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## Newly described and already endangered: a new mammal species endemic to Corsica

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**Abstract:** The *Myotis nattereri* species complex has been the focus of several recent morphological and molecular surveys to assess the species status of various named forms, including three informally referred to as *Myotis* sp. A, *M.* sp. B and *M.* sp. C. The first two forms have now been formally described as distinct biological species, and named *M. crypticus* and *M. zenatius*, respectively, both distinct from the nominotypical *M. nattereri* s. str. and *M. escaleraei*. The latter form, *Myotis* sp. C is known only from Corsica. Here we demonstrate that this form has not only unique mitochondrial haplotypes but also several nuclear alleles that are divergent and not found anywhere else, which emphasizes its long independent evolution. We therefore confirm its specific status and describe it as a new species. Its ecology and rupicolous roosting habits resemble those of the Iberian *M. escaleraei*, but it is otherwise morphologically most similar to *M. crypticus*. This new species is endemic to Corsica and is apparently very rare and essentially localised to mountain forests. Owing to its restricted distribution, its small population size, and limited population connectivity, it seems highly vulnerable to climate change and thus should be classified as endangered.

**Résumé:** *Myotis nattereri* représente un complexe d'espèces qui a mobilisé l'attention récente de morphologistes et de généticiens pour définir le statut spécifique de diverses formes, y compris celles informellement connues sous l'appellation de *Myotis* sp. A, *M.* sp. B et *M.* sp. C. Les deux premières formes ont depuis été reconnues comme espèces à part entière et nommées respectivement *M. crypticus* et *M. zenatius*; elles diffèrent des espèces nominales *M. nattereri* au sens strict et *M. escaleraei*. La dernière forme, *Myotis* sp. C, n'est connue que de Corse. Nous démontrons ici qu'elle possède non seulement des haplotypes mitochondriaux uniques, mais aussi plusieurs allèles nucléaires divergents que l'on ne retrouve nulle part ailleurs et qui démontrent une longue évolution indépendante de ce taxon. Ceci confirme son statut spécifique et nous la décrivons par conséquent comme espèce nouvelle. Son mode de vie et ses affinités rupicoles la rapprochent de l'espèce ibérique *M. escaleraei*, bien que morphologiquement elle soit plus semblable à *M. crypticus*. Cette nouvelle espèce est endémique de Corse et y est apparemment très rare et presque exclusivement localisée aux régions montagneuses boisées. En raison de sa distribution restreinte, de la petite taille de ses populations, et de la connectivité restreinte de ses populations, elle semble très vulnérable au changement climatique et devrait être classée comme espèce en danger.

**Keywords:** *Myotis* - taxonomy - cryptic species - DNA - nuclear loci - Vespertilionidae.

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## INTRODUCTION

Despite over two and a half centuries of taxonomic scrutiny, systematists have only catalogued a small fraction of the living species existing on earth (Mora *et al.*, 2011). As most of this biodiversity was essentially based on morphological characteristics, good biological species differing only minimally by their phenotype (i.e. cryptic species) could have been particularly overlooked (Bickford *et al.*, 2007). During the last decades, this limitation has been largely overcome with the use of molecular data as they carry information that were otherwise not accessible via morphological criteria, that is, the level of reproductive isolation between two populations. This information is critical to delineate species under several species concepts (e.g. De Queiroz, 2007), including the classically used biological species concept. This biological species concept defines species as “groups of interbreeding natural populations that are reproductively isolated from other such groups. The isolating mechanism by which reproductive isolation is effected are properties of individuals” (Mayr, 1996). Given the information it carries, genetic data has been successfully used to split emblematic, common or widespread species into two or more species that are reproductively isolated (e.g. the common pipistrelle split into *Pipistrellus pipistrellus* and *P. pygmaeus*, Barratt *et al.*, 1997; the African elephant split into *Loxodonta africana* and *L. cyclotis*, Roca *et al.*, 2001; the bent-winged bats [*Miniopterus schreibersii*], split into tens of species across the Old World, Demos *et al.*, 2020). When populations of two species are in geographic proximity (e.g. in sympatry or parapatry), appropriate molecular markers can indeed determine how much genetic material they exchange, and hence test hypothetical species’ status. The situation, however, is more complicated when the two entities are found in allopatry, a classic example being continental versus island populations (or island versus other island populations). Indeed, geographic barriers are not properties of individuals and hence are not regarded as an isolating mechanism *per se* and taxonomic decisions about the specific status of such allopatric populations is more contentious (Mayr & Ashlock, 1991; Mayr, 1996).

