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Research Article

Pantelleria island (Sicily, Italy): a biogeographic crossroad for bats between Africa and Europe

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Introduction

Areas at the boundaries of different bioregions represent particularly interesting sets for biogeographers, as they usually feature high species richness levels due to their geographical position. This peculiar geographic context leads to unique biological communities that comprise taxa from different regions, as well as endemic ones (Cracraft, 1985). Among Mediterranean islets, Pantelleria stands out for its isolation, being far from other islands and located 70 and 120 km off the African (Tunisia) and Italian (Sicily) coasts, respectively. Due to its position in the Sicilian Channel, Pantelleria and several other islands (e.g., Malta-Gozo) comprise unique faunal assemblages, featuring affinities with both the European and African communities, as well as endemic taxa. For instance, Pantelleria hosts some typical North African bird species as residents (Corso et al., 2012), such as the North African blue tit (Cyanistes teneriffae), as well as at least 20 endemic invertebrates (Muscarella and Baragona, 2017). Bats are among the few mammals on offshore islands worldwide (Lawlor, 1986) thanks to their long-range dispersal. Across the Mediterranean, many bat species occur on several major islands such as Crete, Sicily, and Sardinia, including some endemic or sub endemic taxa (Mucedda et al., 2002; Benda et al., 2004b). Yet, knowledge of the bat assemblages of smaller islands is mostly an-

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Abstract

Islands often comprise unique faunal assemblages, particularly when they lie at the boundaries of different bioregions or host endemic taxa. Bats are among the few mammals that can regularly be found on islands around the world, yet knowledge on insular bat assemblages is often poor and anecdotal. Here we integrate different approaches to assess the bat assemblage composition on the Mediterranean island of Pantelleria. We found that at least six species occur on the island, including two typically African taxa, thus doubling the numbers known from previous studies. We provide insights into the distribution, biogeography and conservation planning of these bat species, highlighting the importance of studying and conserving insular bat populations.

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ecdotal or completely unavailable, leaving gaps in the biogeographical patterns of several species, as well as in bat conservation policies.

Previous information on the presence of bats on Pantelleria is scarce, and only two studies are available, listing three species: *Plecotus austriacus, Pipistrellus kuhlii* and *Rhinolophus hipposideros* (Felten and Storch, 1970; Zava and Lo Valvo, 1990). Here we present the results of *ad hoc* bat surveys on the island carried out adopting an integrated approach (Brinkley et al., 2021), comprising bioacoustic surveillance, roost inspections, temporary netting sessions, and molecular techniques. By conducting systematic and integrated research, we aim to provide a comprehensive assessment of the bat assemblage on Pantelleria in terms of species composition to improve bat conservation planning on the island.

We pursue the hypothesis that the bat fauna of Pantelleria will follow the same biogeographical patterns of other taxa present on the island and will therefore consist of bat species from both North African and European Mediterranean biogeographical regions.

Materials and methods

Study area

The island of Pantelleria is located in the middle of the Sicilian Channel, covering an area of 83 km^2 . It was formed ca. 320000 years ago (Mahood and Hildreth, 1986) due to volcanism and has high hydrothermal activity. Pantelleria mostly features a mountainous or hilly



Table 1 – Biometric data of bats captured on Pantelleria island; Sex: M=male, F=female; Age: Ad=adult, Juv=juvenile; status: Pl=Post-lactating, Nr=non reproductive, L=lactating; FAL: forearm length (mm); W: weight (g); EL: ear length (mm); TL: tragus length (mm); TW: tragus width (mm); CM³: upper tooth row length (mm); TiL: tibia length (mm); Th: thumb length (mm); III: third finger length (mm); V: fifth finger length (mm).

Bat ID	Species	Sex	Age, status	FAL	W	EL	TL	TW	CM ³	TiL	ThL	ш	\mathbf{V}
P1	Plecotus gaisleri	М	Ad, Nr	39.7	7.5	_	13.6	5.5	5.4	17.9	5.5	60.0	48.3
P2	Plecotus gaisleri	F	Ad, Pl	39.7	8.2	-	13.7	5.6	5.6	18.0	6	62.3	49.0
P3	Plecotus gaisleri	Μ	Ad, Nr	38.6	6.9	-	13.3	5.5	5.6	18.1	5.5	61.0	47.8
M1	Myotis punicus	Μ	Ad, Nr	55.1	21.0	26.6	-	-	-	-	-	88.0	64.0
R1	Rhinolophus mehelyi	F	Ad, Nr	49.4	12.0	-	-	-	-	-	-	-	-
R2	Rhinolophus mehelyi	Μ	Ad, Nr	47.5	11.3	_	-	_	_	-	_	-	-
R3	Rhinolophus hipposideros	Μ	Juv	35.1	3.2	_	-	_	_	-	_	-	_
R4	Rhinolophus hipposideros	F	Ad, L	36.1	3.7	_	-	_	_	-	_	-	_
R5	Rhinolophus hipposideros	F	Ad, Nr	36.1	4.6	_	-	_	_	-	_	-	_
R6	Rhinolophus hipposideros	Μ	Ad, Nr	35.5	4.3	_	-	_	_	_	_	-	_
R7	Rhinolophus hipposideros	М	Juv	32.0	3.4	-	-	-	-	-	-	-	-

profile and reaches a maximum altitude of 834 m a.s.l.; it has a typically Mediterranean climate. The island is mainly covered by shrubland and Mediterranean woods of oaks and native conifers. The two types of dominant crops are vineyards and olive groves.

