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Mediterranean populations of the lesser white-toothed shrew (*Crocidura suaveolens* group): an unexpected puzzle of Pleistocene survivors and prehistoric introductions

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Abstract

An earlier study revealed the strong phylogeographical structure of the lesser white-toothed shrew (*Crocidura suaveolens* group) within the northern Palaearctic. Here, we aim to reconstruct the colonization history of Mediterranean islands and to clarify the biogeography and phylogeographical relationships of the poorly documented Middle East region with the northern Palaearctic. We performed analyses on 998-bp-long haplotypes of the mitochondrial cytochrome *b* gene of 143 samples collected around the Mediterranean basin, including islands and the Middle East. The analyses suggest that the Cypriot shrew belongs to the rare group of relict insular Pleistocene mammal taxa that have survived to the present day. In contrast, the Cretan, Corsican and Menorcan populations were independently introduced from the Middle East during the Holocene. The phylogeographical structure of this temperate Palaearctic species within the Middle East appears to be complex and rich in diversity, probably reflecting fragmentation of the area by numerous mountain chains. Four deeply divergent clades of the *C. suaveolens* group occur in the area, meaning that a hypothetical contact zone remains to be located in central western Iran.

Keywords: *Crocidura suaveolens*, endemism, Holocene, mammals, Mediterranean islands, Middle East, Pleistocene

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Introduction

The Pleistocene fauna of large Mediterranean islands such as Cyprus, Crete, Corsica, Sardinia, Sicily, Malta and Majorca is known for its low mammalian diversity and extremely endemic fauna, characterized by dwarf forms of large herbivores such as elephant and hippopotamus (Azzaroli 1971, 1982; Sondaar 1977; Palombo 1985), as well as giant forms of small mammals such as rodents (Blondel & Vigne 1993). These islands have been isolated from the African and European continents since the Oligocene 30 million years ago (mya), and only the Messinian salinity crisis that occurred about 5.3 mya (Miocene–Pliocene transition) and partially dried out the Mediterranean Sea (Krijgsman *et al*. 1999) permitted colonization of islands by land bridges (Palombo 1985). This event marked the beginning of a prolonged period of evolution of insular mammals in isolation from the continental ones. But, most endemic species became extinct during the Holocene following human colonization (Vigne 1999), and only a handful of small endemic mammals survive today: the Cretan shrew *Crocidura zimmermanni* (Vogel *et al*. 1986), the Sicilian shrew *C. sicula* (Vogel *et al*. 1990), and the Cypriot mouse *Mus cypriacus* (Bonhomme *et al*. 2004; Cucchi *et al*. 2006). These extinction events appear to be a general pattern of the postglacial period, as massive extinction of

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the mammalian megafauna occurred all around the world at that time (Martin 1984). Landscape changes, hunting and colonization by continental species, both competitors and predators, are regularly invoked to have played a major rule in the cascade of extinctions (Vigne & Alcover 1985; Simmons 1991; Hardy *et al*. 1994; Vigne 1999; Davis *et al*. 2003; De Marinis & Masseti 2003).

The chronology of colonization of the Mediterranean islands by continental mammals during the Holocene has been documented in detail from fossil records on most of the large Mediterranean islands; see Marra (2005) for a compilation of field data from numerous authors. Various processes of colonization have been hypothesized, such as rafting and swimming (Simpson 1940), especially for large species at the times that the sea straits were narrow or when land bridges emerged between the continent and nearby islands during periods of lower sea levels associated with Ice Ages (e.g. Schüle 1993). Alternatively, many colonization events probably occurred with passive or active human assistance (Vigne 1992; Dobson 1998; Masseti 1998). In fact, the non-endemic insular small mammalian genera introduced are mainly commensal ones such as *Mus*, *Rattus* or *Acomys*, and synanthropic ones such as *Crocidura*, *Suncus* or *Apodemus*, and these probably colonized the islands by passive boat transport. Other mammals such as domesticated ones (cat, cow, dog, goat, pig, sheep and horse) and their feral derivates, as well as games species such as *Cervus*, *Dama* or *Lepus*, were actively introduced (Vigne 1999).

As a result, recent phylogeographical studies have documented different types of introductions for different taxa. In some cases, the site of geographical origin of introduced populations was relatively close in the adjacent mainland, e.g. the wood mouse *Apodemus sylvaticus* from Baleares and the white-breasted hedgehog *Erinaceus concolor* from Crete (Michaux *et al*. 1998; Seddon *et al*. 2001, 2002). In other cases, the geographically closest and most genetically related populations were localized far away on the continent, probably as the result of human transfer via commercial trading. For instance, the North African white-toothed shrew (*C. ichnusae*) known from Ibiza, Pantelleria and Sardinia, were all introduced from an area encompassing eastern Algeria and Tunisia (Brändli *et al*. 2005; Cosson *et al*. 2005). Concerning the lesser white-toothed shrews (*C. suaveolens*), fossil records indicate introduction to Menorca, Corsica and Crete at least 3000–4000 years ago, and to Cyprus as early as 10 000 years ago (Reumer & Payne 1986; Payne 1995; Vigne 1999). Vogel *et al*. (1986) used allozymic data to demonstrate the close affinity between samples from eastern Turkey and Crete, suggesting a Turkish origin for the latter. Thus, considering these results and the origin of other insular shrew populations, the search for a continental source requires a comparison with different lineages that are localized all around the Mediterranean basin, especially in the Middle East. However, the Phoenicians, who were known as traders and sailed all around the Mediterranean Sea, originated from this part of the world and began their colonial activities approximately 3200 years ago (Negbi 1992), long after the Minoan period and others.

The Middle Eastern fauna is presently poorly known from a biogeographical point of view. Few studies have focused on this area, despite the fact that a complex landscape with numerous mountain chains located at the interface of the European, Asian and African continents has led to complex phylogenetic relationships between taxa and resulting from contact zones (Boursot *et al*. 1996; Gündüz *et al*. 2000; Seddon *et al*. 2001; Veith *et al*. 2003; Koch *et al*. 2006).

Based on mitochondrial DNA (mtDNA) and nuclear DNA, Dubey *et al*. (2006) studied the phylogeographical structure of the Eurasian *Crocidura suaveolens* group from Portugal to Japan, omitting the Middle East and the Mediterranean islands. They identified seven main genetically distinct continental clades, which diverged from the Upper Pliocene to the Lower Pleistocene. The largest clade covers a huge range from Eastern Europe to Mongolia (*C.* s. *suaveolens*). West European clades originated from Iberian (*C.* s. *iculisma*) and Italo-Balkanic refugia (*C.* s. *mimula*). In the Near East, three clades evolved in an apparent hotspot of refugia, represented in western Turkey by an undetermined subspecies of *C. suaveolens*, in southwest of the Caucasus by *C. s. gueldenstaedtii*, and southeast of the Caucasus by *C. s. caspica*.

In the present study, we addressed the following questions: (i) are the insular Mediterranean populations of the lesser white-toothed shrews introduced or endemic? (ii) when did these colonization events occur, and from which mainland area? and (iii) is the Middle East phylogeographical structure as complex as the Near East one, and have these southern populations suffered from the Pleistocene glaciations? To unravel these questions, we sequenced 998 bp of the cytochrome *b* gene (*cyt b*) from a large sample of *C. suaveolens* from the Mediterranean basin and the Middle East, including islands as well as the endemic *C. aleksandrisi* from Lybia (Cyrenaica) that is the only known taxon from northern Africa, which could be a close relative or conspecific to the strictly Eurasian *C. suaveolens* (Hutterer 2005). In addition to these samples, we added haplotypes published by Dubey *et al*. (2006) to our dataset in order to clarify the origin of insular lesser white-toothed shrews and the population history across their southern distribution.

