

First record of *Hypsugo cf darwinii* (Tomes, 1859) in Tuscany, Italy

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Abstract: *Hypsugo darwinii* was originally described in the Canary material supplied by Darwin and attributed later to *Hypsugo savii*, but recent genetic studies have instead highlight edits new systematic position. It is distributed in North Africa, the Canary Islands, Sicily and Sardinia. Research carried out on Montecristo Island (Tuscan Archipelago National Park) in 2015, revealed the presence of this species on this island, the first for Tuscany, thus providing the new northernmost limit of its distribution. Our results also highlight the importance of small isolated islands for the conservation of bat biodiversity, particularly in the Mediterranean basin.

Key words: Cryptic species, 16S, distribution, Montecristo Island.

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Hypsugo cf darwinii (Tomes, 1859) is a bat species originally described for the Canary material and reported for a large portion of North Africa, Canary Islands, Sicily, and Sardinia (Veith et al. 2011). This different lineage was proposed as new species by Mayer et al. 2007, according to mitochondrial DNA sequences, although it is still in need of a modern morphological description. Accordingly, at present time, it is not possible to discriminate between the *taxa* *H. cf darwinii* and *Hypsugo savii* (Bonaparte, 1837) on the only basis of morphology. Genetic markers are necessary for the identification of this species (e.g., 16S or ND1: Veith et al. 2011). Despite the unclear status of its species rank, we followed Mayer et al. 2007, waiting for further studies, e.g. using nuclear markers, that will enlighten the definitive rank of this lineage.

As part of a research on bats of Montecristo Island (Tuscan Archipelago National Park, 43°53'46"N - 11° 5'59"E) (Fig. 1), on 8th June 2015, two individuals were captured with a mist net along a narrow valley with rainwater puddles, at an altitude of about 180 m a.s.l.

Montecristo is a granite, entirely mountainous island of 10.39 sq km located 65 km from the Italian mainland and 60 km from Corsica, with a maximum height of 645 m (Monte della Fortezza).

The female individual, captured at 9.40 p.m., was in an excellent physical condition, with well developed *mammae* and visible nipples (presumably lactating). The following measures were taken: weight 6,2 g; forearm 33,9 mm; length of the fifth finger (D5): 40,7 mm. The male was captured at 9.50 p.m. The following measures were taken: weight 5,3 g; forearm 33 mm; length of the fifth finger (D5): 40,4 mm.

Both individuals had dark skin contrasting with dorsal light brown pelage, and a small reddish brown spot between the ears, the corners of mouth and the shoulders (Fig. 1).

Both individuals were photographed and their ultrasounds recorded when hand-released. Five other recordings were made while individuals were flying. Recordings were made through a Pettersson Elektronik D-240X Ultrasound Detector, connected to an Edirol R-09. The resulting sequences were then analysed (through the software BatSound 3.10) using a sampling frequency of 44.1 kHz and a 512 pt FFT. The comparison with the acoustic measures of the closely related *Hypsugo savii* (Russo & Jones 2002, Papadatou et al. 2008, Barataud 2015) showed that the two *taxa* seem to echolocate using almost identical calls, a factor which may increase the difficulties to define and study *H. cf darwinii* (Table 1). Tissue samples were collected with a sterile biopsy punch of 3 mm in diameter to discriminate correctly the species: a genetic analysis was then performed. Molecular samples of the 2



Fig. 1 – Female of *Hypsugo cf darwinii*, Montecristo Island. The reddish brown spot under the ear is evident.

individuals allowed the taxonomic identification through a comparison with the deposit sequences in GenBank.

We sequenced about 560 bp of the mitochondrial 16S ribosomal gene using the 16SPle1+(5'ACA TCACCTCTAGCATAAAA-3') and 16SPle4–(5'-CCGGTCTGAACTCAGATCACG-3') (Spitzenberger *et al.* 2006). PCR cycling procedure was the following: initial denaturation step: 120 s at 94°C, 37 cycles: denaturation 30 s at 94°C, primer annealing for 90 s at 57,6°C, extension for 180 s at 65°C and final extension for 180s at 65° C.

