On the presence of pipistrelle bats (*Pipistrellus* and *Hypsugo*; Chiroptera: Vespertilionidae) in Sardinia

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Islands are biodiversity hotspots, often containing numerous endemic species. This makes them also hotspots for conservation. Within the Mediterranean region, Sardinia is known for its comparatively high degree of endemism, including cryptic diversity. In this paper we aim to elucidate the variability of pipistrelles (*Pipistrellus* and *Hypsugo*) on Sardinia. More specifically, we ask which species occur on Sardinia and we describe the geographic affiliations of these evolutionary lineages. We sequenced ca. 560 bp of the 16S rRNA gene from 36 pipistrelle specimens representing 17 localities from all major parts of Sardinia. For comparison we added samples from the entire Mediterranean region as well as sequences stored at GenBank. We constructed Bayesian phylogenetic trees and minimum spanning networks to identify which species occur on Sardinia and to infer their genetic affiliation to lineages occurring throughout the Mediterranean and the Canary Islands. We identified five pipistrelle lineages on Sardinia: *Pipistrellus pipistrellus* (haplolineage II), *P. pygmaeus*, *P. kuhlii/desertii*, *Hypsugo savii* s.str. and *H.* cf. *darwinii*. Colonization of Sardinia occurred at different times from different geographical sources, namely Europe and Africa. Some lineages may have invaded Sardinia recently. The Sardinian *H.* cf. *darwinii* may be endemic to the island. Our results highlight the importance of Sardinia as a major Mediterranean hotspot for bat biodiversity. The island harbours a pipistrelle diversity that is higher than that on any other Mediterranean island. Lying geographically at the interface between Europe and Africa, Sardinia combines elements from both continents.

Key words: Mediterranean, cryptic diversity, 16S rDNA, minimum spanning network, Bayesian inference, geographic origin

INTRODUCTION

Islands can harbour extremely large amounts of endemic species, and often such endemics are habitat specialists, making them especially vulnerable to alterations in land use and other anthropogenic changes (Munguira, 1995; Grill *et al.*, 2007). Consequently, islands are not only hotspots for species diversity and rates of endemism, but also for conservation priorities (Myers *et al.*, 2000).

The Mediterranean islands largely differ in their amount of endemics. Sardinia and Corsica, the two large islands in the Tyrrhenian Sea, are known to harbour a comparatively high number of endemics across a variety of taxa (Grill *et al.*, 2007). However, among flying vertebrates only the Marmora's warbler (*Sylvia sarda*) seems to be restricted to Sardinia, Corsica and some Balearic Islands, and even this species leaves these islands in order to migrate to its wintering habitats in Africa. At subspecific level,

Sardinia is known to harbour three endemic taxa: the great spotted woodpecker (*Dendrocopos major harterti*), the great tit (*Parus major ecki*), and the Eurasian jay (*Garrulus glandarius ichnusae*).

It therefore came as a surprise, when Mucedda *et al.* (2002) discovered a cryptic species of long-eared bats on Sardinia, *Plecotus sardus*. Up to know this species has only been found in Sardinia. Based on 16S rDNA molecular distances, this species' evolutionary age is tentatively estimated at ca. 3.6 million years (authors' unpublished data). Given this high age, it is surprising that the species seems to be missing on the nearby island of Corsica. Interestingly, however, Sardinian populations of the brown long-eared bat, *Plecotus auritus*, also comprise a lineage of their own. In contrast, the haplotypes of Sardinian grey long-eared bats, *Plecotus austriacus*, are deeply nested within continental haplotypes (Kiefer, 2007).

The pattern found for long-eared bats in Sardinia shows that our knowledge of the Sardinian bat

fauna still may harbour surprises. This may especially hold for species that are hard to tell apart based on morphology alone, such as the genera *Pipistrellus* and *Hypsugo*. Recently, Hulva *et al.* (2004, 2007) discovered some isolated mitochondrial lineages within this genus, namely within the *P. pipistrellus/pygmaeus* clade. They proposed an allopatric speciation model, with the split between the two species dated to the Pliocene, ca. 4.1–3.1 million years ago. Further locally restricted lineages were discovered from Crete, Cyprus, Sicily and Corsica. It is therefore likely that also other Mediterranean islands may harbour more cryptic, and maybe even endemic, genetic bat diversity than previously thought.

The systematics and taxonomy of the genera *Pipistrellus* and *Hypsugo* is still far from being sufficiently resolved. The number of lineages newly described through molecular analyses is steadily increasing (e.g., Mayer and von Helversen, 2001; Hulva *et al.*, 2004; Ibáñez *et al.*, 2006). For *Pipistrellus*, Mayer *et al.* (2007) mentioned seven species, and Hulva *et al.* (2007) added five further genetic lineages from the Mediterranean region. For a long time, *Hypsugo savii* seemed to be the only representative of the genus in Europe. Only two subspecies were distinguished within the Western Palearctic realm: *H. s. savii* (Bonaparte, 1837) in south-eastern Europe, including Italy and the Alps, and *H. s. ochromixtus* (Cabrera, 1904), probably restricted to the Iberian Peninsula (Horáček *et al.*, 2000). However, molecular data showed that *H. savii* from the Canary Islands is highly divergent from the Iberian *H. s. ochromixtus*. According to Mayer *et al.* (2007), additional lineages within the species are to be found, such as *H. ariel* (Thomas, 1904) from the Near East (see also Horáček *et al.*, 2000).

Prior to this study, in Sardinia only the presence of *P. pipistrellus* (Schreber, 1774), *P. kuhlii* (Kuhl, 1817) and *H. savii* were reported (Mucedda *et al.*, 1999). A recent record of *P. pygmaeus* (Leach, 1825), only known by acoustic identification of a single specimen of the 55 kHz phonic type (Russo and Jones, 2000), has not yet been confirmed. The taxon *P. albolimbatus* (Küster, 1835), first described from the vicinity of Cagliari, is nowadays considered a synonym of *P. kuhlii* (Simmons, 2005).

We here identify the number of pipistrelle taxa of the genera *Pipistrellus* and *Hypsugo* living on Sardinia. Additionally, we use gene trees to gain information on the potential geographic origin of Sardinian haplotypes.