Bat sampling

Captures were conducted in the summers of 2013 and 2019 with one 6 m long mist net (19 mm mesh and five pockets each) positioned near potential roosts and water sites. The net was positioned 30 min before sunset and removed at dawn; in total, we performed twelve mistnetting sessions using one net per session at four roosts and three water sites. On some occasions, we also used hand-nets to catch bats inside the roost, provided this would not imply disturbance to colonies (n roosts=5). Morphological identification of captured bats was conducted following Dietz and von Helversen (2004). We also recorded echolocation calls of captured bats on release, and rhinolophids were recorded in hand to avoid Doppler-shift compensation and obtain diagnostic calls (Russo et al., 2007). In September 2019, we used automatic D500x bat detectors (Pettersson Elektronik AB, Uppsala, Sweden) placed in different island locations and left active from sunset to dawn, for one night. We recorded bat activity at eight sites, equally distributed in four habitat types: water sites, urban areas, Mediterranean scrubland, and woodland. Sites were at least 1500 m apart, and recordings were made once per site; recorders were set to record 5 s files per minute, with a sampling rate of 500 kHz, also applying the builtin high-pass filter, no pre-triggering, and trigger sensitivity at "high". Additionally, we opportunistically recorded bat activity with a handheld D1000x bat detector (Pettersson Elektronik AB, Uppsala, Sweden) while walking or driving for 4 nights for ca. two hours since sunset; the detector was set to record at a 348 kHz sampling rate. Call measurements and species assignment of recorded calls were done using the ver. 4.03 BatSound package (Pettersson Elektronik AB, Uppsala, Sweden) by following Russo and Jones (2002) and using reference recordings.

Molecular analyses

DNA samples were collected using a 3 mm biopsy punch taken from the bat's wing membrane and stored in 98 % ethanol. DNA analysis was performed on a fragment of the mitochondrial 16S rRNA and ND1 genes. DNA extraction, PCR amplification and sequencing protocols were the same as described by Veith et al. (2011) for 16S and Bogdanowicz et al. (2015) for ND1. Sequences were aligned using MEGA (version X; Kumar et al., 2018). For the taxonomic assignment, the sequences obtained were queried against the GenBank database using the BLAST algorithm.

Results

We captured eleven bats (Tab. 1): three *Plecotus* bats (two males and one female from two sites), two *Rhinolophus mehelyi* (one male, one female, from one site), one *Myotis* sp., all with mist-nets positioned

at the entrance of potential roosts, and five *Rhinolophus hipposideros* (three males and two females from four sites) with hand nets inside the roosts.

The two *R. mehelyi* were identified by morphological characters (Fig. 1), and their calls showed a peak resting frequency of 105.7–106.8 kHz. *Rhinolophus hipposideros* were found at different localities across the island, mostly inside abandoned traditional buildings found on the island ("dammusi"), caves and underground tunnels. Echolocation calls of the five captured *R. hipposideros* showed a peak resting frequency of 117.30 \pm 0.7 kHz (n=3; range: 116.3–118.0 kHz) and 122.20 \pm 0.9 (n=2; range: 121.3–123.1 kHz) for males and females, respectively.

The DNA sequences obtained from *Plecotus* tissue samples were unequivocally assigned to *P. gaisleri* (Fig. 1), being homologues of Pindet2 *Plecotus t.* cf. *gaisleri* subsp. n. The *Myotis* we captured was identified genetically as *Myotis punicus* (Fig. 1). Detailed measurements used for morphological identification are reported in Tab. 1. These values support the genetic identification of the species according to Dietz and Kiefer (2016).

We recorded echolocation calls from free-flying bats belonging to four species at ten locations (eight fixed, two corresponding to driven or walked transects) across the island, totalling 302 bat passes (Tab. 2). The species most frequently contacted was *P. kuhlii* (n=228), present at all sites, followed by *Plecotus* sp. (n=39; from 4 sites), *Hypsugo savii* (n=31, from 4 sites) and *Myotis* sp. (n=4, from 2 sites); for all species,



Figure 1 – Some bats and habitats from Pantelleria. 1: The Mediterranean scrubland where acoustic surveys took place; 2. *Myotis punicus*; 3. *Rhinolophus mehelyi*; 4. *Plecotus gaisleri*.

Table 2 – Main variables of echolocation calls recorded from bats in free flight on Pantelleria. FmaxE=frequency of maximum energy (in kHz); SF=start frequency (in kHz); EF=end frequency (in kHz); Dur=call duration (in ms).