Materials and methods

Origin of the material and use of nomenclature

Shrews were collected from 53 localities across the Palaearctic (Fig. 1 and Table 1). The set of samples included material

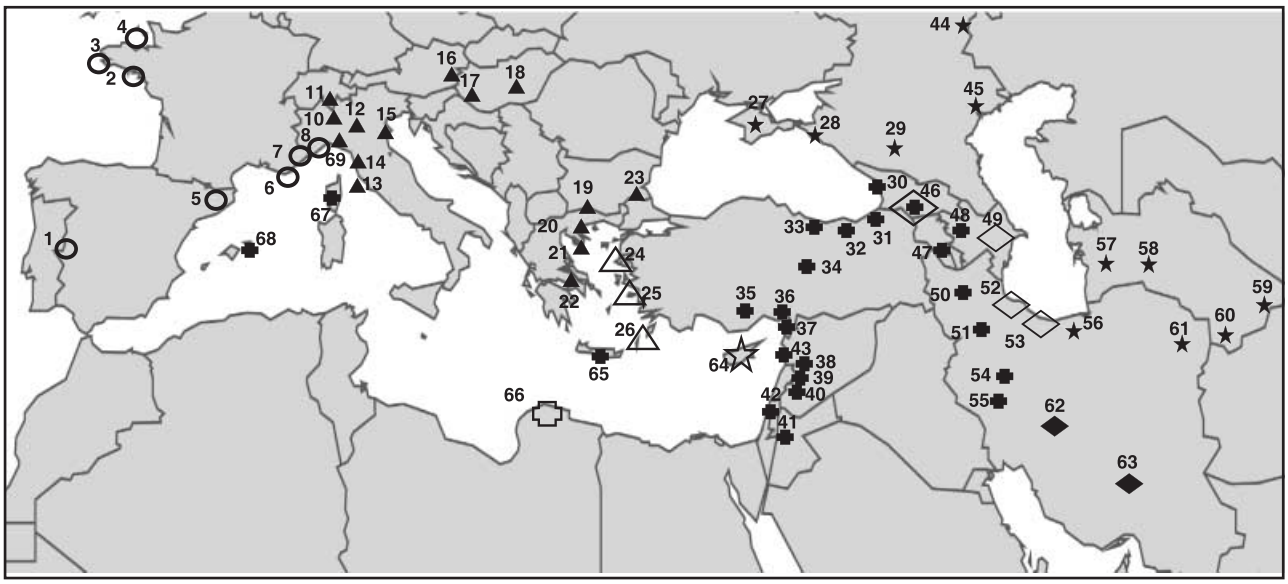

Fig. 1 Locality of samples from our study and from Dubey *et al*. (2006) included in this study (cf. Table 1), and distribution of Clades (II–X) from our results. The closed stars are samples of Clade II, the open rhombus Clade III, the open circle Clade IV, the closed cross Clade V, the open triangle Clade VI, the closed triangle Clade VII, the closed rhombus Clade VIII, the open cross Clade IX and the open star Clade X.

Table 1 Taxa sequenced and used in the present study, geographical origin of samples, haplotype name, locality on Fig. 1, and accession numbers of sequences (*from Dubey *et al*. 2006; + Ohdachi *et al*. 2004; – Poulakakis *et al*. 2005). Country abbreviations are as follows: Austria (AU), Azerbaijan (AZ), Bulgaria (BG), England (GB), France (FR), Georgia (GE), Greece (GR), Hungary (HU), Indonesia (ID), Iran (IR), Israel (IL), Italy (IT), Japan (JP), Libya (LY), Russia (RU), South Korea (KR), Spain (ES), Switzerland (CH), Syria (SY), Tajikistan (TJ), Turkmenistan (TM), Turkey (TR) and Ukraine (UA)

Species or subspecies	Sample locality	Haplotype name	Num. map	Accession number
C. s. aleksandrisi	LY, Cyrenaica, Wadi al Kuf	LY1	66	DQ630061
C. s. aleksandrisi	LY, Cyrenaica, Wadi al Kuf	LY2	66	DQ630060
C. s. caspica	GS, Dusheti	GE1	46	AY843488*
C. s. caspica	AZ	AZ1	49	AY843487*
C. s. caspica	IR, Gilan, Asalem	IR2	52	DQ630055
C. s. caspica	IR, Gilan, Asalem	IR3	52	DQ630057
C. s. caspica	IR, Mazandaran, Churti	IR1	53	DQ630056
C. s. gueldenstaedtii	GE, Sukhumi	GE2	30	AY843496*
C. s. gueldenstaedtii	TR, Rize	TR7	31	AY843498*
C. s. gueldenstaedtii	TR, Altindere	TR3	32	AY843499*
C. s. gueldenstaedtii	TR, Cakalli	TR6	33	AY843502*
C. s. gueldenstaedtii	TR, Kah.–Maraş, Tanir	TR5	34	DQ630085
C. s. gueldenstaedtii	TR, Konya, Balkuşan	TR8	35	DQ630083
C. s. gueldenstaedtii	TR, Adana	TR2	36	DQ630078
C. s. gueldenstaedtii	TR, Hatay, Y. Karafakili	TR4	37	DQ630082
C. s. gueldenstaedtii	SY, Hama, Ash'meiseh	SY6	38	DQ630071
C. s. gueldenstaedtii	SY, Homs, Qattinah	SY2	39	DQ630069
C. s. gueldenstaedtii	SY, As Suwayda, Qanawat	SY7	40	DQ630081
C. s. gueldenstaedtii	IL, Tiberias Lake	IL2	41	DQ630079
C. s. gueldenstaedtii	IL, Akhziv, 15 km N Acre	IL1	42	DQ630075
C. s. gueldenstaedtii	SY, Al Latakiah, Ras al-Bassit	SY1	43	DQ630077
C. s. gueldenstaedtii	SY, Al Latakiah, Rabi'ah	SY3	43	DQ630072
C. s. gueldenstaedtii	SY, Al Latakiah, Slinfeh	SY4	43	DQ630076
C. s. gueldenstaedtii	SY, Al Latakiah, Jablah	SY5	43	DQ630070
C. s. gueldenstaedtii	GE, Dusheti	GE9	46	AY843497*
C. s. gueldenstaedtii	GE, Shulaveri	GE8	47	AY843500*
C. s. gueldenstaedtii	GE, Alazani River	GE6	48	DQ630089