Sequences were aligned and compared using the software Mega version 7 (Kumar *et al.* 2015), including sequences by Veith *et al.*, 2011. The female belonged to the published haplotype “Hsav-II-7 Sicily”; by contrast, the male belonged to a new haplotype, different for only one base (a T substituted by a C) from the “Sicilian” one. Thus, a transition has occurred. Hereafter it will be referred to as “Hsav-II-8 Montecristo”.

The discovery of this species in Tuscany increases the total number of mammal species of this region, enables us to expand the Italian and overall range of *H. darwinii* with its Northernmost report (Fig.2). Furthermore, these observations highlight the value of small islands for the conservation of biodiversity, particularly within the Mediterranean basin.

Since, we can presume that a breeding colony is present on the island as we can infer that the female was lactating, thus ranking Montecristo as the smallest island where a population of this species occur.

Since the island is roughly 60 km away from the closest land, it is very likely that the population living on the island is

a closed population, with scarce arrivals of new individuals. Further researches will highlight if the population is effectively isolated or if further studies on Minimum Viable Population on bats should be deserved.

Considering that *H. savii* shows a broad range of fur colour (Arlettaz *et al.* 1993), it is possible that the lighter fur and the reddish brown spot on the shoulder is one of the possible colours within the normal range of fur patterns also in *H. cf. darwinii* and that it could be present throughout the distribution of this taxon.

However, this kind of reddish brown spot has never been observed in Sardinia (Mucedda & Fichera *pers. com.*), Malta (Mifsud *pers. com.*), Algeria (Mourad *pers. com.*) and Canary Islands (Trujillo & Barone *pers.com.*). On the other hand, it's not possible to exclude a partially seasonal moult, but at the moment no data are available, due to the difficulty to perform a survey all year round.

It is unlikely that these reddish brown spots are due to the facial glands. In *H. savii*, these glands secrete an orange oil to maintain the skin of *patagium* in good conditions, but orange spots have never been observed in Italy mainland and in other Tuscan islands close to the coast (Dondini & Vergari, *personal observation*). *H. savii* has buccal pads at the mouth corner and it has never been reported to have orange secrets (Dondini *et al.* 2003).

The presence of *H. darwinii* in an island located between Corsica and Tuscany, suggests the hypothesis that this *taxon* might also be present in Corsica island and in Italy mainland. Further surveys combined with molecular identification are required to assess the current occurrence of this species in the Tuscan archipelago, where *Hypsugo savii* has been recorded on the basis of morphologic identification (Vergari & Dondini 1998).

Recently a mitochondrial lineage, *Pipistrellus kuhlii desertii* Thomas 1915, after being arisen to a species level, is now ranked as exactly an ancient divergent lineage of *Pipistrellus kuhlii* (Kuhl, 1817), (Andriollo *et al.* 2015, Benda *et al.* 2015). This may also be the case of *Hypsugo cf darwinii*, but it should be valued that the lineage *P. k. desertii* differs ca. 6% from *P. kuhlii* (Coraman *et al.* 2013, Ibáñez *et al.* 2006), where as *H. cf darwinii* 9.6% from *H. savii* in the ND1-gene (Mayer *et al.* 2007).

Beside taxonomic changes, *Hypsugo cf darwinii* is an Evolutionary Significant Units (ESU) (Veith *et al.* 2011) and its occurrence should be taken into account in modern approach to biodiversity conservation. All sequences have been deposited in GenBank. (*the sequences will be deposited after the acceptance of the manuscript*).

Table 1. Descriptive statistics for *Hypsugo cf darwinii* calls in Montecristo Island (Tuscany, Italy). SF= start frequency; EF= end frequency; Fmax= frequency of maximum energy; D= duration; SD= standard deviation.

	SF (kHz)	EF (kHz)	Fmax (kHz)	D (ms)
Mean	42,6	29,6	33,9	8,8
SD	3,54	1,62	0,69	1,51
min-max	37-48	28-32	33-34,9	6,9-11,1
n	7	7	7	7



Fig. 2 – Montecristo record (red circle) and records of *Hypsugo cf darwinii* in Italy, based on Veith *et al.* 2011 (red dots).

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