Following the use of molecular data, vertebrate taxonomy and classification has vastly changed in recent decades (e.g. Springer *et al.*, 2004) especially in the two most species-rich orders, Rodentia and Chiroptera. The number of newly described species is not homogeneously distributed across the globe and surprisingly, some well-studied regions such as Europe have had several species described recently. Furthermore, while islands only represent 5% of the world land area, they host 28% of newly-described species (Reeder *et al.*, 2007), suggesting that islands 1) have been overlooked until now, 2) have more species diversity per surface area, or 3) might have been subject to over-splitting. These explanations are not mutually exclusive.

For European bats, the description of new species has been clearly boosted by the use of mitochondrial DNA markers to detect divergent lineages that could represent separate species. If those diverging lineages occurred in sympatry or parapatry and populations showed some diagnostic morphological differences, they were often elevated to species level (for those already named as subspecies; e.g. *Myotis escaleraei*, Ibañez *et al.*, 2006) or described as novel species (e.g. *Myotis alcathoe*, Von Helversen *et al.*, 2001; *Plecotus sardus*, Mucedda *et al.*, 2002). For populations that were geographically separated by large expanses of sea, such as the Corsican and North African populations of large mouse-eared bats, biparentally inherited, nuclear loci were needed to conclusively identify *Myotis punicus* as a separate species (Castella *et al.*, 2000). The *Myotis nattereri* species complex has been the group with the most changes over the last decades. *Myotis nattereri* was originally described from Hanau in central Germany (Kuhl, 1817) and was traditionally considered as a single polytypic species with several subspecies differing chiefly by size (Corbet, 1978). According to this view, its distribution included North-West Africa and Western Europe, extending east to Turkey, Iraq and Iran and further into Northeast Asia, East Siberia, Korea and Japan. Based on morphological traits, Horáček & Hanák (1984) considered the representatives from the Eastern Palaearctic as a separate species (*M. bombinus* Thomas, 1905), while in the Western Palaearctic, they recognized a further species localised to Armenia and northern Iran (*M. schaubi* Kormos, 1934). A series of DNA surveys based on single mitochondrial markers showed that besides the nominal species (*M. nattereri* s. str.), Western Europe and North Africa was inhabited by at least four other main lineages, one present in the south-western portion of continental Europe (lineage A; now *Myotis crypticus* Ruedi *et al.*, 2019), one endemic to the Maghreb (lineage B; now *Myotis zenatius* Ibañez *et al.*, 2019), one endemic to Corsica (lineage C) and a last one restricted to the Iberian Peninsula and the Eastern Pyrenees (*M. escaleraei* Cabrera, 1904) (Ibañez *et al.*, 2006; Puechmaille *et al.*, 2012; Juste *et al.*, 2018). Bats from these lineages are morphologically extremely similar but were separated in two morpho-groups owing to distinct types of wing insertion to the hind feet (Cabrera, 1904; Puechmaille *et al.*, 2012) and differences in the rows of stiff hairs running along the trailing edge of the uropatagium (Agirre-Mendi & Ibañez, 2012; Puechmaille *et al.*, 2012; Juste *et al.*, 2018). Further studies based on several nuclear genes also showed that the main continental lineages were distinct (Salicini *et al.*, 2011), providing evidence for the existence of significant barriers to gene flow. The fact that several of these continental lineages lived in sympatry provided strong evidence for intrinsic isolation mechanisms, leading to their formal recognition as distinct biological species (Juste *et al.*, 2018). East of the Bosphorus, three taxa of the *Myotis nattereri* species complex have been