Table 1 *Continued*

Species or subspecies	Sample locality	Haplotype name	Num. map	Accession number
C. s. gueldenstaedtii	GE, Alazan Valley	GE3	48	DQ630086
C. s. gueldenstaedtii	GE, Alazan Valley	GE4	48	DQ630087
C. s. gueldenstaedtii	GE, Alazan Valley	GE5	48	DQ630088
C. s. gueldenstaedtii	GE, Alazani River	GE7	48	DQ630090
C. s. gueldenstaedtii	IR, W-Azerbaijan, Bastam	IR13	50	DQ630091
C. s. gueldenstaedtii	IR, W-Azerbaijan, Bastam	IR15	50	DQ630084
C. s. gueldenstaedtii	IR, W-Azerb., Mohammad Yar	IR8	51	DQ630094
C. s. gueldenstaedtii	IR, W-Azerb., Mohammad Yar	IR9	51	DQ630074
C. s. gueldenstaedtii	IR, Hamadan, Alanje	IR10	54	DQ630073
C. s. gueldenstaedtii	IR, Hamadan, Alanje	IR12	54	DQ630092
C. s. gueldenstaedtii	IR, Bahtaran, Bisotun	IR11	55	DQ630093
C. s. gueldenstaedtii	IR, Bahtaran, Bisotun	IR14	55	DQ630080
C. s. gueldenstaedtii	GR, Crete	CR3, H19	65	DQ630099
C. s. gueldenstaedtii	GR, Crete, Almyros	CR20, H13	65	DQ630103
C. s. gueldenstaedtii	GR, Crete, Ano Meros	CR19, H25	65	DQ630109
C. s. gueldenstaedtii	GR, Crete, Bramiana	CR17, H14	65	DQ630105
C. s. gueldenstaedtii	GR, Crete, Malia	CR1, H21	65	DQ630101
C. s. gueldenstaedtii	GR, Crete, Malia	CR11, H14	65	DQ630105
C. s. gueldenstaedtii	GR, Crete, Malia	CR12, H14	65	DQ630105
C. s. gueldenstaedtii	GR, Crete, Malia	CR14, H18	65	DQ630106
C. s. gueldenstaedtii	GR, Crete, Malia	CR2, H20	65	DQ630100
C. s. gueldenstaedtii	GR, Crete, Malia	CR6, H23	65	DQ630104
C. s. gueldenstaedtii	GR, Crete, Malia	CR9, H14	65	DQ630105
C. s. gueldenstaedtii	GR, Crete, Pachia Ammos	CR18, H22	65	DQ630108
C. s. gueldenstaedtii	GR, Crete, Pitsidia	CR16, H13	65	DQ630103
C. s. gueldenstaedtii	GR, Crete, Platanias	CR10, H15	65	DQ630102
C. s. gueldenstaedtii	GR, Crete, Platanias	CR13, H16	65	DQ641270
C. s. gueldenstaedtii	GR, Crete, Platanias	CR15, H17	65	DQ630107
C. s. gueldenstaedtii	GR, Crete, Platanias	CR4, H15	65	DQ630102
C. s. gueldenstaedtii	GR, Crete, Platanias	CR7, H13	65	DQ630103
C. s. gueldenstaedtii	GR, Crete, Platanias	CR8, H13	65	DQ630103
C. s. iculisma	ES, Candelario	ES1	1	AY843492*
C. s. iculisma	FR, Hoedic	FR2	2	AY843490*
C. s. iculisma	FR, Triélen	FR1	3	AY850035*
C. s. iculisma	GB, Sark	GB1	4	AY843489*
C. s. iculisma	ES, La Figueras	ES2	5	AY843491*
C. s. iculisma	FR, Porquerolle	PO1	6	DQ630066
C. s. iculisma	FR, Porquerolle	PO2	6	DQ630067
C. s. iculisma	FR, Porquerolle	PO3	6	DQ630068
C. s. iculisma	IT, Ventimiglia	IT1	7	AY843495*
C. s. iculisma	IT, Ventimiglia	IT10	7	DQ630065
C. s. iculisma	IT, Fraitusa	IT2	8	AY843494*
C. s. iculisma	IT, Fraitusa	IT8	8	AY843493*
C. s. balearica	ES, Minorca	MI1	68	DQ630095
C. s. balearica	ES, Minorca	MI2	68	DQ630097
C. s. balearica	ES, Minorca	MI3	68	DQ630098
C. s. balearica	ES, Minorca	MI4	68	DQ630096
C. s. cypria	CY, Pafos	CY1	64	DQ630050
C. s. cyrnensis	FR, Corsica, Alitone	CO10, H2	67	DQ630111
C. s. cyrnensis	FR, Corsica, Alitone	CO13, H4	67	DQ630114
C. s. cyrnensis	FR, Corsica, Biguglia	CO17, H1	67	DQ630110
C. s. cyrnensis	FR, Corsica, Biguglia	CO20, H1	67	DQ630110
C. s. cyrnensis	FR, Corsica, Biguglia	CO21, H1	67	DQ630110
C. s. cyrnensis	FR, Corsica, Biguglia	CO24, H9	67	DQ630117
C. s. cyrnensis	FR, Corsica, Bonifacio	CO18, H1	67	DQ630110
C. s. cyrnensis	FR, Corsica, Bonifacio	CO25, H8	67	DQ630118
C. s. cyrnensis	FR, Corsica, Bonifacio	CO5, H11	67	DQ630116
C. s. cyrnensis	FR, Corsica, Bonifacio	CO8, H1	67	DQ630110
C. s. cyrnensis	FR, Corsica, Bonifacio	CO9, H2	67	DQ630111

Table 1 *Continued*

Species or subspecies	Sample locality	Haplotype name	Num. map	Accession number
C. s. cyrnensis	FR, Corsica, Cardetou	CO27, H10	67	DQ630120
C. s. cyrnensis	FR, Corsica, Cardetou	CO28, H3	67	DQ630121
C. s. cyrnensis	FR, Corsica, Fango	CO22, H1	67	DQ630110
C. s. cyrnensis	FR, Corsica, Fango	CO23, H1	67	DQ630110
C. s. cyrnensis	FR, Corsica, Fangu	CO11, H6	67	DQ630112
C. s. cyrnensis	FR, Corsica, Muro	CO15, H1	67	DQ630110
C. s. cyrnensis	FR, Corsica, Ogliastro	CO3, H1	67	DQ630110
C. s. cyrnensis	FR, Corsica, Ogliastro	CO7, H1	67	DQ630110
C. s. cyrnensis	FR, Corsica, Olmeto	CO1, H7	67	AY843501*
C. s. cyrnensis	FR, Corsica, Restonica	CO16, H1	67	DQ630110
C. s. cyrnensis	FR, Corsica, Restonica	CO19, H1	67	DQ630110
C. s. cyrnensis	FR, Corsica, Restonica	CO26, H12	67	DQ630119
C. s. cyrnensis	FR, Corsica, Restonica	CO29, H2	67	DQ630111
C. s. cyrnensis	FR, Corsica, Roncier Calca	CO14, H1	67	DQ630110
C. s. cyrnensis	FR, Corsica, St. Eustache	CO12, H24	67	DQ630113
C. s. cyrnensis	FR, Corsica, St-Eustache	CO4, H5	67	DQ630115
C. s. cyrnensis	FR, Corsica, Tuarelli	CO6, H2	67	DQ630111
C. s. cyrnensis	FR, Corsica, U Fangu	CO2, H1	67	DQ630110
C. s. mimula	IT, Elba	EL1	10	DQ630052
C. s. mimula	IT, Vercelli	IT5	10	AY843459*
C. s. mimula	CH, Gordevio	CH1	11	AY843452*
C. s. mimula	IT, San Nicolo	IT7	12	AY843453*
C. s. mimula	IT, Fivizzano	IT6	14	AY843450*
C. s. mimula	IT, Latisana	IT3	15	AY843457*
C. s. mimula	IT, Latisana	IT4	15	AY843456*
C. s. mimula	AU, Wien	AU1	16	AB077280+
C. s. mimula	HU, Fülophasa	HU2	17	AY843451*
C. s. mimula	HU	HU1	18	AY843454*
C. s. mimula	BG, Sandanski	BG1	19	AY843458*
C. s. mimula	GR, Thessaloniki	GR1	20	AY843449*
C. s. mimula	GR, Epanomi	GR3	21	AY843448*
C. s. mimula	GR, Athina	GR2	22	AY843455*
C. s. mimula	BG, Burgas	BG2	23	DQ630051
C. s. mimula	IT, Varazze	IT9	69	DQ630064
C. s. suaveolens	UA, Sevastopol	UA1	27	AY843475*
C. s. suaveolens	RU, Krasnodarsskyi reg.	RU5	28	AY843476*
C. s. suaveolens	RU, Stavropol	RU4	29	AY843467*
C. s. suaveolens	RU, Kazan	RU6	44	AY843483*
C. s. suaveolens	RU, Astrakanskaya reg.	RU3	45	AY843477*
C. s. suaveolens	IR, Mazandaran, Nowkande	IR6	56	DQ630063
C. s. suaveolens	TM	TM4	57	AY843466*
C. s. suaveolens	TM, Firusa	TM1	58	AY843481*
C. s. suaveolens	TM, Kerki	TM2	59	AY843480*
C. s. suaveolens	TM, Badchiz	TM3	60	AY843479*
C. s. suaveolens	IR, Meshed	IR7	61	DQ630062
C. shantungensis	JP, Tsushima Isl.	JP1	/	AY843447*
C. shantungensis	KR, Cheju Isl.	KR1	/	AB077077+
C. shantungensis	KR, Kyungju	KR2	/	AB077079+
C. shantungensis	RU, Popov Isl.	RU1	/	AB077082+
C. shantungensis	RU, Putjatin Isl.	RU2	/	AB077080+
C. suaveolens	IR, Esfahan, Espidan	IR4	62	DQ630058
C. suaveolens	IR, Kerman	IR5	63	DQ630059
C. suaveolens	GR, Lesvos	LE1	24	AY843460*
C. suaveolens	GR, Lesvos	LE2	24	DQ630053
C. suaveolens	GR, Lesvos	LE3	24	DQ630054
C. suaveolens	TR, Vuyukarikisilka	TR1	25	AY843461*
C. suaveolens	GR, Karpathos Island	/	26	AY452174–
C. brunnea	ID, Java	/	/	DQ059025*
C. nigripes	ID, Sulawesi	/	/	DQ059024*

