



Short Note

Recent island colonization by an introduced shrew in the western Mediterranean

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Abstract

Insular ecosystems are sensitive to alien invasive species. The Balearic Islands have been colonized by a large list of invasive species, in most cases due to human activity. White-toothed shrews have been historically recorded in this archipelago and to date four different species have been found. In this study we focus on the white-toothed pygmy shrew (*Suncus etruscus*) at the island of Mallorca, assessing its current status and identifying both its genetic variation and geographic structure. During the last 50 years this species has colonized a large proportion of the island. Haplotype network analyses support the hypothesis of a unique and recent colonization event from a single but unknown source population. Available genetic information suggests the populations from northeast Iberian Peninsula (Catalonia) and southwest France (Camargue) as the most plausible source of the lineage found in Mallorca. The recent and extensive distribution of *Suncus etruscus* on this island is characteristic of a fast growing population at an invasive colonization phase, indicating that it has found in Mallorca suitable ecological conditions to thrive.

The problems presented by invasive species have been increased with the globalization of world trade (Gargan et al., 2016). Large-scale movement of people and goods by land, sea and air has led to many species being accidentally transported to regions out of their natural ranges, with often detrimental consequences for native biodiversity (Hulme, 2009; Vilà et al., 2011). Identifying the origins of these invasions is a critical management strategy in controlling the spread of invasive species (Boissin et al., 2012; Hulme, 2009) and the analyses of genetic data from invasive populations and putative source areas become imperative (Boissin et al., 2012; Estoup and Guillemaud, 2010; Searle, 2008).

Two species of white-toothed shrews (Crocidae) have been historically recorded in the Balearic Islands (Western Mediterranean). *Crocidae pachyura* is present in Eivissa, where was probably introduced by the Carthaginians between 645 and 123 BC, and *C. suaveolens* occurs in Menorca and was introduced in Roman times, between 123 BC and 100 AD (see Alcover, 2010). The occurrence in Mallorca

of two other shrews, namely *C. russula* and *Suncus etruscus*, has been recognized in the past decade (Brover et al., 2012; Pinya et al., 2008). To date, the distribution of these two latter species in the Balearic Islands remains unknown, and few records have been published so far.

White-toothed pygmy shrew, *S. etruscus*, is a south Palearctic species of Asian origin. Currently it is widely distributed all over the Mediterranean area (e.g., Libois and Fons, 1999), but no fossil evidence has been recorded in the whole region (e.g., López-García et al., 2013). *S. etruscus* has colonized different Mediterranean islands transported by humans, and its distribution pattern points to a human-related spread (Dobson, 1998). In the Mediterranean area, it inhabits open areas and is common in olive groves, vineyards, Mediterranean scrubland, and abandoned fields close to urban areas. It can also dwell in oak, pine and chestnut forests.

The first record of *S. etruscus* in Mallorca was published in 1956 based on a skull found inside a pellet of *Tyto alba* (Kahmann and Altner, 1956). Despite the extensive fieldwork and barn owl pellet analyses carried out by several researchers in the 70s and 80s, no additional citations exist for the 20th century. Van den Brink (1971) included Mallorca in a map with the European distribution area of *S. etruscus*, while Al-

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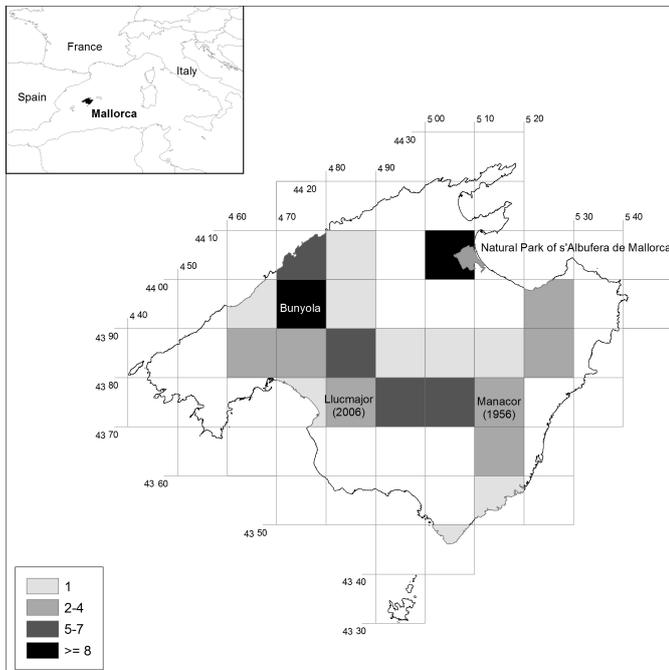