further recognized as largely allopatric species (Çoraman *et al.*, 2019; Uvizl & Benda, 2021): *M. hovei* Harrison, 1964 from the Levant to northern Iraq, *M. tschuliensis* Kuzynkin, 1935 from Crimea, the Caucasus and east to Turkmenistan (Benda *et al.*, 2012), and *M. schaubi* an endemic species from Armenia and north-western Iran (Benda *et al.*, 2012). Despite these recent efforts to understand its systematics, it is still unclear whether the new taxonomic arrangement captures the full extent of genetic diversity in the *M. nattereri* species complex (Razgour *et al.*, in press).

One of the Western European lineage (lineage C), however, remained taxonomically unevaluated both because it was only characterized so far with maternally inherited mitochondrial markers (Puechmaile *et al.*, 2012), hence giving a partial view of its evolution (Ballard & Whitlock, 2004), and because no vouchered specimen could be examined.

Based on new molecular, bi-parentally inherited markers sequenced for *M. nattereri* from Corsica and the continent, we report here the taxonomic evaluation of representatives of the Corsican lineage C and compare them with all the other known species in the *Myotis nattereri* species complex. We integrated these genetic data with morphological evidence to evaluate the taxonomic status of bats from this enigmatic insular lineage.

## MATERIAL AND METHODS

### Molecular methods

#### Sample collection & DNA extraction

Wing biopsy punches preserved in silica-gel or 100% ethanol (Puechmaile *et al.*, 2011) were collected from 83 bats during field surveys with appropriate permits (Arrêté préfectoral no. 2018-s-41, 2018-s-42, 2018-s-42-m1, 2004-06, 2009-11, 2013-04, 2013168002, 2013168003; RIOEW Ruse No. 205/29.05.2009; No. 10752, 1492 & 1698 from the Direction générale des forêts du Ministère de l'Agriculture de la République Tunisienne). Wing, liver or muscle tissue stored in 100% alcohol were also obtained from six specimens preserved in museum collections. In total, we analysed samples from one *Pipistrellus pipistrellus*, one *Myotis alcathoe*, one *M. blythii*, one *M. chinensis*, one *M. punicus*, 11 *M. mystacinus*, one *M. nattereri*, 27 *M. crypticus*, and 45 *M. sp. C* (see Supplementary Table S1). Whole genomic DNA was extracted using the KingFisher cell and tissue kit run on the Flex system (Thermo Fisher Scientific). Extracted DNA concentration was quantified on a NanoDrop 2000c Spectrophotometer (Thermo Fisher Scientific).

#### PCR & sequencing of nuclear introns

To gain information on the relationship between currently described species of the Western Palaearctic