from the following collections: St. Petersburg (ZISP), Russia; Montpellier (JFC) and Banyuls-sur-mer (MAGN), France; Prague (DZCU and NMP), Czech Republic; Heraklion (MHNH), Greece; Lausanne (IZEA), Switzerland; and Tbilissi (IZT), Georgia. Fourty-three additional sequences were taken from Ohdachi *et al*. (2004), Poulakakis *et al*. (2005) and Dubey *et al*. (2006; mentioned in Table 1).

On the basis of the results by Dubey *et al*. (2006), only *C. shantungensis* and *C. suaveolens* are treated as valid species. In fact, genetic clades within these taxa have a parapatric distribution and nothing is known about the degree of genetic isolation between them; this problem cannot be resolved by mitochondrial markers. As long as data on gene flow interruption in contact zones are lacking, we consider all sister clades of *C. shantungensis* to be conspecific to *C. suaveolens*, and consequently treat them as subspecies; i.e. *C. s. suaveolens* (Pallas 1811), *C. s. iculisma* (Mottaz 1908), *C. s. caspica* (Thomas 1907), *C. s. cypria* (Bate 1904), *C. s. gueldenstaedtii* (Pallas 1811) and *C.* s. *mimula* (Miller 1901).

DNA extraction and amplification

Samples (liver) from IZEA collection (Lausanne) were first frozen in the field in liquid nitrogen, then kept for several years at –70 °C, and finally stored in ethanol (80%) until DNA extraction. Samples (liver) from other collections were stored directly in ethanol (80%). DNA extraction was carried out using a QIA Amp DNA Mini Kit (Qiagen). Amplifications of *cyt b* were performed with different combinations of primer pairs L14841/C4, C8/C4, C1/C2, C6/C7, C3/H15915, C5/H15915, L14841/H15915 (Irwin *et al*. 1991; Dubey *et al*. 2006).

PCR products were then electrophoresed on a 1% agarose gel, visualized with ethidium bromide (0.5 μg/mL) staining to verify PCR quality, and purified by centrifugal dialysis using the QIAquick PCR Purification Kit (Qiagen).

Cycle sequencing was performed in 10 μL total volume containing 1–3 μL of amplified DNA, 1 μL of 10 μM primer and 4 μL of ABI PRISM™ Dye Terminator 1 (Perkin–Elmer). Sequence reactions were visualized on an ABI 3100 genetic analyser (Applied Biosystems).

Phylogenetics methods

Nucleotide sequences of *cyt b* were edited with Sequence Navigator (Parker 1997) and aligned by eye. Two methods of phylogenetic analyses were carried out using PAUP Version 4.0b10 (Swofford 1998). Tests were conducted on the total fragments (998 bp), all codon positions were used, and trees were rooted using sequences of the black-footed shrew, *C. nigripes* (DQ059024) and the thick-tailed shrew, *C. brunnea* (DQ059025) from Indonesia — two species that are included within the sister clade of the *C. suaveolens* group (Dubey *et al*. in press). A neighbour-joining (NJ) tree was constructed using Kimura two-parameter genetic distances (K2P; Kimura 1980), which is a distance commonly used that allows to compare the results with others studies. Maximum parsimony (MP) analysis was performed using the following options: heuristic search, stepwise-addition of sequences, 10 replicates of random addition of taxa, and TBR branch swapping (Swofford 1998); all codon positions were equally weighted. For maximum likelihood (ML) analyses, we used PHYML (Guindon & Gascuel 2003), which performs fast ML heuristic searches. The general time-reversible (GTR) model was previously selected with MODELTEST 3.06 according to the protocol of Posada & Crandall (1998), with base frequencies (A = 0.2895, C = 0.2632, G = 0.1291, T = 0.3183) estimated from the data, an unequal distribution of rates at variable sites ($\gamma = 1.682$), and six different substitution types [rate (A-C) = 0.7115, rate (A-T) = 1.0909, rate (C-G) = 0.6899, rate (G-T) = 1.0000, rate (A-G) = 11.4390, rate (C-T) = 16.2446]. NJ, MP, and ML results were compared for congruence of tree topologies. Bootstrap support values were obtained with 1000 pseudoreplicates for MP and NJ with PAUP, and for ML with PHYML (Guindon & Gascuel 2003). Bayesian analyses (BA) were conducted using MRBAYES Version 3.1.2 (Huelsenbeck *et al*. 2001), which performs Metropolis-coupled Markov chain Monte Carlo analysis. A GTR model was used, with an among-site rate variation following a gamma distribution. The Markov chain was run for 1 000 000 generations and sampled once every 100 generations; burning was set at 100 000 generations. To assure convergence in the Bayesian analyses, two independent runs were performed and compared.

Molecular clock

Estimation of divergence time from the molecular data was performed according to the calibration developed for the Soricidae by Fumagalli *et al*. (1999), based on an estimate of 20 million years for the split between Crocidurinae and Soricinae. This calibration was developed considering *cyt b* sequence divergence based on third-position transversions, although it could not be used directly because of the low numbers of observed third-position transversions. Therefore, we used the ML distances, as in Dubey *et al*. (2006).

To identify heterogeneity in the rates of *cyt b* substitutions among different clades, relative-rate tests were conducted between clades. The relative-rate tests were performed using RRTREE Version 1.0 (Robinson *et al*. 1998), which improves upon the test of Wu & Li (1985) by taking into account taxonomic sampling and phylogenetic relationships. Relative-rate tests were performed on the proportions of synonymous (Ks) and nonsynonymous (Ka) substitutions, and synonymous transversions (B4).

Nucleotide diversity and genetic structure

Nucleotide diversity (p) was estimated using the DNASP Version 4.10.3 (Rozas *et al*. 2005). The population genetic structure was determined by an analysis of molecular variance (AMOVA) available in ARLEQUIN Version 2.0 (Schneider *et al*. 2000). This analysis was performed at two different hierarchical levels: among clades and within clades.

