

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

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20

21 **Short note**

22

23 **Abstract**

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

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33 **Keywords:** Biogeography; Chiroptera; *Myotis punicus*; Pantelleria; *Plecotus gaisleri*; *Rhinolophus*
34 *mehelyi*; Sicily

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36

37 **Introduction**

38 Areas at the boundaries of different bioregions represent particularly interesting sets for
39 biogeographers, as they usually feature high species richness levels due to their geographical position.
40 This peculiar geographic context leads to unique biological communities that comprise taxa from

41 different regions, as well as endemic ones (Cracraft, 1985). Among Mediterranean islets, Pantelleria
42 stands out for its isolation, being far from other islands and located 70 and 120 km off the African
43 (Tunisia) and Italian (Sicily) coasts, respectively. Due to its position in the Sicilian Channel,
44 Pantelleria and several other islands (e.g., Malta-Gozo) comprise unique faunal assemblages,
45 featuring affinities with both the European and African communities, as well as endemic taxa. For
46 instance, Pantelleria hosts some typically North African bird species as residents (Corso et al. 2012),
47 such as the North African blue tit (*Cyanistes teneriffae*), as well as at least 20 endemic invertebrates
48 (Muscarella & Baragona, 2017). As flying mammals, bats are among the few mammals on offshore
49 islands worldwide (Lawlor, 1986) thanks to their long-range dispersal. Across the Mediterranean,
50 many bat species occur on several major islands such as Crete, Sicily, and Sardinia, including some
51 endemic or sub endemic taxa (Mucedda et al., 2002; Benda et al., 2004). Yet, knowledge of the bat
52 assemblages of smaller islands is mostly anecdotal or completely unavailable, leaving gaps in the
53 biogeographical patterns of several species, as well as in bat conservation policies.

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

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

65

66 **Materials and Methods**

67 *Study area*

68 The island of Pantelleria is located in the middle of the Sicilian Channel, covering an area of 83 km².
69 It was formed ca. 320,000 years ago (Mahood and Hildreth, 1986) due to volcanism and has high
70 hydrothermal activity. Pantelleria mostly features a mountainous or hilly profile and reaches a
71 maximum altitude of 834 m a.s.l.; it has a typically Mediterranean climate. The island is mainly
72 covered by shrubland and Mediterranean woods of oaks and native conifers. The two types of
73 dominant crops are vineyards and olive groves.

74

75 *Bat sampling*

76 Captures were conducted in the summer of 2013 and 2019 with one 6-m long mist net (19 mm mesh
77 and five pockets each) positioned near potential roosts and water sites. The net was positioned 30 min
78 before sunset and removed at dawn; in total, we performed twelve mist-netting sessions using one net
79 per session at four roosts and three water sites. On some occasions, we also used hand-nets to catch
80 bats inside the roost, provided this would not imply disturbance to colonies (n roosts=5).
81 Morphological identification of captured bats was conducted following Dietz & von Helversen
82 (2004). We also recorded echolocation calls by captured bats on release, and rhinolophids were
83 recorded in hand to avoid Doppler-shift compensation and obtain diagnostic calls (Russo et al., 2007).
84 In September 2019, we used automatic D500x bat detectors (Pettersson Elektronik AB, Uppsala,
85 Sweden) placed in different island locations and left active from sunset to dawn, for one night. We
86 recorded bat activity at eight sites, equally distributed in four habitat types: water sites, urban areas,
87 Mediterranean scrubland, and woodland. Sites were at least 1,500 m apart, and recordings were made
88 once per site; recorders were set to record 5s files per minute, with a sampling rate of 500 kHz, also
89 applying the built-in high-pass filter, no pre-triggering, and trigger sensitivity at “high”. Additionally,
90 we opportunistically recorded bat activity with a handheld D1000x bat detector (Pettersson Elektronik
91 AB, Uppsala, Sweden) while walking or driving for 4 nights, for ca. two hours since sunset; the
92 detector was set to record at a 348 kHz sampling rate. Call measurements and species assignment of
93 recorded calls were done using the ver. 4.03 BatSound package (Pettersson Elektronik AB, Uppsala,
94 Sweden) by following Russo & Jones (2002) and using reference recordings.

95

96 *Molecular analyses*

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

103

104 **Results**

105 We captured eleven bats (Table 1): three *Plecotus* bats (two males and one female from two sites),
106 two *Rhinolophus mehelyi* (one male, one female, from one site), one *Myotis* sp., all with mist-nets
107 positioned at the entrance of potential roosts, and five *Rhinolophus hipposideros* (three males and
108 two females from four sites) with hand nets inside the roosts.