*M. nattereri* species complex and individuals of *M. sp. C* from Corsica, we selected the following five informative mammalian-specific intronic markers that offered independent representation of the nuclear genome: SLC38A7, ABHD11, ACOX2, COPS7A, and ROGDI (Igea *et al.*, 2010; Salicini *et al.*, 2011). These loci were chosen based on their known variability in Vespertilionidae and because comparative sequence data exist for many samples in this species complex (Salicini *et al.*, 2011). Primer details are given in Supplementary Table S2, as are alignment lengths and number of variable and parsimony informative sites for each locus, as calculated in MEGA v. 7.0 (Kumar *et al.*, 2016). PCRs were conducted in 10 µl reaction volumes containing 1 µl of DNA (10 ng/µl), 1x Platinum Taq Green Hot Start polymerase (Invitrogen, USA), 1x Green PCR Buffer without Mg, 1.5 mM MgCl<sub>2</sub>, 0.4 µM of each primer and 0.2 mM dNTPs. PCRs were carried out on an AB 2720 thermocycler (Applied Biosystems, CA, USA) using a touchdown procedure with annealing temperatures decreasing from 65 to 55 °C: 95 °C for 2 min; 2 cycles of 95 °C for 15 s, 65 °C for 30 s, 72 °C for 1 min; followed by 2 cycles each at annealing temperature in 2 °C decrements from 65 °C (i.e. 63 °C–57 °C); 30 cycles of 95 °C for 15 s, 55 °C for 30 s, 72 °C for 1 min; 72 °C for 5 min. PCR products were purified using Exonuclease I and FastAP Thermosensitive Alkaline Phosphatase (Thermo Fisher Scientific) following the manufacturer's protocol. Amplicons were Sanger sequenced by Macrogen Inc. (Seoul, South Korea) using the forward PCR primer for each locus. Sequences were edited and aligned using CODONCODE ALIGNER v. 4.2.7 (CodonCode Corporation) and MUSCLE in MEGA v. 7.0.

#### Recovery of published nuclear introns

We downloaded from GenBank all homologous nuclear intron data published to date for the *M. nattereri* species complex, including those from Salicini *et al.* (2011), later corrected in Salicini *et al.* (2018), and from Çoraman *et al.* (2019). To avoid problems associated with missing data, we restricted the data to individuals with a minimum of four loci in common with our study. This resulted in data from 19 *M. crypticus*, 15 *M. escaleraei*, 5 *M. hovei*, 35 *M. nattereri*, 6 *M. schaubi*, 5 *M. tschuliensis*, and 4 *M. zenatius*. Using BLAST, we also extracted these introns from the full genomes of *Myotis myotis* (Jebb *et al.*, 2020) and *M. brandtii* (Seim *et al.*, 2013). Further information about these data and specimens are detailed in Supplementary Table S1.

#### Phylogenetic reconstructions

The most appropriate model of substitution for each nuclear locus (see Supplementary Table S2) was selected based on the Bayesian Information Criterion (BIC) in jMODELTEST v. 2.1.10 (Darriba *et al.*, 2012). Loci were treated as unlinked partitions in a Bayesian analysis in BEAST v. 1.10.4 (Suchard *et al.*, 2018)



using two independent MCMC chains of 50 million generations each, sampled every 1000 generations. Priors were: Yule speciation, lognormal relaxed clock and a UPGMA starting tree with IUPAC ambiguity codes used as informative characters. Convergence was assessed in TRACER v. 1.7.2 (Rambaut *et al.*, 2013). The runs were combined after removal of 10% burn-in with LOGCOMBINER and a maximum clade credibility tree was generated with TREEANNOTATOR and visualised with FIGTREE v. 1.4.4 (<http://tree.bio.ed.ac.uk/software>). Individual gene trees were generated in the same way, though with shorter chains of 10 million generations.

### PCR & genotyping of nuclear microsatellite loci

To get a better insight into the population structure of *M. sp. C* in Corsica, we amplified nine microsatellites for 45 individuals sampled from five locations. Geographically, *M. crypticus* is the closest taxon to *M. sp. C*. Therefore, to assess the uniqueness of *M. sp. C*, a subset of 17 individuals belonging to *M. crypticus* were genotyped with the same microsatellites for comparison. Primers used to amplify the nine microsatellite loci are described in the Supplementary Table S3. Reaction volumes were 9  $\mu$ l for each multiplex, using 1x Type-It Master Mix (Qiagen, Hilden, Germany), 1  $\mu$ l DNA (10 ng/ $\mu$ l), and primer concentrations as indicated in Supplementary Table S3. Amplification conditions were as follows: 95  $^{\circ}$ C for 5 min; 30 cycles of 95  $^{\circ}$ C for 30 s, 60  $^{\circ}$ C for 90 s, 72  $^{\circ}$ C for 30 s; 60  $^{\circ}$ C for 30 min. PCR products were run on an ABI Prism 3130xl Genetic Analyser (Applied Biosystems, CA, USA). Amplified regions were sized with an internal lane standard (LIZ 600) and the software GENEMAPPER v. 5.0 (Applied Biosystems, CA, USA).