Expansion time

We undertook several tests to verify the hypothesis of recent population growth from low-diversity founder populations in insular samples of Crete and Corsica (Clade V) as well as in the continental source population. Three methods were implemented in DNASP and one in ARLEQUIN. The first method, Ramos-Onsins & Rozas's (2002) R_2 statistic, is based on the difference between the number of singleton mutations and the average number of nucleotide differences. Lower values of R_2 are expected under a scenario of recent population growth. The second method, Fu's (1997) F_S statistic, tests the probability of having no fewer than the number of observed alleles in the sample, given that q (heterozygosity per sites) = p. This statistic tends to be negative when there is an excess of recent mutations (or rare alleles). The third method, Tajima's (1989) D statistic, tests the null hypothesis that two estimates of the neutral mutation parameter, one derived from the average number of pairwise nucleotide differences and the other based on the number of segregating sites in the sample, are equal. In the fourth test, pairwise mismatch distributions among individuals were plotted and tested for goodness-of-fit against a model of sudden expansion using parametric bootstrapping with 1000 replicates (Schneider & Excoffier 1999). Expansion time following the bottleneck was estimated from the mismatch distribution (t; Rogers 1995) and uncorrected distances (p). The evolutionary rate for uncorrected distance (p) was estimated using the molecular clock developed by Fumagalli *et al*. (1999).

Network

The method of statistical parsimony (Templeton *et al*. 1992), implemented in TCS Version 1.21 (Clement *et al*. 2000), first defines the uncorrected distance (p) above which there is a > 5% probability that the parsimony criterion is violated (parsimony limit). All connections are then established among haplotypes starting with the smallest distances and ending either when all haplotypes are connected or when the distance that corresponds to the parsimony limit has been reached.

Results

Phylogenetic relations between clades

We found 116 different haplotypes of 998 bp in the 143 samples from the *C. suaveolens* group and the two outgroup samples analysed, which showed 338 variable sites of which 262 were parsimony-informative. No insertions or deletions were observed. As the four phylogenetic methods gave identical arrangements of the main branches, the relationship between haplotypes is given only for the ML analysis (Fig. 2).

Ten well-defined major clades were observed, supported by bootstrap values of 100% for ML, MP and NJ, and a posterior probability of 1.0 for BA, excepted for the Cypriot one. Seven of these clades are those found in our previous study on the phylogeography of the northern range of the *C. suaveolens* group (Dubey *et al*. 2006). For simplicity, we maintain the same numbering for these clades; i.e. Clades I to VII.

Clade I corresponds to the morphotype *C. shantungensis* and retains its basal position. Clade II, which in our previous study included all haplotypes from Russia and central Asia and a specimen from the type locality of *C. suaveolens*, is represented here with two additional new samples from northern Iran. Clade III includes specimens of *C.* s. *caspica*, from Georgia, Azerbaijan and northwestern Iran, in which two new samples from northwestern Iran are added. This clade is closely related to Clade II. Clade IV comprises haplotypes from Western Europe, including new Ligurian (Italy) and insular samples from Porquerolle (France). Clade V contained the haplotypes of *C.* s. *gueldenstaedtii* from northern and eastern Turkey and Georgia. In addition, numerous samples from Turkey, Israel, western Syria and western Iran are included here, as well as all the insular samples from Corsica, Menorca and Crete. Clade VI includes the haplotypes from Lesvos Island (Greece) and western Turkey. One additional sample from Karpathos Island (AY452174; Greece) described by Poulakakis *et al*. (2005) belongs to this clade (result not shown), but was not used for phylogenetic analysis due to

Fig. 2 Phylogeny of the 998 bp *cyt b* fragment analysed using a maximum likelihood (ML) procedure and the GTR model of substitution. Values in branches are indices of support for the major branches for Bayesian (BA), ML, maximum parsimony (MP), and distance (NJ) analyses (percentage of 1000 replications for ML, MP, and NJ, and posterior probabilities based on 1 000 000 generations for BA). Codes are as in Table 1. Haplotypes in bold style are samples from the present study and haplotypes in regular style from Ohdachi *et al*. (2004) and Dubey *et al*. (2006).

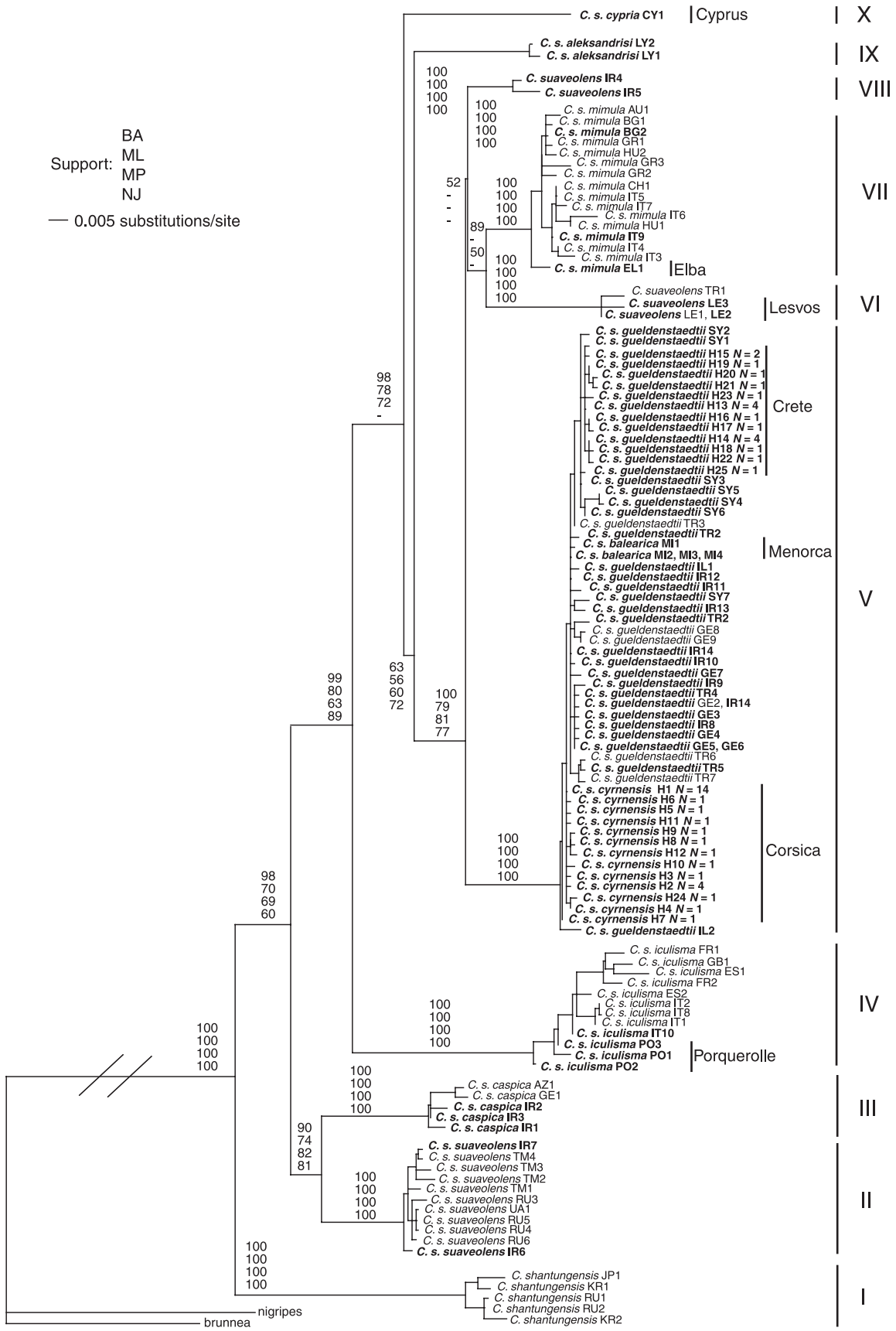

Table 2 Mean corrected pairwise sequence divergences between the 10 clades (Kimura two-parameter distances). Paired numbers in the diagonal row are percentage of mean pairwise sequence divergence (K2P) and nucleotide diversity within clades

	I	II	III	IV	V	VI	VII	VIII	IX	X
I	1.2/1.2									
II	0.091	0.7/0.6								
III	0.091	0.056	0.9/0.8							
IV	0.107	0.087	0.094	1.5/1.4						
V	0.112	0.085	0.094	0.084	0.6/0.7					
VI	0.115	0.088	0.089	0.088	0.058	0.8/0.5				
VII	0.113	0.086	0.087	0.081	0.051	0.047	0.9/0.9			
VIII	0.111	0.083	0.088	0.075	0.046	0.051	0.039	0.9/0.9		
IX	0.110	0.081	0.084	0.086	0.059	0.058	0.058	0.057	0.4/0.4	
X	0.107	0.087	0.091	0.084	0.077	0.081	0.074	0.066	0.063	—/—

the small size of the DNA fragment (around 300 bp). Clade VII comprises the haplotypes of *C. suaveolens* from Southern and Central Europe (Italy, Switzerland, Austria, Hungary, Bulgaria and Greece) from our previous study, along with new samples from Bulgaria and Elba Island (Italy).