Materials and Methods

We analysed 36 Sardinian samples of the genera *Pipistrellus* and *Hypsugo* from 17 samples sites (Fig. 1 and Appendix). To study their geographic affiliations, we further included own and GenBank sequences from all over Europe, the Canary Islands, Northern Africa and the Near East, as well as some samples from Corsica and Sicily (Appendix). For outgroup rooting we included sequences of *Myotis bechsteinii* and *Vespertilio murinus*.

With the exception of some freshly deposited faeces samples, bats were caught at the entrances of their roosts or mist-netted in foraging areas. Tissue samples were collected with a sterile biopsy punch (3 mm diameter) from plagiopatagium and preserved in 98% ethanol until processing. Animals were kept in separate textile bags, measured, sexed, aged and released directly at the site of capture.

We sequenced approximately 560 bp of the mitochondrial 16S ribosomal gene using the primers 16SA (light chain; 5'-CGC CTG TTT ATC AAA AAC AT-3') and 16SB (heavy chain; 5'- CCG GTC TGA ACT CAG ATC ACG T-3') of Palumbi *et al.* (1991). PCR cycling procedure was as follows: initial denaturation step: 90 s at 94°C, 33 cycles: denaturation 45 s at 94°C, primer annealing for 45 s at 55°C, extension for 90 s at 72°C. Sequences were aligned with Mega 4.1 (Tamura *et al.*, 2007) using the clustal W algorithm. In hypervariable regions the alignment was slightly refined by eye.

We run MrModeltest (version 2.3 — Nylander, 2004) to find the best fitting substitution model. Phylogenetic trees were calculated using maximum likelihood (Mega 5b7 — Tamura *et al.*, 2007) and Bayesian inference (MrBayes, version 3.1.2 — Huelsenbeck and Ronquist, 2001). Maximum likelihood trees were calculated using the model suggested by Mr-Modeltest, with 2000 bootstrap replicates, eight discrete gamma categories, an automatically generated starting tree and the nearest neighbor interchange (NNI) branch swapping algorithm. All sites were included in the calculation. In MrBayes we performed four runs with four chains, each for 1.000.000 generations. This was sufficient to let the average standard deviation drop below 0.01. The burn-in was set to 20%. Since all runs converged at similar likelihood levels we calculated Bayesian posterior probabilities from the 8,000 trees of one randomly chosen run.

For three species with numerous haplotypes (*Pipistrellus pipistrellus*, *P. pygmaeus* and *Hypsugo savii*) we calculated minimum spanning haplotype networks based on statistical parsimony (TCS version 1.18 — Clement *et al.*, 2000). To enhance connection of highly divergent haplotypes to the networks we set the parsimony level to 90%. This did, in our case, not alter topologies obtained under a 95% parsimony level. Average levels of nucleotide divergence (*p*-distance) among groups of haplotypes were calculated with Mega 4.1, with standard errors inferred from 1,000 bootstrap replicates.

Results

MrModeltest selected the GTR+I+G model based on the Akaike Information Criterion (AIC = 5799.8589, -lnL = 2889.9294). Our samples of the genus *Pipistrellus* form several larger monophyletic groups with sufficient support in the Bayesian

consensus tree (Fig. 1; Bayesian posterior probabilities ≥ 0.95): (i) *P. pipistrellus*, *P. hanaki* (Benda *et al.*, 2004*a*) and *P. pygmaeus*, (ii) *P. kuhlii/desertii*, *P.* cf. *lepidus* (Blyth, 1845) and *P. maderensis* (Dobson, 1878), and (iii) *P. nathusii*.

We here preliminarily name the Pkuh-I- and Pkuh-II-lineages as *P. kuhlii/desertii* (Fig. 1; for details see the discussion section). The haplotype of two specimens from Oujda/Morocco, which morphologically clearly represent the small desert form
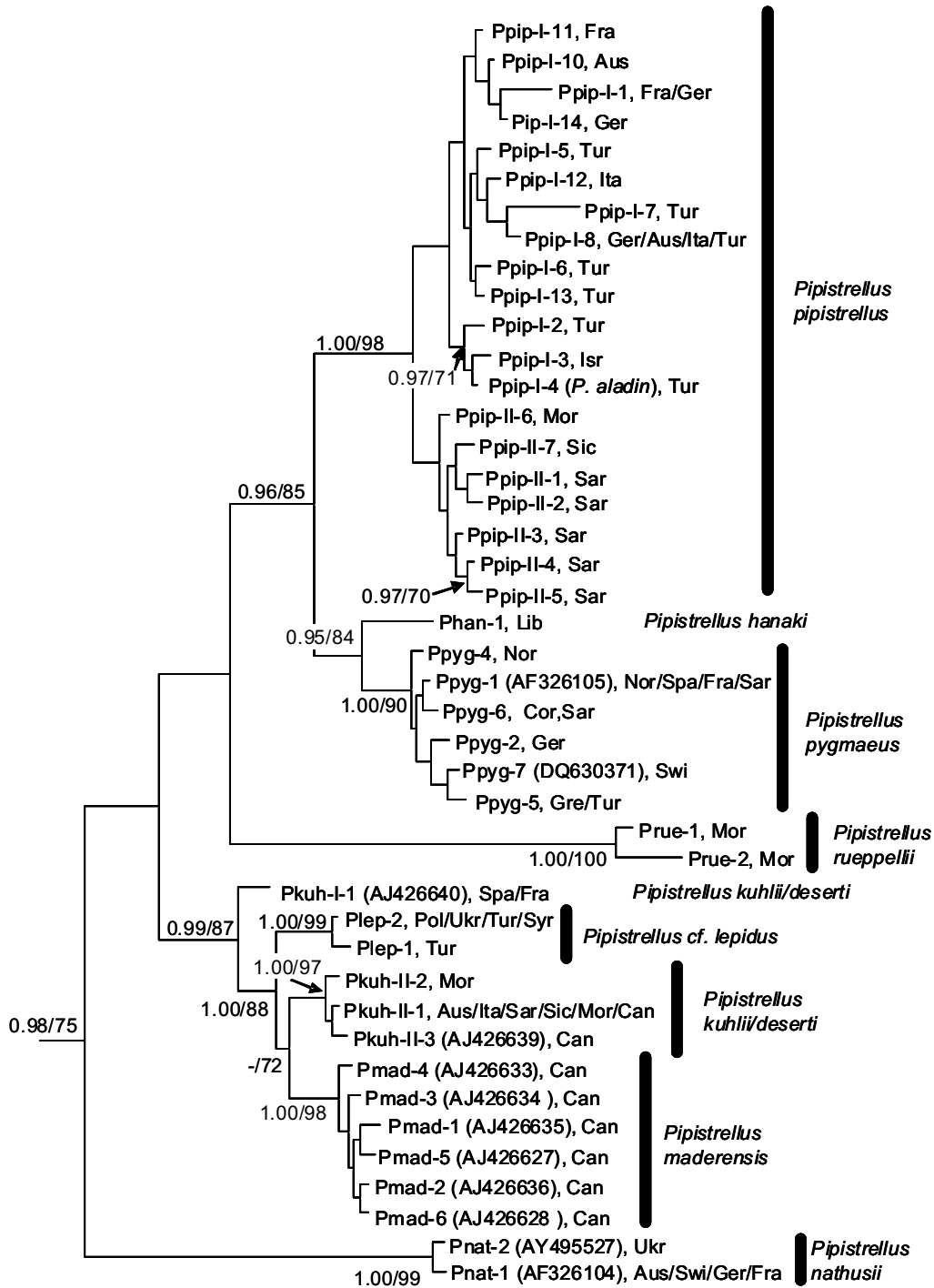