### Microsatellite analysis

The 62 samples included in the study originated from southern France ( $n=17$ ; *M. crypticus*), and Corsica ( $n=45$ ; *M. sp. C*). As the samples of *M. crypticus* were geographically dispersed across southern France, we considered all individuals as a single population. For *M. sp. C*, two locations were represented by over 10 individuals (Ghisoni [ $n=27$ ] and Zonza [ $n=12$ ], see Fig. 1) while three others (Asco, Albertacce, Evisa; max 16 km apart) had few individuals only and hence were grouped as a single population named AAE ( $n=6$ ). Deviations from Hardy–Weinberg and linkage equilibrium were tested using the genepop R package v. 1.2.2 (Rousset, 2008). Observed heterozygosity and within population gene diversity were calculated in the hierfstat R package v0.5.11 (Goudet, 2005).  $F_{st}$  and significance were tested in the genepop package. Comparisons were made between *M. crypticus* and *M. sp. C*, and between the three populations of *M. sp. C*. Tests were carried out in R v. 4.2.1 patched (R Development Core Team, 2022). Population structure was studied using Principal Component Analysis (PCA; Jombart, 2008) implemented

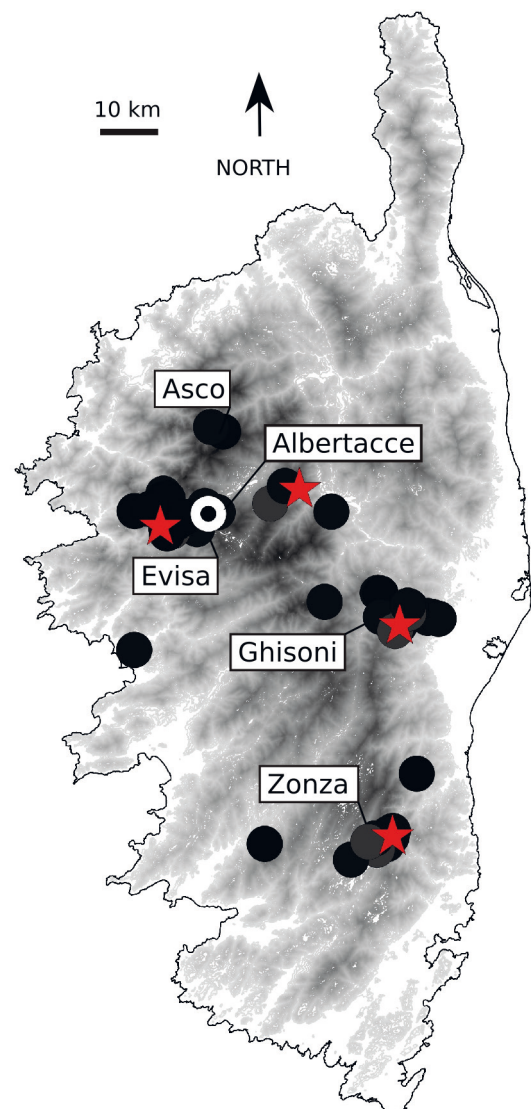


Fig. 1. Topographic map of Corsica (France) showing the current records of *M. nustrale* sp. nov. on this Mediterranean island (Courtois *et al.*, 2011; Groupe Chiroptères Corse, 2019). The black dots represent capture sites, while the red stars indicate the presence of lactating females, including those living in the only known nursery roost of Ghisoni. A double circle indicates the location where the holotype of *M. nustrale* sp. nov. was found. Shades of grey represent areas above 100 m a.s.l.