In terms to the new clades, Clade VIII comprises two samples from central Iran, and clusters with Clades VI and VII; Clade IX comprises two samples of *C. aleksandrisi* from Libya, and is basal to Clades V–VIII. As this taxon is included in the *C. suaveolens* group, we consider it to be a subspecies of *C. suaveolens*. The name *C. s. aleksandrisi* is therefore used in the following sections and in Table 1 and Fig. 2. Finally, Clade X comprises one sample of *C.* s. *cypria* from Cyprus, and is close to Clades V–IX.

The AMOVA shows that the majority of the total mtDNA variation (89.51%) is distributed among the 10 clades, with just a low percentage of this variation (10.49%) observed within clades. Mean pairwise K2P distances between clades range from 3.9% (VII and VIII) to 11.5% (I–VI; Table 2).

Within clades, K2P distances range from 0.4% (IX) to 1.5 (IV), and nucleotide diversities range from 0.004 (IX) to 0.014 (IV; Table 2). With regard to insular samples (Clade V), their nucleotide diversities are lower (0.0022 for Crete and 0.0013 for Corsica) than the continental population of the same clade (0.006).

Molecular clock

Relative-rate tests revealed that Clade V evolved more rapidly than Clades VII and VIII ($P < 0.05$). We found no significant differences in evolutionary rates between any other clades ($P > 0.05$). We therefore decided to exclude Clade V from the molecular clock analysis.

The split between the Cypriot sample and Clades VI–IX took place approximately 1.54 mya [95% CI: 1.26–2.20]; i.e. during the Lower Pleistocene (Ogg 2004). The split between *C. s. aleksandrisi* (Clade IX) and Clades VI–VIII 1.21 mya [95% CI: 0.98–1.56] occurred next during the same period, and finally the split between Iranian samples (Clade VIII) and clades of Central Europe (VII) and western Turkey (VI) occurred at the end of the Lower Pleistocene (0.83 mya [95% CI: 0.67–1.07]). For more details about the other splits, see Dubey *et al*. (2006).

Network

Only Clade V, which included continental and insular populations, was analysed using the method of statistical parsimony implemented in the TCS program (Clement *et al*. 2000) to clarify the origin of insular shrews. The central haplotype of the obtained network appeared to be a Menorcan, around which the others are articulated (Fig. 3). We also noted a unique origin for the insular samples of Corsica and Crete; the first are connected together in a star-like topology, distinct from other continental haplotypes but directly connected to the central Menorcan one. The Cretan haplotypes show a close affinity to the Syrian samples, and the relationships between haplotypes remained more complex with three frequently interconnected haplotypes.

Expansion time

We observed a nonsignificant P value for the mismatch distribution test of goodness-of-fit for the insular (Crete and Corsica) and continental populations of Clade V ($P > 0.4$; Table 3), with the frequency distribution of the mean pairwise difference between haplotypes showing a bell-shaped distribution (Fig. 4). All Fu's F_S statistics were negative and significant ($P < 0.001$). Tajima's D values were also all negative, and only the continental and Corsican populations obtained significant P values ($P < 0.01$). The R_2 statistic was only significant for the continental and Corsican populations ($P < 0.05$), and only marginally so for the Cretan population ($P = 0.10$). Consequently, we inferred a scenario of expansion for the continental and Corsican populations. Although a scenario of expansion

Table 3 Mean pairwise differences between haplotypes, goodness-of-fit test probability, Fu's *Fs* test probability, Tajima's test probability, R_2 statistic probability, estimated τ-value, and expansion time for continental and insular (Crete and Corsica) populations of Clade V

Clade (V)	Mean pairwise difference	Goodness-of-fit test (*P*)	Fu's *Fs* test		Tajima's test		R_2 statistic (*P*)	τ	Expansion time
			Fs	*P*	Tajima's *D*	*P*			
Continent	5.73	0.93	–27.98	< 0.001	–2.17	< 0.01	< 0.05	4.73	19 500 years ago (95% CI: 17 300–22 100)
Crete	1.94	0.50	–9.98	= 0.001	–1.09	= 0.14	= 0.10	2.05	8400 years ago (95% CI: 7500–9500)
Corsica	1.31	0.78	–9.21	< 0.001	–2.22	< 0.01	< 0.05	1.45	6000 years ago (95% CI: 5300–6800)

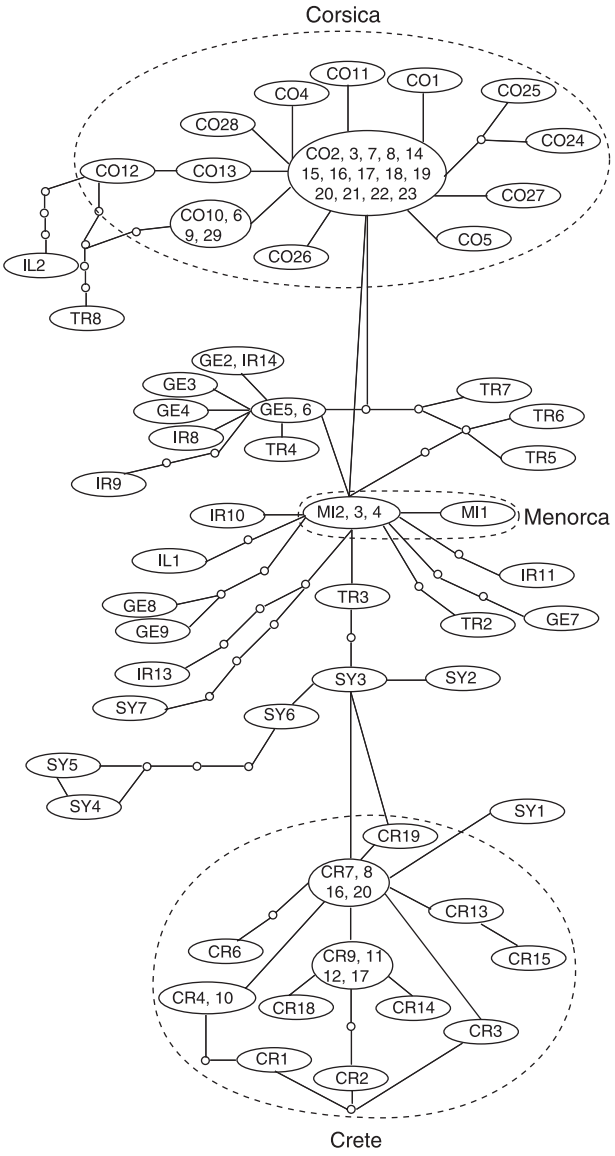

Fig. 3 Statistical parsimony network of Clade V. Codes are as in Table 1.

was expected in the Cretan population, the results were less clear, perhaps due to smaller sample sizes. Nevertheless, we estimated the expansion date of this population.

