et al., 1993; cf. Carretero et al., 2009) and Aeolian Islands

(AEO5; Solano et al., 2013). The two haplotypes observed in Lampedusa by Solano et al., (2013) cluster together with those from Linosa, France, and Northern Italy in the Hg1 and Hg2 haplogroups (Solano et al., 2013: Figure 2).

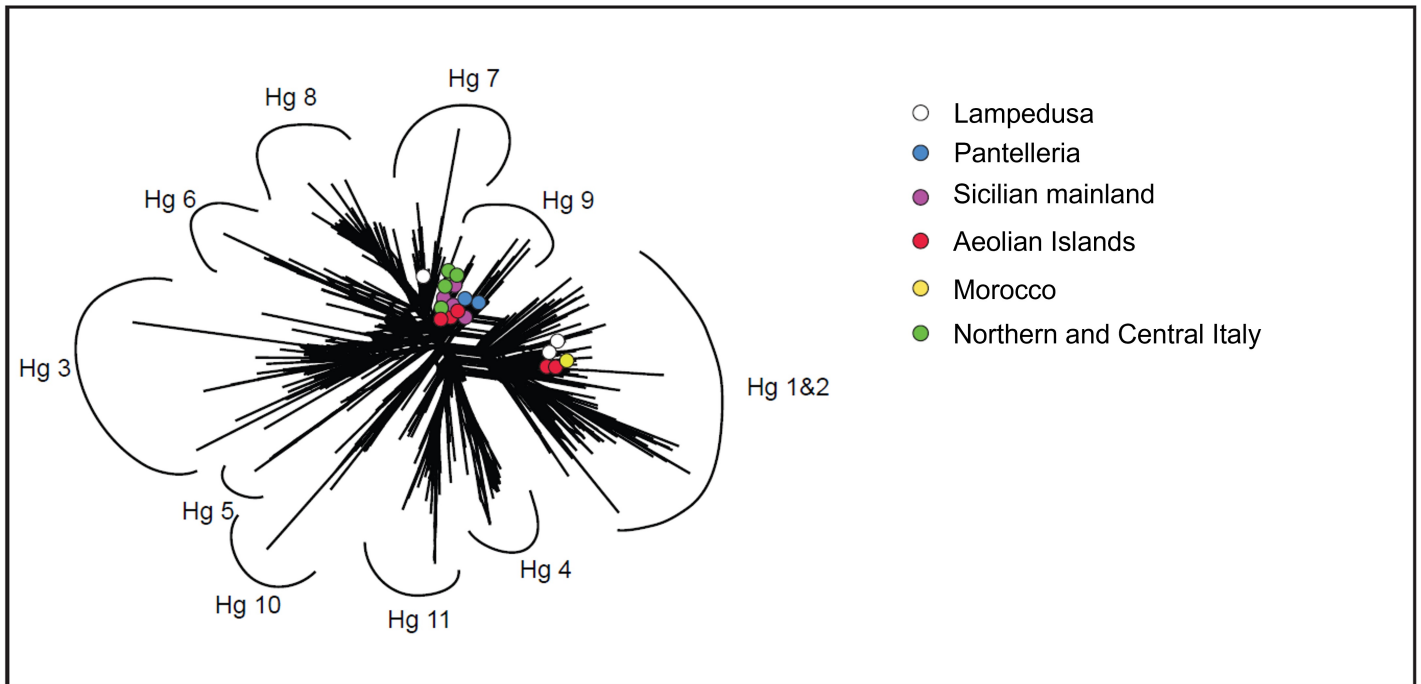


Figure 2. Neighbor-net network showing the relationship among *Mus domesticus* haplotypes from Lampedusa (white dots) and Pantelleria (blue dots) and the ones from previous phylogeographic analyses of house mouse population from Mediterranean area. Dots of different colours represent Mediterranean haplotypes. The acronym Hg refers to haplogroups identified in previous works (Bonhomme et al., 2011).

### *Rattus* spp.

The BLAST results show that one of the two haplotypes from Lampedusa blasts with *Rattus rattus* (identity = 99%; 1 mutation over 1039 bp). The second sequence from the same island blasts (100% identity over 1057bp) with three GenBank sequences assigned to *R. norvegicus* (KY986748, KY697996, and KM820832) and therefore this individual can be assigned to this last species. The new haplotype from

Pantelleria blasts with several *Rattus rattus* specimens (100% identity over 1024 bp). The TCS network evidenced that the new *R. rattus* haplotype from Pantelleria corresponds to the most common Mediterranean haplotype (HapC1; Colangelo et al., 2015), which is present in Italy and Egypt, but also occurs outside the Mediterranean area, from Australia to America (Figure 3).