in the adegenet R package v. 2.1.10 (Jombart, 2008). Given the very clear results of this analysis (see Results), we used a discriminant analysis of principal components (DAPC) to assess the extent to which the genetic data from the microsatellites could be used to assign individuals to their population of origin (Corsican versus continental populations). We therefore constructed a DAPC by discriminating between two groups (Corsican versus continental). We then tested the accuracy of this DAPC using the ‘leave-one-out’ procedure, which

consists of constructing a DAPC on a defined number of individuals (here 61) and testing it on the remaining individual. This procedure was repeated as many times as there were individuals in the dataset ( $n=62$  times).

### Morphological data

To characterise the morphological attributes of the Corsican *Myotis*, we based our comparisons on a range of specimens from continental Europe which were previously used in a revision of the *M. nattereri* species complex (Juste *et al.*, 2018). All these comparative specimens are deposited in the collections of the Muséum d'histoire naturelle de Genève, Switzerland (MHNG), the Hungarian Natural History Museum of Budapest, Hungary (HNHM) and the Estación Biológica de Doñana Seville, Spain (EBD) and represent all four species currently known from this region. Details about their origins can be found in Juste *et al.* (2018). In addition, we obtained a single specimen from Corsica that was found dead in Albertacce, Haute-Corse. This specimen is deposited in the collection of the Muséum National d'Histoire Naturelle in Paris (MNHN) under voucher number MNHN-ZM-2023-12.

Specimens were characterized with the following external mensural characters: body weight (W, expressed in grams); forearm length (FA); head and body length (HB); tail length (TL); hindfoot length, including claws (HF); tibia length (TIB); ear length (EAR) and tragus length (TRA). These characteristics were measured with a digital calliper to the nearest 0.1 mm. In addition, we also report some important qualitative external characters, including the type of wing (plagiopatagium) insertion to the feet (Cabrera, 1904; Puechmaile *et al.*, 2012), the shape of tragus and ears (Çoraman *et al.*, 2019), the pilosity along the trailing edge of the uropatagium (Czech *et al.*, 2008; Juste *et al.*, 2018), and the colouration of the lower lip.

The following 16 craniodental characters were also measured with a digital calliper (to the nearest 0.01 mm) to characterise skull dimensions: greatest length of skull, excluding incisors (GTL); condylo-basal length (CBL); condylo-canine length (CCL); maxillary toothrow length (CM3); width across the upper molars (M3M3W); width across the upper canines (CCW); zygomatic breadth (ZB); braincase width (BCW); breadth of skull measured across mastoids (MAB); least postorbital constriction (POC); rostral length taken from the rostral margin of the orbits to the anterior tip of the skull, without incisors (ROL); upper molars length (M1M3); mandible length, without incisors (ml); mandibular toothrow length (cm3L), lower molars length (m1m3L); and least height of the coronoid process (coh). More precise definitions of these measurements are given in Ruedi *et al.* (2012).

Because only a single female specimen (MNHN-ZM-2023-12) was available from Corsica for detailed morphological assessment, we did not perform any morphological analysis but only used these data for comparative purposes. We thus simply projected

the 16 craniodental characters onto the discriminant morphospace of the Western European and North African species elaborated in a previous study (Juste *et al.*, 2018).

### Distribution

Based on previous studies conducted in the field in Corsica (Courtois *et al.*, 2011; Groupe Chiroptères Corse, 2019), we calculated the extent of occurrence and area of occupancy of *M. sp. C* with the 'EOO.computing' and 'AOO.computing' functions, respectively, via the ConR package v. 1.3.0 (Dauby *et al.*, 2017) in R.