109 The two *R. mehelyi* were identified by morphological characters (Figure 1), and their calls showed a
110 peak resting frequency of 105.7-106.8 kHz. *Rhinolophus hipposideros* were found at different

111 localities across the island, mostly inside abandoned traditional buildings found on the island
 112 (“dammusi”), caves and underground tunnels. Echolocation calls of the five captured *R. hipposideros*
 113 showed a peak resting frequency of 117.3 ± 0.7 kHz (n=3; range: 116.3-118.0 kHz) and 122.2 ± 0.9
 114 (n=2; range: 121.3-123.1 kHz) for males and females, respectively.

115 The DNA sequences obtained from *Plecotus* tissue samples were univocally assigned to *P. gaisleri*
 116 (Figure 1), being homologues of Pindet2 *Plecotus t. cf. gaisleri* subsp. n. The *Myotis* we captured was
 117 identified genetically as *Myotis punicus* (Figure 1). Detailed measurements used for morphological
 118 identification are reported in table 1. These values support the genetic identification of the species
 119 according to Dietz & Kiefer 2014.

120 We recorded echolocation calls from free-flying bats belonging to four species at ten locations (eight
 121 fixed, two corresponding to driven or walked transects) across the island, totalling 302 bat passes
 122 (table 2). The species most frequently contacted was *P. kuhlii* (n=228), present at all sites, followed
 123 by *Plecotus* sp. (n=39; from 4 sites), *Hypsugo savii* (n=31, from 4 sites) and *Myotis* sp. (n=4, from 2
 124 sites); for all species, the highest activity levels were recorded at the only artificial freshwater pond
 125 present on the island.

126

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

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

131

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

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

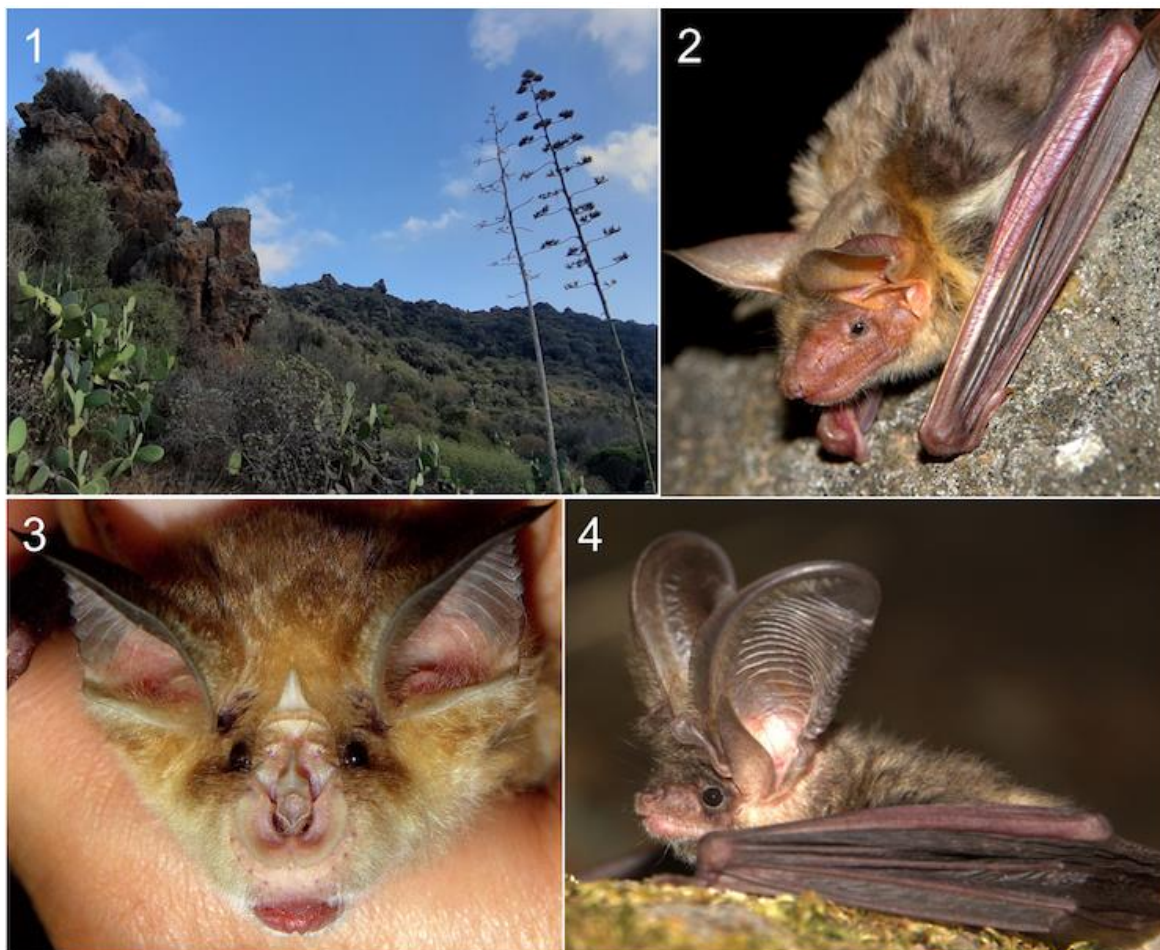
135 ²Molecular evidence from the present work assigns our only record of *Myotis* sp. to *M. punicus*.