Figure 1 – Distribution map of *Suncus etruscus* records during the period 1956-2015, expressed in 10 km² grids.

cover (1979) considered that the remains documented by Kahmann and Altner (1956) could have been transported by a barn owl that hunted an individual of *S. etruscus* on mainland, flew to Mallorca and produced there a pellet with its remains. This first record remained isolated until 2006, when two specimens were filmed in a southern Mallorcan scrubland (Alcover, 2010). In order to expand our knowledge about this species in the Balearic Islands we (i) assess both the current status and distribution of the white-toothed pygmy shrew on the island of Mallorca through a compilation of records and (ii) investigate the genetic diversity and source of origin of Mallorca's population.

Records of *S. etruscus* were obtained from three different sources: social participation, literature revision and barn owl pellet analysis. So-

cial participation consisted on obtaining information from 23 naturalists from Mallorca which kindly reported information of date, locality and number of specimens of *S. etruscus* found. The literature review consisted in retrieving available information from the Balearic Environmental Agency on *S. etruscus* distribution available at the *Bioatles* project (www.bioatles.caib.es), as well as in checking the records of the wildlife recovery centre *Consorti per a la Recuperació de la Fauna de les Illes Balears* (COFIB) and scientific literature. In addition to distribution data, we also obtained information regarding date, locality and number of specimens found. Finally, information regarding barn owl pellets with positive content of *S. etruscus* remains was obtained from a study on distribution of micromammals at the Natural Park of s'Albufera carried out during an environmental teaching camp (spring and autumn from 2007–2014).

Genetic analyses were performed on 20 shrews from Mallorca that were captured by cats or found dead in gardens or pools. In addition, we also obtained tissue from five specimens from Catalonia provided by IRBio from the University of Barcelona. All these samples were preserved in absolute ethanol at -22°C until DNA extraction. The right foot of each specimen was excised, air-dried and fragmented in small pieces. Tissue samples were incubated in 180 μl homogenization buffer (ATL buffer, Qiagen) and 20 μl proteinase K 20 mg/ml overnight at 56°C . DNA was extracted using a spin column containing anion-exchange purification matrix following manufacturer's instructions (DNasy extraction kit, Qiagen, Valencia, CA, USA). Purified DNA was eluted in 200 μl Tris 10 mM buffer in the final protocol step. PCR reactions were performed using primer pair C3 Forward (5'-TATTCTCCCCAGACATATTAGG-3', Dubey et al., 2006) and H15915 Reverse (5'-AACTGCAGTCATCTCCGTTTACAA GAC-3', Brändli et al., 2005) amplifying a 419 bp fragment of the mitochondrial cytochrome b (cytb) gene (including a short fragment of tRNA-Thr, 31 bp). One microlitre of template DNA (50–100 ng) was used in each reaction in a final volume of 25 μl containing: 1 \times PCR BioTaq Buffer (Bioline, London, UK), 3.75 mM MgCl₂, 0.1 μM from each primer, 0.2 mM dNTPs, 0.275 U BioTaq (Bioline, London, UK), and sterile water. PCR cycling conditions were: initial denaturation at 94°C for 3 min; 40 cycles of denaturation at 94°C for 30 s; primer annealing at 45°C for 45 s; elongation at 72°C for 1 min; and a final elongation step at 72°C for 10 min. PCR products were inspected by

Table 1 – Details of the *Suncus etruscus* specimens from Mallorca and Catalunya used in this paper. (*) Pinya et al., 2008.