## RESULTS

### Phylogenetic analyses

The five sequenced nuclear introns (SLC38A7, ABHD11, ACOX2, COPS7A and ROGDI) resulted in a total alignment of 2,131 bp. The proportion of parsimony informative sites varied little among loci (0.05-0.11, Table 2). Individual gene trees are available in the supplementary information (Figs S1-S5). The Bayesian tree resulting from the concatenation of the five nuclear introns ( $n=125$  individuals, Fig. 2) recovered most species as monophyletic with high support while support for more basal nodes was often low.

A basal divergence of the clade composed of *M. alcaethoe* and *M. mystacinus* distinguishes it from all other *Myotis* species included in the study. Within the second large clade, *M. brandtii* occurs in a basal position followed by the large-bodied *Myotis* (*M. chinensis*, *M. blythii*, *M. punicus* and *M. myotis*). All samples of Corsican *Myotis* were monophyletic and formed a strongly supported clade (BPP 1) that is basal to all remaining taxa in the *M. nattereri* species complex. This basal position, however, was only moderately supported (BPP 0.86). All other species were also monophyletic and very distinct from *M. sp. C*. The single exception to species monophyly was caused by two *M. crypticus* individuals (MNA0094\_IT\_ORIS, ITAO\_MspA) appearing more closely related to members of the *M. nattereri s. str.* clade than to other *M. crypticus*.

### Microsatellites

No significant deviations from linkage equilibrium were detected (data not shown) among the specified individuals or populations. No significant deviations from Hardy-Weinberg were detected for any loci in any population of *M. sp. C* (Table 1), while deviations were significant when considering the single population of *M. crypticus* from southern France (exact test,  $p<0.01$ ). This deviation was not detected at single loci but only when information from all loci were combined together (Fisher's method,  $p<0.01$ ), suggesting that the observed deviations in *M. crypticus* are an artefact of our grouping of individuals from non-panmictic populations. We therefore kept all