Species	N sites	N passes	N calls	FmaxE	SF	EF	Dur
Pipistrellus kuhlii	10	228	65	40.7 ± 2.1	59.3 ± 9.9	37.5 ± 3.2	5.5 ± 2.1
Plecotus sp. ¹	4	39	62	33.2 ± 1.7	44.8 ± 2.3	24.9 ± 1.5	2.9 ± 0.3
Hypsugo savii	4	31	13	34.1 ± 3.3	44.7 ± 2.1	29.9 ± 2.2	5.5 ± 1.8
Myotis sp. ²	2	4	14	49.8 ± 4.5	68.5 ± 11.1	29.7 ± 5.9	5.1 ± 1.4

¹ Molecular evidence from the present work and Ancillotto et al. (2020) assigns long-eared bats on Pantelleria to *P. gaisleri*.

² Molecular evidence from the present work assigns our only record of *Myotis* sp. to *M. punicus*.

the highest activity levels were recorded at the only artificial freshwater pond present on the island.

the risk of frequency overlap with sympatric *R. mehelyi* (Russo et al., 2007).

Discussion

The bat fauna of Pantelleria features a unique set of species with ranges covering very different biogeographical areas within the Palearctic realm (sensu Vigna Taglianti et al., 1992). In agreement with our hypothesis, the bat assemblage found on the island comprised species with very different distributions, including Central European (R. hipposideros), as well as Central European/Mediterranean (P. kuhlii and H. savii), purely Mediterranean (R. mehelyi), and Western Mediterranean/North African (M. punicus and P. gaisleri) species. Moreover, two of our records (R. mehelyi and M. punicus) add to the currently known range of these species in Italy, being the southernmost Italian observations. Both species are rare in Italy, being recorded almost exclusively in Sardinia and Sicily, and thus our findings highlight the importance of Pantelleria, and the National Park therein, for future management and conservation choices. Rhinolophus mehelyi is a new addition to the bat assemblage of Pantelleria, increasing the knowledge of the species' range, which in Italy is restricted to Sardinia and three caves in Sicily, thus deserving particular conservation efforts. Moreover, our study confirms the univocal identity of the long-eared bats from Pantelleria as P. gaisleri, and reports on the first evidence of reproduction by this species on the island. Despite the relatively close distance between Pantelleria and Africa, migration of Plecotus bats between the island and the continent is unlikely, since these bats are non-migratory and do not tend to cross the sea actively; for example, no species seem to cross the short distance (ca. 11 km) between the islands of Sardinia and Corsica (Mucedda & Fichera pers. obs.). The confirmation of the identity of long-eared bats from Pantelleria (Ancillotto et al., 2019; this work) and Malta-Gozo (Batsleer et al., 2019) as P. gaisleri (Benda et al., 2004a) also supports the statement that the species is the only long-eared bat present on these islands, which was debated in the past (Ancillotto et al., 2019). Yet, P. gaisleri is still awaiting to be included in the Italian mammal checklist (Loy et al., 2019), and in the list of the bat species protected by the EUROBATS Agreement. Myotis punicus is also a new species for Pantelleria, a record that increases the known Italian and European distribution of this species, previously limited to Sardinia, Corsica, Malta and possibly Sicily (Bogdanowicz et al., 2015).

Acoustically, the bat species from Pantelleria mostly resemble their continental conspecifics; for example, calls of *R. mehelyi* fall within the variability of those known for Sardinia (Russo et al., 2007) and Tunisia (Puechmaille et al., 2012; Dalhoumi et al., 2016), yet their frequencies are lower than those recorded in Sicily (whose peak frequency is 111.4–113.2 kHz; Mucedda and Pidinchedda, 2016). Similarly, calls of *P. gaisleri* are also comparable to those from African populations of the species (Puechmaille et al., 2012). Calls by *R. hipposideros* from Pantelleria differ from those from Europe and North Africa, reaching the highest call frequency known for the species to date: 123.1 kHz (Lanza, 2012). Such difference may probably be related to the generally smaller size of *R. hipposideros* from Pantelleria, that Felten and Storch (1970) assigned to the *R. h. minimus* subspecies, which typically leads to higher frequencies (Russo et al., 2018), and minimizes

We remark that the bat assemblage found on Pantelleria deserves particular attention for future research efforts. For instance, the mechanisms that allowed colonisation of the island by species with contrasting dispersal abilities (Moussy et al., 2013) from different geographic regions are yet to be clarified. Further efforts should also be made on roost search and protection against disturbance, due to the general rarity of *R. mehelyi*, *M. punicus* and *P.gaisleri* and the small numbers of roosts known from their Italian ranges. The high numbers of tourists and the accessibility of most roosting sites make insular bat populations such as those of Pantelleria particularly sensitive and prone to extinction, besides other well-known threats to insular bats in general, such as climate change, wildfires, and free-ranging cats (Conenna et al., 2017; Ancillotto et al., 2021; Oedin et al., 2021). All such factors call for greater protection efforts to guarantee the long-term conservation of this highly valuable biodiversity heritage. *[%]*

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