

Short Note

Evidence for a recent introduction of *Crocidura russula* (Mammalia, Eulipotyphla, Soricomorpha) in Mallorca (Balearic Islands, western Mediterranean Sea)

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Barceló i Combis (1872, 1875) reported on the presence of *Crocidura aranea* in Mallorca. He mentioned the sighting of two specimens west of Palma in May 1870 (Barceló i Combis 1872), indicating that he collected a male (Barceló i Combis 1872: 87). This is the sole claimed direct evidence for the presence of *Crocidura* in this island. Despite an effective and extensive sampling by mammalogists during the past 140 years no specimen of *Crocidura* was obtained again in Mallorca, and not a sole inhabitant of the island, except for Barceló i Combis (1872, 1875), reported its presence on the island. The shrews are unknown to the local farmers and skeletal remains from owl pellets have never been obtained in Mallorca. Consequently, the report by Barceló i Combis (1872), although incorporated by Thomas (1901) and Cabrera (1914), was later (see Alcover 1979) considered unreliable (like the reference for the presence of *Arvicola amphibius* in Mallorca by the same author – a misidentification of *Rattus norvegicus*). Nowadays there is a general consensus on the absence of *Crocidura* on the island. By contrast, a population of *Crocidura suaveolens*, a species introduced in the Roman period (Reumer and Sanders 1984), inhabits Menorca, and *Crocidura pachyura*, a species of North African origin (Beaucournu and Alcover 1984, Cosson et al. 2005) probably introduced by the Carthaginians (Alcover 2010), inhabits the island of Eivissa. The taxonomic status of the Eivissa population, considered as an endemic subspecies of *Crocidura russula*, changed recently after the study of Cosson et al. (2005), which allowed Hutterer (2005) to identify it as *Crocidura*

ichnusae, a name subsequently emended to *C. pachyura* by Turni et al. (2007).

Crocidura pachyura is abundant on Eivissa. Among mammals, the Mediterranean shrew *C. pachyura* was the most captured species by barn owls in two Eivissa samples from the 1970s (>55%; Alcover 1977), while it was less abundant (38.4%) in a recent study by Sommer et al. (2005). Whether these differences are related to annual changes in abundance or to other factors remains unresolved. On Menorca, *Crocidura suaveolens* is scarce and never reaches percentiles >10% in the barn owl pellets (de Pablo 2000).

Here we report on the capture of a specimen of *Crocidura* on Mallorca (Figure 1, top). The specimen was captured by a dog in a country road called Camí de ses Gleasses (Alcúdia, North of Mallorca; UTM 509638/4410882) on 20 February 2011. It was submitted by Mrs Noemí Eva Saura to the wildlife recovery centre Consorci per a la Recuperació de la Fauna de les Illes Balears (COFIB) and the specimen was later sent to the Mediterranean Institute for Advanced Studies (IMEDEA, UIB-CSIC) for further analysis and taxonomical identification. The specimen was prepared by water maceration and its skeleton and skin are currently kept at the Vertebrate Collection of the IMEDEA, under the accession number IMEDEA-95600. Its measurements are as follows: head and body length, 64 mm; tail length, 38 mm; foot length, 12 mm, ear length, 6 mm; weight, 8.37 g.

The fur coloration and skeleton of the specimen was compared with skins and skulls of *Crocidura suaveolens* from the



Figure 1 Specimen of *Crocidura russula* (top) obtained in Mallorca (IMEDEA 95600) in comparison with a specimen of *Suncus etruscus* (bottom) from the same island.