FIG. 1. Bayesian consensus tree of 43 16S rDNA haplotypes of *Pipistrellus*; node support values: Bayesian posterior probabilities ≥ 0.95 / maximum likelihood bootstrap values ≥ 70%; Aus = Austria, Can = Canary Islands, Cor = Corsica, Fra = France, Ger = Germany, Gre = Greece, Isr = Israel, Ita = Italy, Lib = Libya, Mor = Morocco, Nor = Norway, Pol = Poland, Sar = Sardinia, Sic = Sicily, Spa = Spain, Swi = Swiss, Syr = Syria, Tur = Turkey, Ukr = Ukraine

P. desertii (Thomas, 1908), are identical to our *P. kuhlii/desertii* haplotype Pkuh-II-1. The latter is widely distributed across the Mediterranean region (including Sardinia) and even occurs in the Canary Islands (Pestano *et al.*, 2003). *Pipistrellus pipistrellus* consists of two subclades which we named Ppip-I and Ppip-II.

Within *Hypsugo* (Fig. 2), the samples provisionally named by us as *H. savii* comprise three subclades, two of which can be affiliated to *H. savii* s.str. and *H.* cf. *darwinii* (Tomes, 1859), respectively (following Mayer *et al.*, 2007). The third subclade is represented by only one haplotype from Israel (Hsav-III); Mayer *et al.* (2007) consider it a currently undescribed species, *Hypsugo* sp.

The *P. pipistrellus* haplotype network consists of two clades, I and II (Fig. 3). Clade I consists of haplotypes restricted to Europe and Turkey, with one haplotype (Ppip-I-8) being widespread across Germany, Austria, Italy and Turkey (Fig. 1). The Sardinian haplotypes of Ppip-II are widespread across the island (Fig. 6) and are genealogically connected to haplotypes from Morocco and Sicily.

The six *P. pygmaeus* haplotypes form a three ring network and are mainly restricted to Europe and Sardinia/Corsica, with the deeply nested haplotype Ppyg-5 (Fig. 4) also occurring in Turkey (Fig. 4). This is indicative of a European affiliation of the Sardinian *P. pygmaeus* haplotypes.

We only found three *P. kuhlii/desertii* haplotypes, one of which is widespread over Europe, including Sardinia and Sicily, as well as North-Africa and the Canary Islands. Interestingly, the two remaining haplotypes also occur in the Canary Islands and Northern Africa, respectively (Appendix).

Two *Hypsugo* haplolineages, Hsav-I- and Hsav-II, occur in Sardinia (= *H. savii* s.str. and *H.* cf. *darwinii*, respectively; Fig. 1, and 5). Haplotype Hsav-I-1 forms a network with two European haplotypes, while Hsav-II-1 is, together with a Sicilian haplotype, connected to a North-African/Canarian group of haplotypes. This is indicative of European and African affiliations, respectively. The single haplotype from Mt. Hermon in Israel (*Hypsugo* sp.) forms a clade of its own and is differentiated at ca. 5% *p*-distance from the two other haplolineages, which are similarly differentiated from each other and occur in sympatry on Sardinia.

Of the five *Pipistrellus* and *Hypsugo* lineages found in Sardinia (altogether ten haplotypes) three also occur on the European or African mainland with identical or almost identical haplotypes
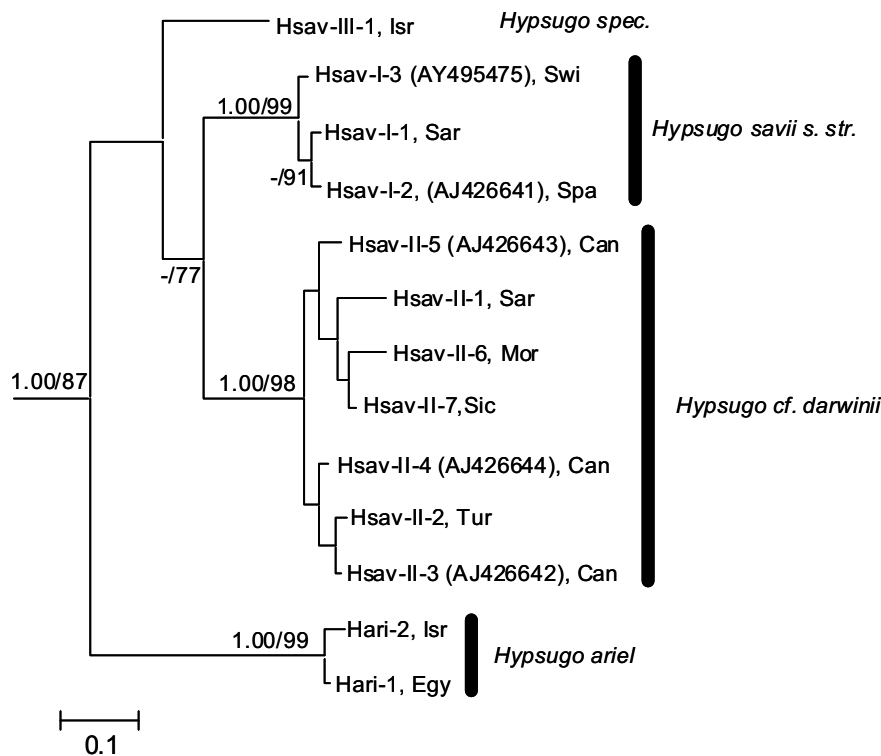