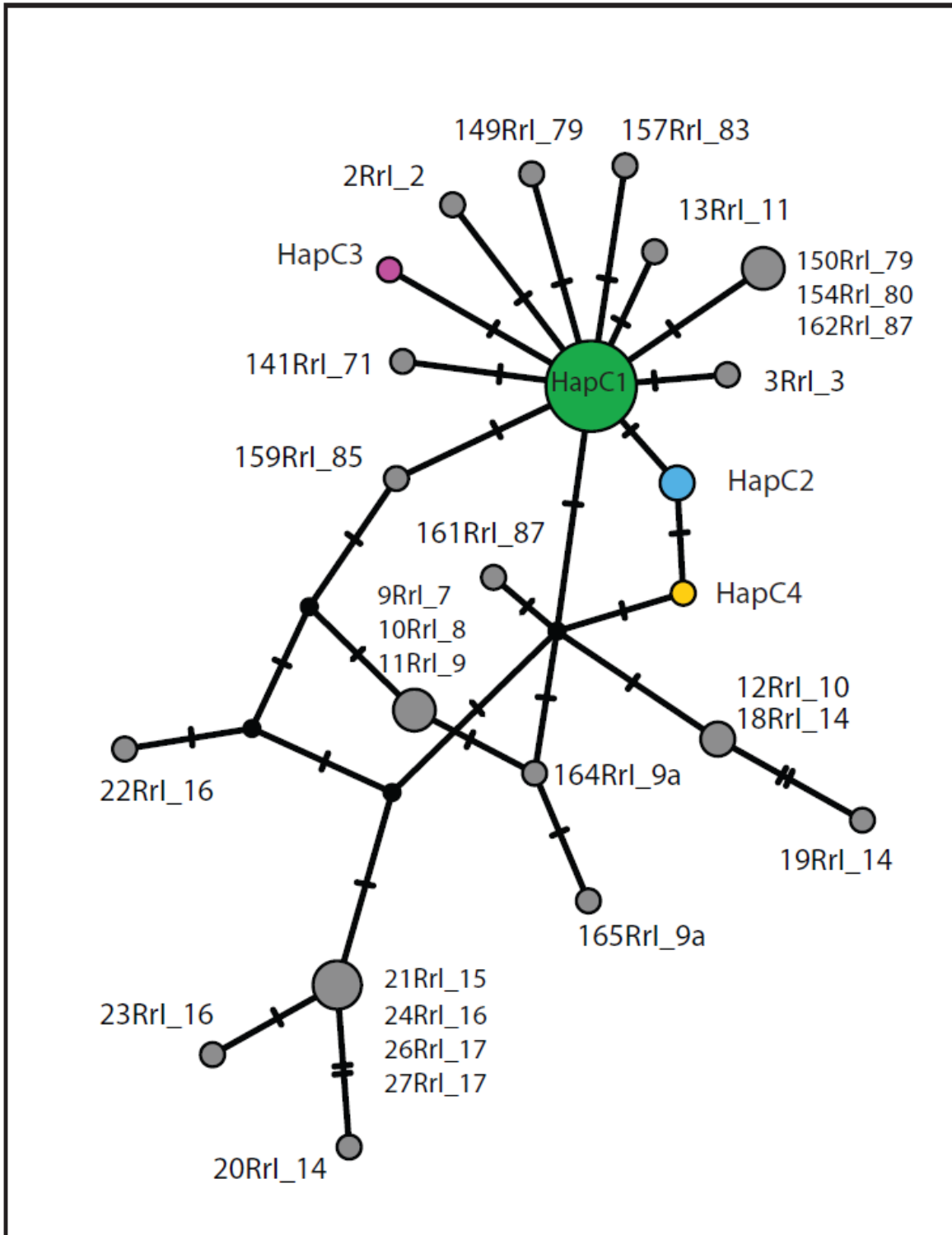


Figure 3. *Rattus rattus* TCS parsimony Network showing the relationship among the new and previously described haplotypes. Circles represent different haplotypes. The green circle represents a haplotype from several localities worldwide (called HapC1 in previous literature) including *Rattus rattus* from Pantelleria of the present study. Other haplotypes are named as in the original papers: HapC (Colangelo et al., 2015), Rrl (Aplin et al., 2011). Dot size is proportional to the number of samples for each haplotype.