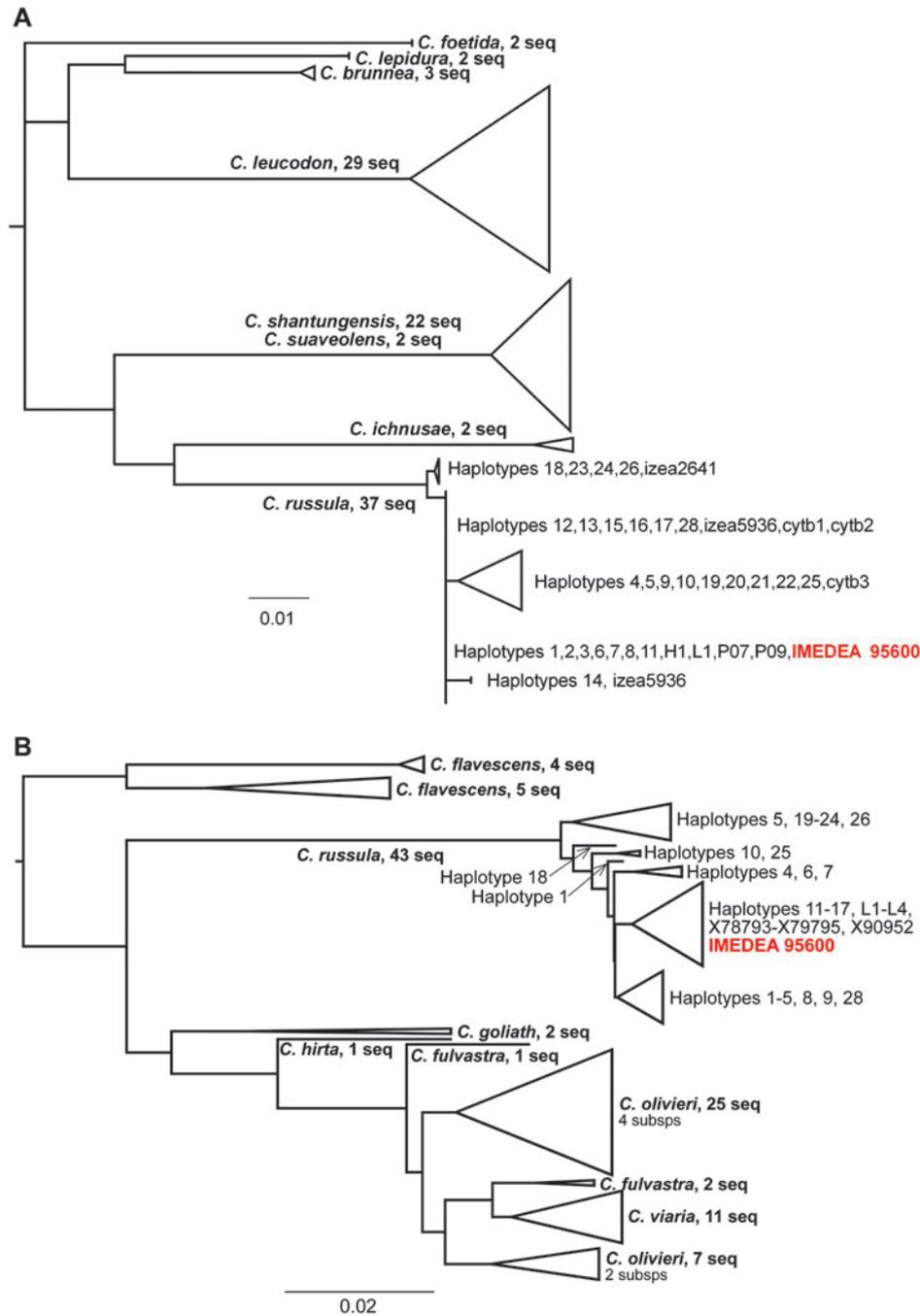


Figure 2 Neighbor-Joining tree built with the sequences of the best 100 blastn hits obtained with the (A) cytochrome b (cyt b) and (B) control region (CR) sequences from individual IMEDEA 95600. Triangles represent cluster of sequences from other *Crocidura* species. The specimen was preserved in ethanol 96% at 4°C until DNA extraction. Right foot was excised, air-dried, fragmented in tiny pieces and then incubated in homogenization buffer and proteinase K overnight at 55°C. DNA was purified with a spin column containing anion-exchange purification matrix following manufacturer's instructions (DNasy extraction kit, Qiagen, Valencia, CA, USA). Polymerase chain reaction (PCR) amplification of a partial sequence of about 350 bp of the mitochondrial cyt b gene coding for the protein cytochrome b was performed using the primer pair L14841-C4 and PCR conditions described in Dubey et al. (2006). We also amplified a fragment of the mitochondrial CR of about 900 bp employing the protocol described elsewhere (Brändli et al. 2005). PCR fragments were sequenced in both directions using the ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction kit v. 2.0 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) and then compared with those deposited on Genbank using the blastn algorithm (<http://www.ncbi.nlm.nih.gov/blast/Blast.cgi>) to determine which sequences (and taxa) were most similar to our queries.