Fɪɢ. 2. Bayesian consensus tree of 13 16S rDNA haplotypes of *Hypsugo*; node support values: Bayesian posterior probabilities ≥ 0.95 / maximum likelihood bootstrap values ≥ 70 %; Can = Canary Islands, Egy = Egypt, Isr = Israel, Mor = Morocco, Sar = Sardinia, Sic = Sicily, Spa = Spain, Swi = Swiss, Tur = Turkey
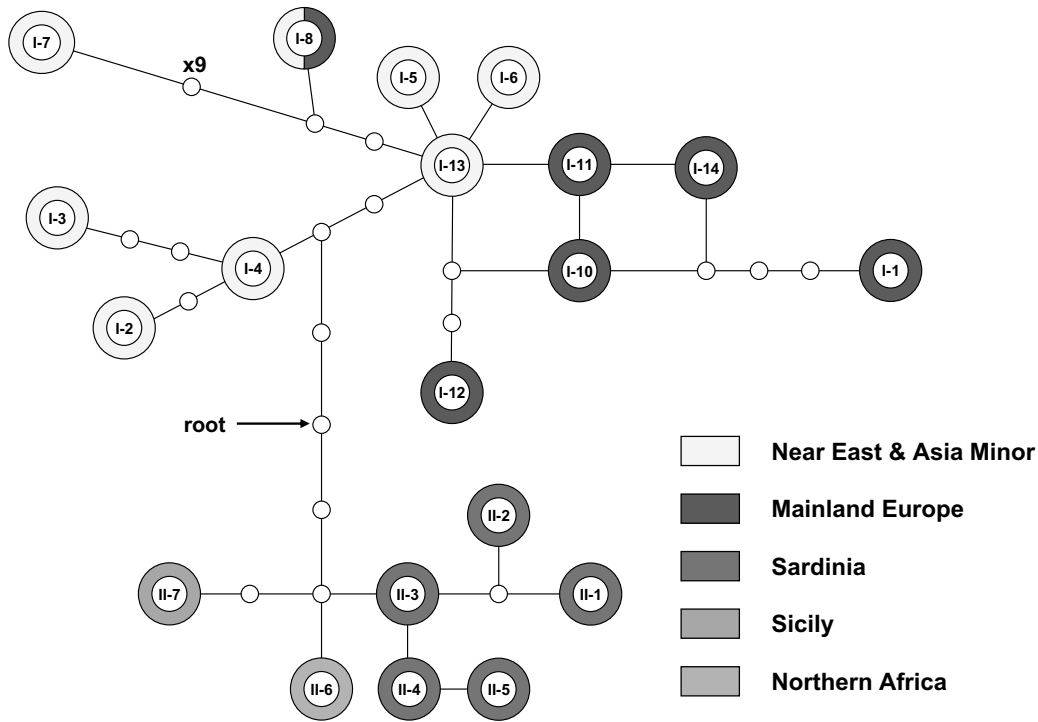
Fig. 3. 90% parsimonious minimum spanning network of 20 *P. pipistrellus* 16S rDNA haplotypes; the tentative position of the root was inferred from the Bayesian tree in Fig. 1

(Table 1). Only one (Ppyg-6) is shared with Corsica. The intra-Sardinian richness of *P. pipistrellus*-II haplotypes shows an intermediate degree of differentiation from Northern African and Sicilian lineages. The highest differentiation of any Sardinian haplotype from a non-Sardinian haplotype is found in *H.* cf. *darwinii*, with six substitutions discriminating it from its closest relative on Sicily.

DISCUSSION

Sardinia harbours only a slightly richer species community of pipistrelle bats than previously thought. *Pipistrellus pipistrellus*, *P. kuhlii* and *Hypsugo savii*, all identified by us on the basis of molecular data, are already listed in the recent compilation of Mucedda (2008). We here add information on the presence of *H.* cf. *darwinii* and genetically confirm a previously published bio-acoustical hint on the occurrence of *P. pygmaeus* (Russo and Jones, 2000). However, none of the lineages we identified from Sardinia is cryptic, as was the case with the Sardinian long-eared bat.

Pipistrellus pipistrellus lineage II is the most diverse pipistrelle of Sardinia. We analysed samples from all over the island (Fig. 6), resulting in
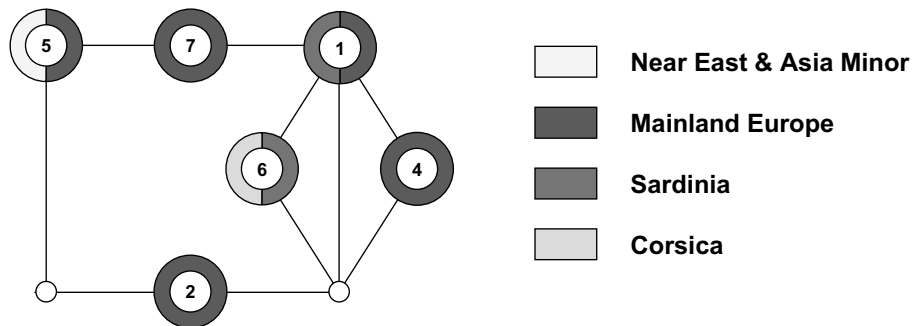