The timing of expansions was estimated from the mismatch distribution (Fig. 3) according to the method of Rogers (1995). τ-values for the Corsican, Cretan and continental populations were 1.45, 2.05 and 4.73, respectively. Assuming no saturation of uncorrected distances (p), as shown in Dubey *et al*. (2006), distance was 0.0612 per million years (95% CI: 0.054–0.069). With a generation time of half a year (Vohralík 1988), the population expansion time is estimated to be 6000 years ago (95% CI: 5300–6800) for the Corsican population, 8400 years ago (95% CI: 7500–9500) for the Cretan population, and 19 500 years ago (95% CI: 17 300–22 100) for the continental population.

The others clades were not tested, either because they were treated in Dubey *et al*. (2006) or were of insufficient sample size.

Discussion

Distribution and biogeography of Middle East continental clades

Our study, based on numerous samples from the southern range of the *C. suaveolens* group, enabled us to define two continental clades (VIII and IX) that were previously undetected in the northern Palaearctic (Dubey *et al*. 2006), and enabled us to form a better understanding of the southern distribution of clades (Fig. 1). The newly identified continental clades were found to be restricted to southern mountainous areas of desert regions, and to comprise samples of the endemic *C. s. aleksandrisi* from the Akhdar Mountains, Libya (Clade IX), and two samples from the Zagros and Qohrud Mountains, central Iran (Clade VIII). These clades differentiated in the Lower Pleistocene 1.21 and 0.83 mya, respectively.

The taxon *C. s. gueldenstaedtii* (Clade V) distributed in Turkey and Georgia in Dubey *et al*. (2006), extended

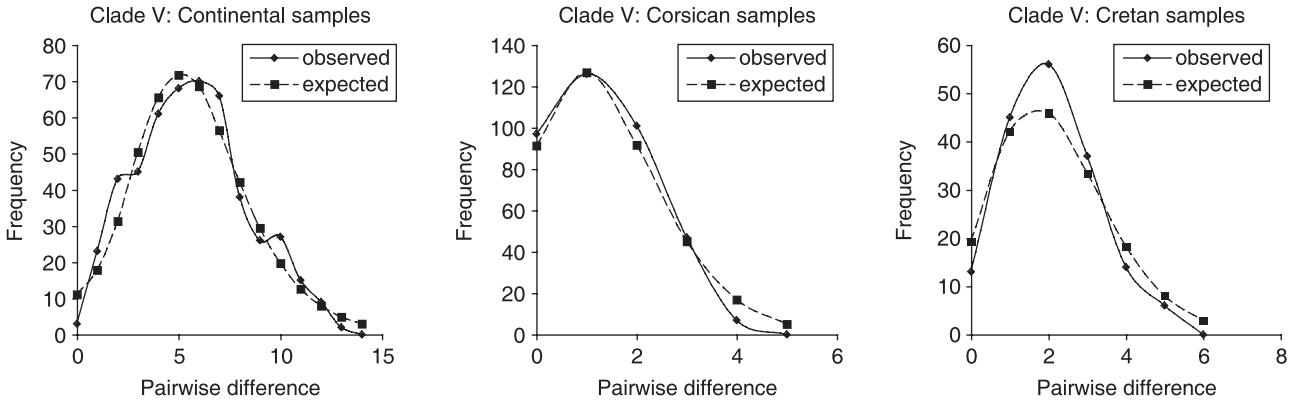

Fig. 4 Observed (solid line) and expected (dot-line) mismatch distributions for a sudden expansion of insular (Cretan and Corsican) and continental samples of Clade V.

southwards in Israel, confirming Catzeflis *et al*. (1985), in western Syria and western Iran. Consequently, it is presently the most widely distributed clade in the southern range of the *C. suaveolens* group (Fig. 1). In addition, it clearly suffered from global cooling during the Upper Pleistocene, where it underwent an expansion following a bottleneck approximately 19 500 years ago (95% CI: 17 300–22 100, Table 3 and Fig. 3). The confidence interval for our dating includes the period of the maximum extension of ice sheets in Europe, i.e. between 20 000 and 18 000 years ago (Frenzel *et al*. 1992; Boulton *et al*. 2004), as well as the end of this severe cooling. Consequently, it is more likely that this expansion event occurred after this last glacial maximum.

In conclusion, our data from the southern distribution of the white-toothed shrew highlight a complex evolutionary history within the Near East and the Middle East, which can be qualified as a hotspot of diversity in the *C. suaveolens* group. This could be a general pattern for many species, as far as our shrew can be considered as a representative member of the temperate Palaearctic biota. Likewise, several studies focusing on the Middle East have evidenced similar complex patterns for other taxa, like the mice from genus *Mus* (Boursot *et al*. 1996; Gündüz *et al*. 2000) and the long-legged wood frog *Rana macrocnemis* (Veith *et al*. 2003). In a more general context, the study of others small temperate mammal species in these poorly documented areas would be most useful to highlight the effect of Pleistocene glaciations on the Middle East biota.

Origin of insular samples

The Mediterranean islands can be roughly classified into two main groups: small islands and/or those close to the continent and which were connected to it during the glacial intervals of the Upper Pleistocene (Van Andel & Shackleton 1982; Shackleton *et al*. 1984), and large and distant islands that have not been connected to the continent since the Messinian salinity crisis (Krijgsman *et al*. 1999), which partially dried out the Mediterranean Sea in the late Miocene (5 mya).

Samples from small islands or those close to the continent, i.e. shrews from Porquerolles in France (Clade IV), Elba in Italy (Clade VII) and Lesvos in Greece (Clade VI), appear to belong to the same clade as the proximate continental population, suggesting a natural colonization during the last glacial maximum when these islands were part of the mainland due to low sea levels. Consequently, these small insular populations were isolated from the mainland populations during the postglacial rise of the sea level. However, more recent introduction by man or other means from adjacent continental localities cannot be strictly excluded. Natural colonization and anthropic introduction were also both invoked to explain the repartition of species of white-toothed shrews in Atlantic continental islands off the coast of France (Cosson *et al*. 1996). The close association of many *Crocidura* species to farm houses (Genoud & Hausser 1979) makes an accidental transfer with agricultural goods very plausible. In nature, pairs share a nest (Cantoni & Vogel 1989) and it was demonstrated experimentally that the introduction of a single pair in habitat islands resulted in the presence of descendants in more than 50% of all cases (Vogel 1999).

The origin of insular populations of large and distant islands is of great interest because natural colonization by land bridges has not been possible since the Messinian crisis of the late Miocene (5 mya). The status of these insular populations has often been the subject of great controversy, as shown below for the Cypriot shrew. This white-toothed shrew was originally described as subspecies of the greater white-toothed shrew, *C. russula cypria* by Bate (1904); later it was considered as an endemic species, *C. cypria* by Spitzenberger (1978) based on morphology. Catzeflis (1983) investigated the karyotype and allozymes and showed close relationships with *C. suaveolens*, but proposed to include it as a basal subspecies of the latter only after analysis of more geographical samples (Vogel *et al*. 1986). More recently, Bronze Age specimens with smaller body sizes have been attributed to a different subspecies, *C. suaveolens praecypria* (Reumer & Oberli 1988), which

was also considered to be an introduced taxon. Based on the data of Catzeflis (1983) and our *cyt b* data, confirming the high divergence with all other continental and insular *C. suaveolens* populations (Fig. 2, Table 2), we conclude that the shrew from Cyprus is highly divergent from all other continental populations. Thus, the shrews from Cyprus can be considered to belong to the rare group of relict insular Pleistocene mammal taxa, even if equally old corresponding fossils are lacking. Consequently, it survived to the present day, beside the extinct species such as the endemic Cypriot hippo and elephant fauna (Simmons 1991; Simmons & Jochim 1999). This finding is of great importance, as prior to the present study only the Cretan shrew *C. zimmermanni* (Vogel *et al*. 1986), the Sicilian shrew *C. sicula* (Vogel *et al*. 1990), and the Cypriot mouse *Mus cypriacus* (Bonhomme *et al*. 2004; Cucchi *et al*. 2006) were known to have survived to the Holocene colonization of Mediterranean islands by men. Based on its divergence with other *C. suaveolens* clades, we admit that the shrew from Cyprus probably colonized the island during the Lower Pleistocene (1.54 mya [95% CI: 1.26–2.20]), which is considerably earlier than the estimation for the Cypriot mouse, *Mus cypriacus*: 0.5–1.0 mya by Bonhomme *et al*. (2004) or 0.53 mya by Cucchi *et al*. (2006). This event probably occurred by rafting, as since that time the island had not been connected to the continent (Simmons 1991).