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

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

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Discussion

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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.*

150 *mehelyi* and *M. punicus*) add to the currently known range of these species in Italy, being the
151 southernmost Italian observations. Both species are rare in Italy, being recorded almost exclusively
152 in Sardinia and Sicily, and thus our findings highlight the importance of Pantelleria, and the National
153 Park therein, for future management and conservation choices. *Rhinolophus mehelyi* is a new addition
154 to the bat assemblage of Pantelleria, increasing the knowledge of the species' range, which in Italy is
155 restricted to Sardinia and three caves in Sicily, thus deserving particular conservation efforts.
156 Moreover, our study confirms the univocal identity of the long-eared bats from Pantelleria as *P.*
157 *gaisleri*, and reports on the first evidence of reproduction by this species on the island. Despite the
158 relatively close distance between Pantelleria and Africa, migration of *Plecotus* bats between the island
159 and the continent is unlikely, since these bats are non-migratory and do not tend to cross the sea
160 actively; for example, no species seem to cross the short distance (ca. 11 km) between the islands of
161 Sardinia and Corsica (Mucedda & Fichera pers. obs.). The confirmation of the identity of long-eared
162 bats from Pantelleria (Ancillotto et al., 2019; this work) and Malta-Gozo (Batsleer et al., 2019) as *P.*
163 *gaisleri* (Benda et al. 2004) also supports the statement that the species is the only long-eared bat
164 present on these islands, which was debated in the past (Ancillotto et al., 2019). Yet, *P. gaisleri* is
165 still awaiting to be included in the Italian mammal checklist (Loy et al., 2019), and in the list of the
166 bat species protected by the EUROBATs Agreement. *Myotis punicus* is also a new species for
167 Pantelleria, a record that increases the known Italian and European distribution of this species,
168 previously limited to Sardinia, Corsica, Malta and possibly Sicily (Bogdanowicz et al., 2015).
169 Acoustically, the bat species from Pantelleria mostly resemble their continental conspecifics; for
170 example, calls of *R. mehelyi* fall within the variability of those known for Sardinia (Russo et al., 2007)
171 and Tunisia (Puechmaille et al., 2012; Dalhoumi, Aissa & Aulagnier, 2016), yet their frequencies are
172 lower than those recorded in Sicily (whose peak frequency is 111.4-113.2 kHz; Mucedda &
173 Pidinchetta, 2016). Similarly, calls of *P. gaisleri* are also comparable to those from African
174 populations of the species (Puechmaille et al., 2012). Calls by *R. hipposideros* from Pantelleria differ
175 from those from Europe and North Africa, reaching the highest call frequency known for the species
176 to date: 123.1 kHz (Lanza, 2012). Such difference may probably be related to the generally smaller
177 size of *R. hipposideros* from Pantelleria, which Felten & Storch (1969) assigned to the *R. h. minimus*
178 subspecies, which typically leads to higher frequencies (Russo et al. 2018), and minimizes the risk of
179 frequency overlap with sympatric *R. mehelyi* (Russo et al. 2007).
180 We remark that the bat assemblage found on Pantelleria deserves particular attention for future
181 research efforts. For instance, the mechanisms that allowed colonisation of the island by species with
182 contrasting dispersal abilities (Moussy et al., 2013) from different geographic regions are yet to be
183 clarified. Further efforts should also be made on roost search and protection against disturbance, due
184 to the general rarity of *R. mehelyi*, *M. punicus* and *P. gaisleri* and the small numbers of roosts known

185 from their Italian ranges. The high numbers of tourists and the accessibility of most roosting sites
186 make insular bat populations such as those of Pantelleria particularly sensitive and prone to extinction,
187 besides other well-known threats to insular bats in general, such as climate change, wildfires, and
188 free-ranging cats (Conenna et al. 2017; Ancillotto et al. 2021; Oedin et al. 2021). All such factors call
189 for greater protection efforts to guarantee the long-term conservation of this highly valuable
190 biodiversity heritage.

191

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201

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