Fig. 4. 90% parsimonious minimum spanning network of six *P. pygmaeus* 16S rDNA haplotypes; due to the three-ring ambiguity we abstained from tentatively assigning a root
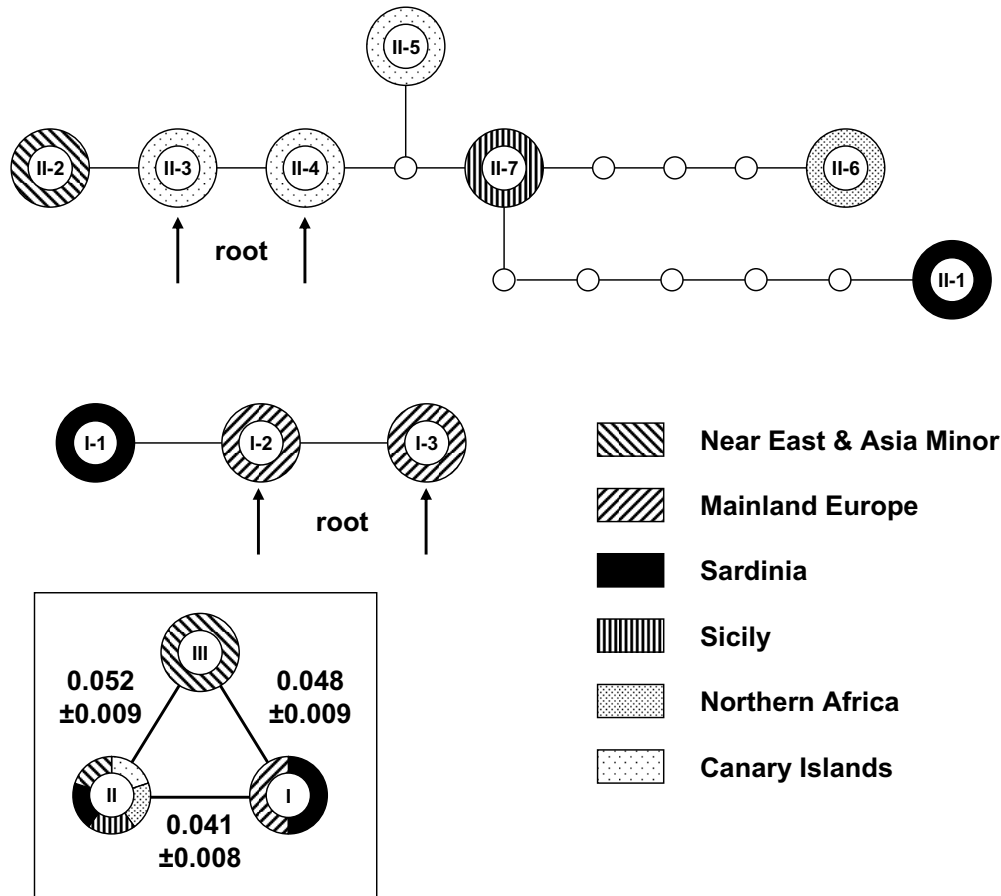
FIG. 5. 90% parsimonious minimum spanning network of 10 *H. savii* s.str. (Hsav-I) and *H*. cf. *darwinii* (Hsav-II) 16S rDNA haplotypes; the tentative positions of the roots were inferred from the Bayesian tree in Fig. 2; the lower left inlet gives the average pairwise *p*-distances between the three haplotype clusters

five haplotypes. All other lineages are currently only represented by 1–2 haplotypes. Like in our study, Hulva *et al*. (2007) described two major lineages within *P. pipistrellus*. One comprised mainly samples from eastern Europe and the Near East (*P. pipistrellus* s.str.), and the second comprised samples from Morocco, Sicily and Corsica. This distribution is almost identical to the pattern found by us. We therefore assume that our Sardinian *P. pipistrellus* lineage is identical to their second *P. pipistrellus* lineage, for which they suggest discussion of its specific status. Unfortunately, both studies indicate allopatry of these forms, with a molecular divergence being slightly lower than what some authors regard to be a good indication of species level differentiation (e.g., Mayer *et al.*, 2007). In our opinion, assigning species status to this *P. pipistrellus* lineage is therefore premature.

Inference of a solid spatio-temporal scenario for the evolution of the analysed lineages must await a geographically much larger circum-Mediterranean sampling. However, already from our sampling two

major patterns of geographic affiliation of Sardinian pipistrelles emerge. Sardinian haplotypes are either linked to or embedded in European/Asian Minor (*P. pygmaeus*, *H. savii*-I) or African/Canary Island/ Sicilian clades (*P. pipistrellus*-II, *P. kuhlii/deserti*-II, *H*. cf. *darwinii*). This well reflects the geographically intermediate position of Sardinia between Europe and Africa.

Based on the number of substitutions between Sardinian haplotypes and their closest non-Sardinian relatives we can assume that their split from the respective closest relatives occurred at different times (we are well aware that this approach gives only a very rough indication that colonization and lineage separation seems to have occurred at different times). We found the presumably oldest tractable pipistrelle relationship in *H*. cf. *darwinii*, with six substitutions distinguishing the Sicilian from the Sardinian haplotypes. Since, as far as our restricted sampling is concerned, we found the latter only on Sardinia, this may in fact later on be assessed as an endemic haplotype which potentially colonised the

● *Pipistrellus pipistrellus* II
○ *Pipistrellus pygmaeus*
▲ *Pipistrellus kuhlii/desertii*
■ *Hypsugo savii s. str.*
□ *Hypsugo cf. darwinii*

Fig. 6. Occurrence of genetically identified *Pipistrellus* and *Hypsugo* lineages in Sardinia

island from Africa/Sicily. The potential colonisation by *P. pipistrellus* (lineage II, 2–3 bp difference), again from an African/Sicilian origin, as is supported by the pattern and polarity emerging from the haplotype network, seems to be younger. *Hypsugo savii* s.str. is likely to have colonised Sardinia from mainland Europe.