| Species | DNA # | Locality 1 | Locality 2 | Code/N° Collection | Date |
|--------------------|-------|---------------|------------|-------------------------------|-------------------------------|
| <i>S. etruscus</i> | #1 | Lluçmajor | Mallorca | | 2006, June |
| <i>S. etruscus</i> | #2 | Porreres | Mallorca | | No date |
| <i>S. etruscus</i> | #3 | Sant Llorenç | Catalunya | Tardor 6–5 | 2006, November 30 |
| <i>S. etruscus</i> | #4 | Sant Llorenç | Catalunya | Tardor 93.1 | 2006, November 30 |
| <i>S. etruscus</i> | #5 | Sant Llorenç | Catalunya | Tardor 44.4 | 2006, November |
| <i>S. etruscus</i> | #6 | Sant Llorenç | Catalunya | 93–4 | 2007 |
| <i>S. etruscus</i> | #7 | Sant Llorenç | Catalunya | 27–2 | 2007 |
| <i>S. etruscus</i> | #8 | Bunyola | Mallorca | MCBN 13473 | 2008, March ^(*) |
| <i>S. etruscus</i> | #9 | Bunyola | Mallorca | MCBN 13474 | 2008, August 3 ^(*) |
| <i>S. etruscus</i> | #10 | Bunyola | Mallorca | MCBN 13475 | 2008, June 10 ^(*) |
| <i>S. etruscus</i> | #11 | Bunyola | Mallorca | MCBN 13476 | 2008, June 10 ^(*) |
| <i>S. etruscus</i> | #12 | Algaida | Mallorca | COFIB 1658/09 IMEDEA 91939 | 2009, August 10 |
| <i>S. etruscus</i> | #13 | Algaida | Mallorca | COFIB 579/10 IMEDEA 91940 | 2010, September 9 |
| <i>S. etruscus</i> | #14 | Bunyola | Mallorca | COFIB 600/10 IMEDEA 91941 | 2010, September |
| <i>S. etruscus</i> | #16 | Manacor | Mallorca | COFIB 579/10 IMEDEA 91943 | 2010, September |
| <i>S. etruscus</i> | #17 | Algaida | Mallorca | IMEDEA 91944 | 2010, September |
| <i>S. etruscus</i> | #24 | Algaida | Mallorca | | 2010 Autumn |
| <i>S. etruscus</i> | #25 | Algaida | Mallorca | | 2010 Autumn |
| <i>S. etruscus</i> | #26 | Algaida | Mallorca | | 2010 Autumn |
| <i>S. etruscus</i> | #27 | Santa Eugènia | Mallorca | COFIB 162/11 | 2011, March 3 |

electrophoresis in a 1% agarose gel stained with ethidium bromide and then visualized under UV light. PCR products were purified using the MSB Spin PCRapace kit (Invitex, Berlin, Germany) following manufacturer’s protocol. PCR fragments were sequenced in both directions using the ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction kit v. 3.1 and analyzed on an ABI 3100 automated sequencer (Applied Biosystems, Foster City, CA, USA). Sequences were trimmed and edited with CodonCode Aligner (CodonCode Corporation, Dedham, MA, USA). In addition to the newly obtained cytb sequences from the Balearic Islands and from Catalonia, we also collected GenBank data from the rest of the world distribution area (see Tab. 2 for accession number and details). Multiple sequence alignment was performed using MAFFT 7 online version (<http://mafft.cbrc.jp/alignment/server/>; Katoh and Standley, 2013) using the default FFT-NS-1 algorithm. The sequences were compared to investigate the source region of the Mallorcan populations. To do so, the genealogical relationships at the intraspecific level of the cytb sequences of *S. etruscus* were analyzed using the software PopART (Leigh and Bryant, 2015) and implementing the TCS method of haplotype network estimation (Clement et al., 2000).

A total of 84 specimens of *S. etruscus* were recorded at different localities all over Mallorca. Temporal distribution of records showed two main chronological groups separated by a gap of 50 years, being the first the single 1956 record isolated from all the rest (2006-2015, see supplementary information). The second group of observations included 22 different localities (Fig. 1) and agreed with the previously published data on habitat use (Libois and Fons, 1999; López-Fuster, 2007), especially in agricultural and periurban areas.

Eight out of the 383 barn owl pellet analyses (2007–2014) at the Natural Park of s’Albufera de Mallorca corroborated the presence of *S. etruscus*, with all positive results from samples collected since 2010.

From the 20 samples for genetic analyses, 15 yielded positive PCR products for the cytb primers that could be sequenced by Sanger chemistry (Accession Number LN908795, see Tab. 1). In addition, cytb sequences were obtained for the five *S. etruscus* individuals from Catalonia (Accession Number LN908796).

All the Mallorcan specimens showed identical sequence for the 419 bp fragment of cytb (Accession Number LN908795), and just showed a single mismatch to the again identical sequence obtained from the five Catalonia specimens (Accession Number LN908796) and the GenBank sequence DQ630396 from Camargue, France (see Fig. 2). Mallorcan *S. etruscus* individuals has a G in position 64 whereas the Catalanian and Camargue specimens display a transition to A. Accordingly, a very close genealogical relationship between the Mallorcan *S. etruscus* population and the individuals from Catalonia and Camargue is supported by the haplotype network reconstruction (Fig. 2). The remainder of the European samples are less related to the Mallorcan haplotype than the Catalanian ones, differing in two and three mutational steps with the

Table 2 – List of cytb sequences of *Suncus etruscus* used in the genetic analysis of this paper.