## DISCUSSION

Our analyses provide the first genetic evidence that *R. norvegicus* occurs on Lampedusa island. The presence of the species was previously confirmed only through direct observation on both islands (reviewed in Angelici et al., 2009). This finding concurs with some recent evidence that this species may colonize islands characterized by a dry and barren environment, apparently quite unsuited for the species (Castiglia et al., 2019). Particularly, this species may have colonized Lampedusa from North Africa (cf. Carretero et al., 2009).

Moreover, *R. rattus* was confirmed to be present both on Pantelleria and Lampedusa. For this species, the haplotype found in the frame of this study does not diverge from the ones previously found in the islands. Its similarity to common and widespread haplotypes does not allow us to infer some indication about the origin of these specimens. Therefore, for *R. rattus*, we cannot go beyond species identification and the origin of the Lampedusa and Pantelleria populations of these animals remains obscure.

In *M. domesticus*, the newly collected haplotype from Pantelleria is very similar (0,24% divergence) to the other one previously described from the island (Solano et al., 2013). These haplotypes cluster with haplotypes from mainland Sicily in the haplogroup Hg9 (Bonhomme et al., 2011), which is distributed mainly in the Middle East and Italy. That association indicate an origin of this haplotype probably from Italy or from a Middle East wave of colonization (Bonhomme et al., 2011). The new haplotype from Lampedusa diverges from the ones previously described (1.5%) and clusters with haplotypes from North Africa and from Middle East, Hg7 (D1 in Jones et al., 2011), indicating a colonization route that likely originates from the south of Mediterranean basin. Since Hg7 is very rare in Italy, we can speculate that this haplotype can be of African rather than Italian origin. Conversely, the two haplotypes previously

described in the islands were part of the Hg1 and Hg2 haplogroups (Solano et al., 2013) which are widely distributed especially in Italy and Sicily, suggesting colonization from Italy. These data, that deserve further investigation, suggest that in Lampedusa at least two distinct events of colonization took place, in agreement with the peculiar geographical position of the island, located between the two mainland masses.

Recent studies have highlighted how the Neolithization process, which began in the Middle East about 14,000 years ago, laid the foundations for links between men and animals (Cucchi et al., 2020). Links exist not only at the level of human exploitation with sheep, goats, and cattle but also for those species which have adapted to coexist and exploit human resources, e.g. mice and rats. Among these, the earliest species known to have become commensal with man is undoubtedly the house mouse, which is associated since early times with humans (Bona 2020).

The expansion of the Neolithic lifeway from the Middle East to the west led the house mouse to colonize all of Europe, with timing and migration routes not yet fully understood. Cucchi et al. (2020) proposed the probable presence of two expansion ways. The first one was from the Mediterranean via sea transport, which saw the colonization of Cyprus as its first stop during the Late Pleistocene, but which does not seem to reach the Aegean islands before the early Bronze Age (about 4000 years ago) (Cucchi et al., 2005, 2012). The second route of colonization may have involved Caucasian Europe towards the end of the Neolithic, about 6500 years ago. Recently, during new excavations in Northern Italy at the late Neolithic site – dating to about 6,000 years ago – of Tosina di Monzambano (province of Mantua) the remains of a probable *M. musculus* have been identified, posing new questions about the timing of the expansion of the house mouse in the area (Bona, 2020). Among other species which took advantage of contact with man and human trade, rats (*R. rattus* and *R.*



*norvegicus*) also have oriental origins (Masseti, 2008). Archaeozoological data on the presence of *R. rattus* shows that it arrived in Europe at the latest during the Iron Age but most likely earlier than that (Audoin-Rouzeau and Vigne 1994; Masseti 2008; Ruffino and Vidal 2010). Conversely, *R. norvegicus* was not known in Western Europe before the 16<sup>th</sup> century (Masseti 2008).

The presence of the house mouse on the islands of Pantelleria and Lampedusa could have arisen from contact with the local populations who transported Neolithic lifeways from the east to the west by the sea. The fossil form of Lampedusa *Mus lopadusae* (Burgio and Catalisano 1994) could also represent an endemic form of North African origin, originating from *M. spretus*. Similarly, the presence of the two species of rats suggests some interesting questions for future research.

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