All other affiliations of Sardinian haplotypes are characterised by only one or no substitution. Some of them occur even widespread outside Sardinia. This may be indicative of recent movements of these haplotypes throughout the Mediterranean from different sources (Table 1, Appendix). *Pipistrellus kuhlii/desertii* seems to be exceptionally mobile across large parts of the Mediterranean and adjacent

regions, since its haplotype Pkuh-II-1 occurs in Sardinia, Sicily, mainland Italy, Austria, Morocco and even the Canary Islands (see Appendix). Unfortunately, it is currently not possible to decide, which of our two *P. kuhlii* lineages represent *P. kuhlii* s.str. *Pipistrellus kuhlii* has originally been described from Friuli-Venezia Giulia/Italy; however, topotypical samples have never been studied. In contrast, we found only one *P. kuhlii/desertii* haplotype to occur all over Sardinia, including a locality close to Cagliari, the type locality of *P. albolimbatus*. We, therefore, can neither rule out that Sardinian *P. kuhlii* are in fact *P. kuhlii* s.str. (then the Iberian *P. kuhlii* would represent an undescribed taxon) nor that they represent *P. albolimbatus*, a species that can genetically (by means of mtDNA) not be distinguished from the North African *P. desertii*. We even cannot exclude a further case of a mitochondrial genome replacement as it has been described for other pairs of bat species (Mayer and von Helversen, 2001; Berthier *et al.*, 2006; Currat *et al.*, 2008).

In conclusion, our data show that there is no common pattern when and from where different pipistrelle bat species may have colonised Sardinia. Since the end of the Messinian Salinity Crisis 5.33 million years ago (Krijgsman *et al.*, 1999) Corsica and Sardinia remained separated from Europe and Northern Africa. Colonization was only possible across the sea. Nevertheless, the genus *Plecotus* repeatedly colonised Sardinia since the Pliocene (Kiefer, 2007). Long-eared bats comprise species with similar wing aspect ratios and wing-loadings (Norberg and Rayner, 1987). This also holds true for the pipistrelle species, which, like long-eared bats, arrived at Sardinia at different times and from different geographic sources. This suggests that eco-morphology seems not to be the major predictor for the probability of a bat species to colonise Sardinia (or any other Mediterranean island — see also García-Mudarra *et al.*, 2009).

DNA sequence analysis has been proven to be a powerful tool to detect cryptic diversity in bats (e.g., Mayer *et al.*, 2007). The number of European bat species is steadily increasing, and our knowledge on intraspecific diversity will help to newly define conservation priorities e.g. through the delimitation of evolutionary significant units (ESUs) for management, as may hold for the Sardinian *H.* cf. *darwinii* haplotype. However, it should be kept in mind that taxonomic conclusions, which are solely based on mitochondrial genes, may be misleading due to effects such as incomplete lineage sorting, introgression, and selection on

TABLE. 1. Differentiation of Sardinian haplotypes from their genealogically closest non-Sardinian relatives. [1] — one indel

Taxon/haplolineage	Sardinian haplotype	Closest relative	Occurrence of the closest relative	n substitutions	GTR+I+G distance
P. pistrellus II	Ppip-II-3	Ppip-II-6	Northern Africa	2	0.00188
P. pistrellus II	Ppip-II-3	Ppip-II-7	Sicily	3	0.00379
P. pygmaeus	Ppyg-1	Ppyg-1	Mainland Europe	0	0.0
P. pygmaeus	Ppyg-6	Ppyg-6	Corsica	0	0.0
H. savii s.str.	Hsav-I-1	Hsav-I-2	Mainland Europe	1[1]	0.0
H. cf. *darwinii*	Hsav-II-1	Hsav-II-7	Sicily	6	0.00722
P. kuhlii/deserti	Pkuh-II-1	Pkuh-II-1	Mainland Europe, Sicily, Northern Africa	0	0.0

mitochondrial loci (e.g., Ballard and Whitlock, 2009; Degnan and Rosenberg, 2009; see Hulva *et al.*, 2010 for *Pipistrellus*).

Taking *Plecotus*, *Pipistrellus* and *Hypsugo* together, we identified eight evolutionary significant units (ESUs) living on Sardinia (three *Plecotus* ESUs, three *Pipistrellus* ESUs and two *Hypsugo* ESUs), with four being recognised through DNA analysis only. Also other recent studies (e.g., Benda *et al.*, 2004a, 2004b; Hulva *et al.*, 2004, 2007; Ibáñez *et al.*, 2006; García-Mudarra *et al.*, 2009) suggest that an extension of intense sampling throughout the entire Mediterranean region, an area which has been prioritised through the IUCN, will substantially increase the number of species and ESUs known for this part of the western Palearctic realm, with important implications for conservation (Gippoliti and Amori, 2006). We therefore think that a thorough molecular analysis of all Sardinian bat species, with special emphasis on the inclusion of large island-wide sample to increase the probability of detecting cryptic lineages, will foster conservation prioritisation of Sardinian bats.

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APPENDIX

Sample identification, sample localities, haplotypes and GenBank accession numbers (GB acc. no.); NMP = National Museum in Prague, IZEA = Institut de Zoologie et d'Écologie Animale, Lausanne

Species	Haplotype	Country, island	Locality	GB acc. no.	Provider/reference	Comment
			Hypsugo			
savii s.str.	Hsav-I-1	Italy, Sardinia	Gadoni	HQ848774	Mauro Mucedda	
savii s.str.	Hsav-I-1	Italy, Sardinia	Bolotana		M. Mucedda	
savii s.str.	Hsav-I-2	Spain	Zaragoza	AJ426641	GenBank	
savii s.str.	Hsav-I-3	Switzerland	Valais region	AY495475	GenBank	IZEA3586
cf. *darwinii*	Hsav-II-1	Italy, Sardinia	Urzulei	HQ848775	M. Mucedda	
cf. *darwinii*	Hsav-II-1	Italy, Sardinia	Anela		M. Mucedda	
cf. *darwinii*	Hsav-II-1	Italy, Sardinia	Santadi		M. Mucedda	
cf. *darwinii*	Hsav-II-2	Turkey	Göreme	HQ848776	Andreas Kiefer	faeces
cf. *darwinii*	Hsav-II-3	Spain, Canary Islands	Gran Canaria	AJ426642	GenBank	
cf. *darwinii*	Hsav-II-4	Spain, Canary Islands	La Gomera	AJ426644	GenBank	
cf. *darwinii*	Hsav-II-5	Spain, Canary Islands	El Hierro	AJ426643	GenBank	
cf. *darwinii*	Hsav-II-6	Morocco	Georges du Dades	HQ848777	Christian Dietz	
cf. *darwinii*	Hsav-II-7	Italy, Sicily	Cefalù	HQ848778	M. Mucedda	
sp.	Hsav-III-1	Israel	Mt. Hermon	HQ848779	C. Dietz	
ariel	Hari-1	Egypt	Sinai	HQ848781	C. Dietz	
ariel	Hari-1	Egypt	Sinai		C. Dietz	
ariel	Hari-2	Israel	not available	HQ848780	C. Dietz	