| Species | Locality | Accession Number | Reference |
|--------------------|-------------------|------------------|-----------------------------------|
| <i>S. etruscus</i> | Mallorca (Spain) | LN908795 | This paper |
| <i>S. etruscus</i> | Catalunya (Spain) | LN908796 | This paper |
| <i>S. etruscus</i> | Gard (France) | JF817396 | Omar et al., 2011 |
| <i>S. etruscus</i> | Camargue (France) | DQ630396 | Dubey et al., 2007 |
| <i>S. etruscus</i> | Fivizzano (Italy) | DQ630397 | Dubey et al., 2007 |
| <i>S. etruscus</i> | Sri Lanka | FJ716836 | Megaaskumbura and Schneider, 2008 |
| <i>S. etruscus</i> | Vietnam | KF110756 | Stanley et al., 2013 |
| <i>S. etruscus</i> | India | JN556043 | Megaaskumbura et al., 2012 |



Figure 2 – Haplotype network of the mitochondrial cytb region of *S. etruscus* constructed with the TCS method. Colored symbols represent the observed haplotypes and black circles represent unobserved, inferred haplotypes. Mutational steps between alleles are indicated with numbers above the connecting lines. Map adapted from <https://upload.wikimedia.org/wikipedia/commons/e/e8/BlankMap-World6-Equirectangular.svg> under a Creative Commons CC0 1.0 Universal Public Domain Dedication. Full terms at: <https://creativecommons.org/publicdomain/zero/1.0/deed.en>.

specimens from the Gard (France) and the Fivizzano (Italy), respectively.

Our results indicate that *S. etruscus* colonized Mallorca (Fig. 1) in the last 60 years, spreading over a large part of the island. The current known distribution is highly connected, since most of the grids of 100 km² where *S. etruscus* has been reported are in contact. The only isolated grid corresponds to the Natural Park of s’Albufera de Mallorca, separated to the rest of the grids by approximately 10 km. Through the barn owl pellet analyses in this Natural Park *S. etruscus* was first recorded in 2010, suggesting that an actual connection exists and that this region was only recently colonized.

The temporal distribution of the records shows a gap between the first record reported by Kahmann and Altner (1956) and the beginning of new records started in 2006 until nowadays. This gap may indicate either (i) two different episodes of introduction, being the first a possible failed attempt since no additional citations have been recorded in the subsequent 50 years, or (ii) a single colonization event with a latency period between the arrival of the species and its wide visibility since 2006.

S. etruscus distribution has been enhanced by human activity (Dobson, 1998). It has been transported to Mallorca by humans, probably involuntarily, as happened in other Mediterranean islands such as Elba, in the Tuscan archipelago (Szpunar et al., 2008) or Lipari, in the Aeolian islands (Lo Cascio and Masseti, 2004, 2007), both in Italy. Our genetic data show the lack of genetic divergence among all sampled Mallorcan samples, supporting for a single and recent colonization event from one locality. Although the genetic information available in GenBank regarding cytb sequences of *S. etruscus* is limited, our haplotype network analysis indicates that the Mallorcan population would come from a single colonization event but from an unknown source. Nonetheless, our results suggest the northeast Iberian Peninsula (Catalonia) and southwest France (Camargue) as the most plausible region of origin to the Mallorcan specimens. However, additional data from other geographical sources are needed, particularly from other Mediterranean territories. The genetic results fit into the most complex panorama of artificial colonization carried out by several species of micro-mammals, mainly shrews, on the Mediterranean islands, through human mediation. A closer case is the shrew, traditionally referred to *Crocidura russula*, that today is dispersed on the European islands of Ibiza (Spain), Sardinia and Pantelleria (Italy), (Lo Brutto et al., 2004; Cosson et al., 2005).

It is hard to predict the invasive or non-invasive character of *S. etruscus* and its ecological consequences. The impact of *S. etruscus* on the Mallorcan small invertebrates, including endemic species, is totally unknown, although it could be important. The recent and extensive distribution of *S. etruscus* on this island is characteristic of a fast grow-

ing population at an invasive colonization phase, thus indicating that it has found in Mallorca suitable ecological conditions to thrive. Furthermore, the ecological impacts of introduced species on islands often become apparent only after decades and remain hidden until it is too late to address their adverse effects adequately (e.g., White and Harris, 2002). Therefore, major attention needs to be focused on this species to determine and quantify its ecological impact on island diversity. Besides, the difficulty of sampling this elusive shrew species encourages the inclusion of social participation as an important source of both ecological information and samples for genetic research. ☞

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Supplemental information

Additional Supplemental Information may be found in the online version of this article:

Table S1 Records from *S. etruscus* in Majorca during the period 1956–2015.