The other insular populations of the lesser white-toothed shrew were first described as separate species, i.e. *C. cyrnensis* from Corsica, *C. balearica* from Menorca and *C. caneae* from Crete. Fossil records document their presence in Crete 3700 years ago and in Corsica 3000 years ago, with a hypothetical introduction certainly in excess of 4700 years ago in the latter island (Reumer & Payne 1986; Reumer 1996; Vigne *et al*. 1997; Vigne 1999). Our results confirm this timing of introduction and provide more precise information about the origin of these populations. According to our analysis they belong unequivocally to Clade V, widely distributed in the Middle East. The large distances separating these islands from the Middle East make natural colonizations by over-water dispersal such as rafting or other hypothetical means unlikely. In addition, the fact that these introductions took place during the Holocene and were contemporary with island colonizations by human, suggests that they resulted from boat transfers from the Middle East, as shown for the badger *Meles meles* (Marmi *et al*. 2006). Following such recent introduction we would expect the signature of a demographic bottleneck followed by a period of expansion. Moreover, the date of this demographic expansion should be contemporary with their introduction and subsequent to human colonization. This proposal has been confirmed for the Corsican shrews, for which a scenario of recent expansion is clearly indicated by the bell-shaped curve of the mismatch distribution analysis (Fig. 3), the statistical tests (Table 3) and the star-like topology of the parsimony network (Fig. 4). This event, dated from the Middle Neolithic, 6000 years ago (95% CI: 5300–6800). Consequently, it is relatively close to the hypothetical date for the introduction of the species, estimated to be at least 4700 years ago, but no older than the early Neolithic (6800 years ago; Vigne *et al*. 1997; Vigne 1999). In addition, it coincided with the first fossil record of the synanthropic wood mouse *Apodemus sylvaticus* and the hedgehog *Erinaceus europaeus* in Corsica.

Therefore, this is younger than the first colonization of Corsica by humans, dated at 10 500 years ago (Costa *et al*. 2003), and the beginning of the Neolithic colonization, which took place during the first half of the 8th millennium BC. But, interestingly, it is situated well before the increase of the trade exchanges between east and west at the Minoan times (Cucchi *et al*. 2005), and before the Phoenician colonization of the Mediterranean islands from the Middle East, which took place about 3200 years ago (Negbi 1992). In contrast, the situation is more complex for the Cretan population because statistical tests of population expansion are not fully consistent with the expected demographic expansion. This discrepancy is also reflected in the parsimony network, where the samples do not form a true star-like topology with a single frequent haplotype; instead, we observe three equally dominant haplotypes connected in a network pattern. An alternative explanation that is consistent with the haplotype pattern of variation in Crete is multiple introductions from the same population source within the Middle East. The 'multiple introduction' hypothesis is a plausible scenario for Crete, as the geographical distances between the island and the continental source populations are less than those for Corsica. There was also more frequent and earlier exchange between the Middle East and Crete throughout the Holocene (Jarman 1996). In addition, the Cretan spiny mouse *Acomys minous*, introduced into Crete during antiquity, was found to represent two distinct maternal lineages of *cyt b*, clustering with those from populations of spiny mice from Cyprus and Asia Minor (Barome *et al*. 2001). This is also suggestive of multiple introductions. Thus, Crete is more likely to have been colonized several times by shrews originating from the Middle East. Taking into account these results, the date of colonization of Crete by *C. suaveolens* should be considered with caution; results from our mismatch analyses are 8400 years ago (95% CI: 7500–9500). Nevertheless, the data obtained suggests an introduction (here probably accidental) by the first Neolithic populations from the eastern Mediterranean shores, where proofs of sea faring are attested as old as 12 000 years ago (Perlès 1979). In addition, it corresponds to the first fossil record of the Badger *Meles meles* (8000 years ago; Vigne 1999), which was also introduced from the Middle East (Marmi *et al*. 2006).

This type of colonization from Middle East to Western Europe has already been demonstrated for the domestic

caprines that originated from this part of the world and were thereafter successively introduced to Crete, Corsica and Mallorca between approximately 8000 and 5500 years ago (Vigne 1999). The same westward colonization is observed in the house mouse *Mus musculus domesticus*, which colonized the shores of the eastern Mediterranean from 14 000 years ago, and was hypothetically introduced to Crete 9000 years ago. On the other hand, it colonized the western Mediterranean only tardily, 2500 years ago; a pattern linked to probable competition with the wood mouse *Apodemus* sp., which was more adapted to the landscape with fewer anthropic changes than Western Europe, thus preventing establishment of the house mouse (Cucchi *et al*. 2005).

The greater genetic diversity of the Cretan population compared to that of the Corsican, and thus the earlier timing of expansion of the first arrivals (bearing in mind that this result could be skewed by several independent introductions to Crete), suggests a similar pattern of island colonization throughout the region, with the first colonization occurring in eastern Mediterranean islands, followed by western islands.

Nevertheless, the progressive colonization of the domestic caprines and the house mouse occurred with a lot of stop on the mainland peninsulas and not by direct transports. For that reason, it is difficult to imagine a similar type of westward progression for *C. suaveolens*, as the mainland was already occupied by other *C. suaveolens* clades (IV and VII) that would have caused an admixture of these clades (IV, V and VII), and consequently would have greatly decreased the probability of a single and eastern origin of the insular introduced populations. Moreover, no continental sample from Western and Central Europe revealed to have a mitochondrial haplotype that originated from the Middle East. Thus, a scenario of colonization with a direct translocation from the Middle East should be preferred, despite the discrepancy with the present day knowledge about Neolithic exchanges in the Mediterranean area. Interestingly, the Menorcan, Corsican and Cretan insular shrew populations all seem to have different and unique origins (Figs 2 and 3), suggesting the colonization of each island from different localities. The central position of Menorcan haplotypes in the statistical parsimony network (Fig. 3) probably results from the fact that they belong to an unsampled continental haplotype from which they originated.

Conclusions

Our molecular study suggests the presence of a new and unexpected endemic and insular mammal taxon of the Mediterranean basin that has survived from the Pleistocene until the present. Only three other taxa are currently known: the recently discovered Cypriot mouse (Cucchi *et al*. 2006) and two other shrews. This raises the possibility that a few other insular small mammals currently considered to be introduced and for which we do not have good evidence of an introduction during the Holocene, could in fact also prove to be endemic. Thus, molecular screening of such species should be a priority, as they could be limited to highly restricted areas and thereby be endangered (Gippoliti & Amori 2006)

We also note the influence of *Homo sapiens* on current mammal distributions examined in the present study, as determined by the process of neolithization of the Mediterranean basin. Actual Cretan population results probably from multiple introductions, whereas Corsican from only one. Nevertheless, we cannot advocate that no other lineages, nowadays extinct, could have existed through the Holocene.

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S. Dubey is interested in the biogeography and the evolution of the Soricidae. This work was part of his PhD on the evolution of shrews. V. Vogel is a population genetist. V. Vogel was also S. Dubey's PhD director.