APPENDIX. Continued

Species	Haplotype	Country, island	Locality	GB acc. no.	Provider/reference	Comment
			Pipistrellus			
kuhlii/deserti	Pkuh-I-1	Spain	Valladolid	AJ426640	GenBank	
kuhlii/deserti	Pkuh-I-1	France	not available		Laurent Arthur	
kuhlii/deserti	Pkuh-II-1	Morocco	Oujda	HQ848768	Petr Benda	NMP90082
kuhlii/deserti	Pkuh-II-1	Morocco	Oujda		P. Benda	NMP90083
kuhlii/deserti	Pkuh-II-1	Spain, Canary Islands	Tenerife	AJ426637	GenBank	
kuhlii/deserti	Pkuh-II-1	Austria	Steiermark		Guido Reiter	
kuhlii/deserti	Pkuh-II-1	Italy, Sardinia	Bolotana		M. Mucedda	
kuhlii/deserti	Pkuh-II-1	Italy, Sardinia	Bolotana		M. Mucedda	
kuhlii/deserti	Pkuh-II-1	Italy, Sardinia	Dorgali		M. Mucedda	
kuhlii/deserti	Pkuh-II-1	Italy, Sardinia	Santadi		M. Mucedda	
kuhlii/deserti	Pkuh-II-1	Italy, Sardinia	Bolotana		M. Mucedda	
kuhlii/deserti	Pkuh-II-1	Italy, Sardinia	Villaputzu		M. Mucedda	
kuhlii/deserti	Pkuh-II-1	Italy, Sardinia	Gadoni		M. Mucedda	
kuhlii/deserti	Pkuh-II-1	Italy, Sardinia	Meana Sardo		M. Mucedda	
kuhlii/deserti	Pkuh-II-1	Italy, Sardinia	Seulo		M. Mucedda	
kuhlii/deserti	Pkuh-II-1	Italy, Sicily	Sortino		M. Mucedda	
kuhlii/deserti	Pkuh-II-1	Italy	Portofino		Gianna Dondini, Simone Vergari	
kuhlii/deserti	Pkuh-II-1	Italy	Prato		G. Dondini, S. Vergari	
kuhlii/deserti	Pkuh-II-1	Italy	Fiesole		G. Dondini, S. Vergari	
kuhlii/deserti	Pkuh-II-1	Italy	Sa Palma		G. Dondini, S. Vergari	
kuhlii/deserti	Pkuh-II-2	Morocco	Anagam	HQ848769	P. Benda	NMP90059
kuhlii/deserti	Pkuh-II-3	Spain, Canary Islands	Tenerife	AJ426639	GenBank	
hanaki	Phan-1	Libya	Wadi al Kuf	HQ848761	P. Benda	NMP49897
hanaki	Phan-1	Libya	Wadi al Kuf		P. Benda	NMP49903
lepidus	Plep-1	Turkey	Manavgat	HQ857598	Reinhardt Vowinkel	
lepidus	Plep-2	Syria	Tell Sheikh Hamad	HQ857597	P. Benda	
lepidus	Plep-2	Poland	Zawiercie		Konrad Sachanowicz	
lepidus	Plep-2	Ukraine	Knarkiv region		K. Sachanowicz	
lepidus	Plep-2	Ukraine	Knarkiv region		K. Sachanowicz	
lepidus	Plep-2	Ukraine	Knarkiv region		K. Sachanowicz	
lepidus	Plep-2	Ukraine	not available		K. Sachanowicz	
lepidus	Plep-2	Ukraine	not available		K. Sachanowicz	
lepidus	Plep-2	Ukraine	not available		K. Sachanowicz	
lepidus	Plep-2	Ukraine	not available		K. Sachanowicz	
lepidus	Plep-2	Ukraine	not available		K. Sachanowicz	
lepidus	Plep-2	Turkey	Manavgat		R. Vowinkel	
maderensis	Pmad-1	Spain, Can. Islands	La Palma	AJ426635	GenBank	
maderensis	Pmad-2	Spain, Can. Islands	La Palma	AJ426636	GenBank	
maderensis	Pmad-3	Spain, Can. Islands	La Gomera	AJ426634	GenBank	
maderensis	Pmad-4	Spain, Can. Islands	Tenerife	AJ426633	GenBank	
maderensis	Pmad-5	Spain, Can. Islands	Tenerife	AJ426627	GenBank	
maderensis	Pmad-6	Spain, Can. Islands	Tenerife	AJ426628	GenBank	
nathusii	Pnat-1	Switzerland	Vaud	AF326104	GenBank	IZEA2830
nathusii	Pnat-1	Switzerland	Vaud	AY495526	GenBank	IZEA3406
nathusii	Pnat-1	France	Bourges	HQ848773	A. Kiefer	
nathusii	Pnat-1	Germany	Altrip		A. Kiefer	
nathusii	Pnat-1	Germany	Bad Kreuznach		A. Kiefer	
nathusii	Pnat-1	Germany	Altrip		A. Kiefer	
nathusii	Pnat-1	Austria	Salzburg		Maria Jerabek,	
nathusii	Pnat-1	Austria	Salzburg		Ulli Hüttmeier, M. Jerabek	
nathusii	Pnat-2	Ukraine	Chernobyl district	AY495527	GenBank	
nathusii	Pnat-2	Ukraine	Chernobyl district	AY495528	GenBank	
pipistrellus	Ppip-I-1	Germany	Mayen	HQ848741	A. Kiefer	
pipistrellus	Ppip-I-1	Germany	Seibersbach		A. Kiefer	
pipistrellus	Ppip-I-1	France	Herault, Larzac		Rudolf Zahner	
pipistrellus	Ppip-I-1	France	Herault, Larzac		R. Zahner	
pipistrellus	Ppip-I-2	Turkey	Cappadocia	HQ848742	A. Kiefer	faeces
pipistrellus	Ppip-I-3	Israel	not available	HQ848743	Asaf Tsoar	
pipistrellus	Ppip-I-4	Turkey	Van	HQ848744	Ahmet Karatash	
pipistrellus	Ppip-I-5	Turkey	Milet	HQ848745	C. Dietz	
pipistrellus	Ppip-I-6	Turkey	Milet	HQ848746	C. Dietz	