neighboring island Menorca and of *Crocidura pachyura* from Eivissa, and with skulls of *Crocidura russula* from Catalonia. The coloration of IMEDEA-95600 is brown in the upper parts, darker than the coloration of the Eivissan *C. pachyura* (brown pale), while the color of the Menorcan *C. suaveolens* is gray dark. The morphology of the teeth clearly differs from *C. suaveolens* and is more similar to *C. pachyura* and *C. russula*. The morphology of the teeth clearly differs from *C. suaveolens* in the relative size of the last two upper unicuspid in relation to the parastyle of the fourth premolar, being more similar to *C. pachyura* and *C. russula*. As the latter two species are morphologically very similar and considered as sibling species (Cosson et al. 2005), a genetic test was performed. The cytochrome b (cyt b) sequence (304 bp, accession number HE575249) was 100% identical to several cyt b haplotype sequences of *C. russula*, and control region (CR) sequence (868 bp, accession number HE576689) showed 99% similarity to those from the former species. The Neighbor-Joining tree (Jukes & Cantor distance method) (Figure 2) shows how similar were our cyt b and CR sequences to the *C. russula* haplotypes obtained elsewhere, but also the divergence to sequences of other species of the same genus (see accession numbers in Appendix 1). The cyt b sequence obtained from IMEDEA 95600 indicates that the specimen displays the European haplotype of *C. russula* recorded in Spain, France, Portugal, Germany and Switzerland, according to data of Pfunder et al. (2004), Brändli et al. (2005), and Fontanillas et al. (2005). CR sequence indicates that the Mallorcan specimen displays the European haplotype of *C. russula* recorded in Switzerland (Fumagalli et al. 1996, Ehinger et al. 2002, Brändli et al. 2005, Fontanillas et al. 2005).

Besides the existing early report on the presence of *Crocidura* on Mallorca in the 19th century (Barceló i Combis 1872), we consider that the reported presence of the species corresponds to a recent arrival, probably a very recent one. It is highly improbable that the species was present on the island and remained invisible for more than 140 years. It is remarkable that the specimen was captured at a distance of nearly 2.5 km from an important harbour, Port d'Alcúdia. Although the main activities of this harbour lie within Menorca, it also receives trade vessels from different places in the Iberian mainland and south France. Recent trade on Mallorca includes an import of large trees for garden equipment and for harvesting. The recent introduction of different snakes and the Pigmy shrew has been associated with this trade (e.g., Pinya et al. 2008, Pinya and Carracedo 2011).

Crocidura russula is probably now established on Mallorca. Shrews of the genus *Crocidura* are non-indigenous in the Balearic Islands. Currently it is hard to predict its "invasive" or "non-invasive" character (considering as invasive a non-indigenous species that spreads and becomes so abundant that it causes an ecological harm; see Kolar and Lodge 2001). In the past, from the Messinian to the human arrival, a lineage of shrews belonging to the endemic genus *Nesiotites* inhabited Mallorca and Menorca (see Bover and Alcover 2008, for the timing and causes of its extinction). Although the impact of the recently introduced *C. russula* on invertebrates is

unpredictable, this species could be considered as an ecological equivalent to the extinct shrews, and therefore a remarkable effect on the endemic invertebrate fauna it is not to be expected.

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Appendix 1

List of GenBank accession number of the best 100 hits found with blastn of the species of *Crocidura* used in this paper. Identity coverage and e-values are available on demand.

Cytochrome b

Crocidura brunnea (DQ630385, EF524729, FJ814030); *Crocidura foetida* (EF524750, EF524751); *Crocidura ichnusa* (EF524715, EF524727); *Crocidura lepidura* (FJ814022, FJ814023); *Crocidura leucodon* (DQ994744-DQ994755, DQ994770-DQ994772, DQ994774, DQ994775, DQ994779-DQ994787, EF417543-EF417545); *Crocidura russula* (AF003766, AJ000467, AJ000468, AY332697, AY332698, AY769263, AY769264, AY918373-AY918398, AY918400, DQ975238, DQ975239, EF524791); *Crocidura shantungensis* (AB077076-AB077082, AB077149, AB077151, AB077152, AB077278, DQ630395, EU742584-EU742590, EU742592-EU742594); *Crocidura suaveolens* (AB066257, AB066259).

Control region

Crocidura flavescens (DQ305146-DQ305148, DQ305150-DQ305152, DQ305191-DQ305193); *Crocidura fulvastra* (DQ305155, DQ305156, DQ305194); *Crocidura goliath* (DQ305165, DQ305166); *Crocidura hirta* (DQ305167); *Crocidura olivieri* (DQ305145, DQ305157-DQ305164, DQ305168-DQ305190); *Crocidura russula* (AF343009-AF343017, AY769263, AY769264, AY918341-AY918366, AY918368, X78793-X78795, X90952); *Crocidura viaria* (DQ305198-DQ305208).

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