APPENDIX. Continued

Species	Haplotype	Country, island	Locality	GB acc. no.	Provider/reference	Comment
pipistrellus	Ppip-I-7	Turkey	Dadia	HQ848747	C. Dietz	
pipistrellus	Ppip-I-8	Turkey	Dadia	HQ848748	C. Dietz	
pipistrellus	Ppip-I-8	Germany	Wiesbaden		A. Kiefer	
pipistrellus	Ppip-I-8	Germany	Mainz		A. Kiefer	
pipistrellus	Ppip-I-8	Italy	Pesina		A. Kiefer	
pipistrellus	Ppip-I-8	Austria	Köttmansdorf, Carinthia		M. Jerabek, G. Reiter	
pipistrellus	Ppip-I-8	Austria	Köttmansdorf, Carinthia		M. Jerabek, G. Reiter	
pipistrellus	Ppip-I-8	Austria	Winkel im Rosenthal, Carinthia		M. Jerabek, G. Reiter	
pipistrellus	Ppip-I-10	Austria	Salzburg	HQ848749	M. Jerabek	
pipistrellus	Ppip-I-11	France	Theillay	HQ848750	L. Arthur	
pipistrellus	Ppip-I-12	Italy	Pesina	HQ848751	A. Kiefer	
pipistrellus	Ppip-I-13	Turkey	Titryengöl	HQ848752	R. Vowinkel	
pipistrellus	Ppip-I-14	Germany	Mayen	HQ848753	A. Kiefer	
pipistrellus	Ppip-II-1	Italy, Sardinia	Monti	HQ848754	C. Dietz	
pipistrellus	Ppip-II-1	Italy, Sardinia	Burgos		C. Dietz	
pipistrellus	Ppip-II-1	Italy, Sardinia	Urzulei		M. Mucedda	
pipistrellus	Ppip-II-1	Italy, Sardinia	Lotzorai		M. Mucedda	
pipistrellus	Ppip-II-1	Italy, Sardinia	Dorgali		M. Mucedda	
pipistrellus	Ppip-II-1	Italy, Sardinia	Urzulei		M. Mucedda	
pipistrellus	Ppip-II-1	Italy, Sardinia	Sorso		M. Mucedda	
pipistrellus	Ppip-II-1	Italy, Sardinia	Sorso		M. Mucedda	
pipistrellus	Ppip-II-1	Italy, Sardinia	Villaputzu		M. Mucedda	
pipistrellus	Ppip-II-1	Italy, Sardinia	Gadoni		M. Mucedda	
pipistrellus	Ppip-II-1	Italy, Sardinia	Seulo		M. Mucedda	
pipistrellus	Ppip-II-1	Italy, Sardinia	Lotzorai		M. Mucedda	
pipistrellus	Ppip-II-2	Italy, Sardinia	Anela	HQ848755	M. Mucedda	
pipistrellus	Ppip-II-2	Italy, Sardinia	Bolotana		M. Mucedda	
pipistrellus	Ppip-II-3	Italy, Sardinia	Domusnovas	HQ848756	M. Mucedda	
pipistrellus	Ppip-II-4	Italy, Sardinia	Santadi	HQ848757	M. Mucedda	
pipistrellus	Ppip-II-5	Italy, Sardinia	Santadi	HQ848758	M. Mucedda	
pipistrellus	Ppip-II-6	Morocco	Bekrite	HQ848759	P. Benda	NMP90033
pipistrellus	Ppip-II-6	Morocco	Tinehir		P. Benda	NMP90074
pipistrellus	Ppip-II-7	Italy, Sicily	Cefalù	HQ848760	M. Mucedda	
pygmaeus	Ppyg-1	Spain	Barcelona region	AF326105	GenBank	IZEA3403
pygmaeus	Ppyg-1	Italy, Sardinia	Santadi	HQ848762	M. Mucedda	
pygmaeus	Ppyg-2	Germany	Hessen district	HQ848763	Frieder Mayer	
pygmaeus	Ppyg-4	France	Plobsheim	HQ848764	L. Arthur	
pygmaeus	Ppyg-4	France	Plobsheim		L. Arthur	
pygmaeus	Ppyg-4	Norway	not available		Tore Christian Michaelsen	
pygmaeus	Ppyg-4	Norway	not available		T. C. Michaelsen	
pygmaeus	Ppyg-4	Norway	not available		T. C. Michaelsen	
pygmaeus	Ppyg-4	Norway	not available		T. C. Michaelsen	
pygmaeus	Ppyg-5	Greece	Pardali, Pelepones	HQ848765	A. Kiefer	
pygmaeus	Ppyg-5	Turkey	Dadia		C. Dietz	
pygmaeus	Ppyg-6	France, Corsica	Col de Saltu	HQ848766	A. Kiefer	
pygmaeus	Ppyg-6	Italy, Sardinia	Lotzorai		M. Mucedda	
pygmaeus	Ppyg-6	Italy, Sardinia	Sorso		M. Mucedda	
pygmaeus	Ppyg-6	Italy, Sardinia	Sorso		M. Mucedda	
pygmaeus	Ppyg-7	Switzerland	Preverenges	DQ630371	GenBank	
rueppellii	Prue-1	Morocco	Anagam	HQ848771	P. Benda	NMP900057
rueppellii	Prue-2	Morocco	Taourirt	HQ848772	P. Benda	NMP900081
Myotis						
bechsteinii		Germany	Boos	HQ848782	A. Kiefer	
Vespertilio						
murinus		Switzerland	Valais Prov.	AY395866	GenBank	IZEA3599