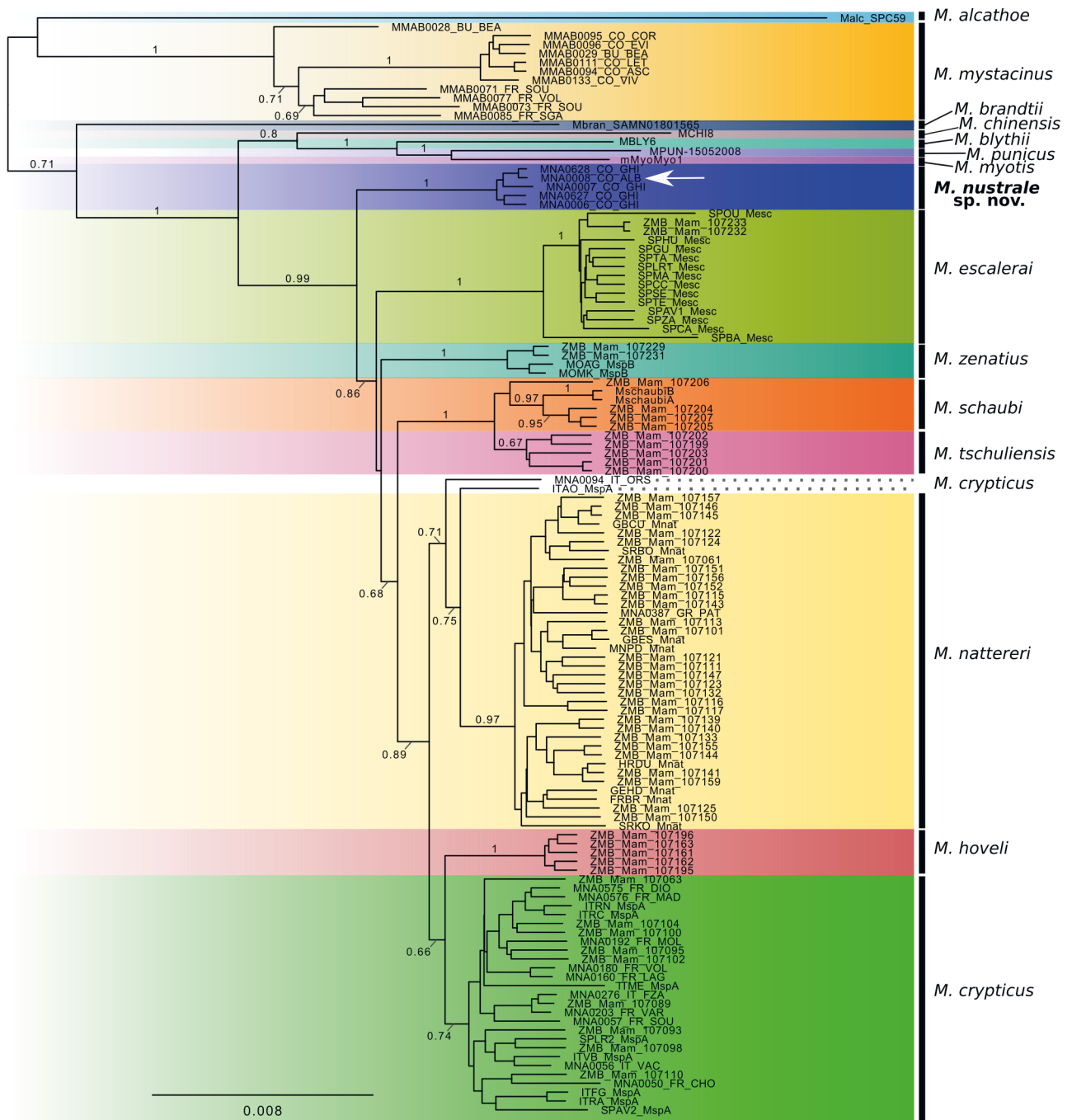


Fig. 2. Bayesian topology based on five partitioned nuclear introns (SLC38A7, ABHD11, ACOX2, COPS7A and ROGDI). The outgroup (*Pipistrellus pipistrellus*) is not shown. Bayesian posterior probabilities (BPP) < 60 are not shown, neither are within species BPP values. The holotype of *Myotis nustrale* sp. nov. (sample number MNA008\_CO\_ALB) is indicated by a white arrow.

markers and populations for subsequent analyses. The PCA showed a clear separation between *M. crypticus* and *M. sp. C* individuals. Interestingly, the first axis which explained nearly a quarter of the total variance (23.9%) was already sufficient to separate both species (Fig. 3). The *F<sub>st</sub>* index confirmed this separation with highly significant values of 0.25, 0.25 and 0.28 for AAE, Zonza and Ghisoni, respectively, when compared to continental

*M. crypticus* (exact G test, *p*<0.001). The DAPC assigned each individual to their original population (Corsican versus continental) with a probability of one. When we restricted the analyses to the different populations within Corsica, a clear differentiation was visible on the PCA between the two most densely sampled locations and the composite AAE population. Accordingly, the AAE population had the highest *F<sub>st</sub>* values (0.17 and 0.14)

Table 1. Diversity measures (Ho: observed heterozygosity; Hs: within population gene diversity) for three populations of *M. sp. C* (Ghisoni [n=27], Zonza [n=12], and AEE [n=6]) and *M. crypticus* (Mcry; n=17) genotyped for nine microsatellite loci. After correction for multiple testing, no significant deviation from Hardy-Weinberg equilibrium were detected for any loci in any population.

|          | Ho   |         |       |      | Hs   |         |       |      |
|----------|------|---------|-------|------|------|---------|-------|------|
|          | Mcry | Ghisoni | Zonza | AAE  | Mcry | Ghisoni | Zonza | AAE  |
| D15      | 0.92 | 0.56    | 0.42  | 0.67 | 0.82 | 0.56    | 0.36  | 0.67 |
| EF15     | 0.42 | 0       | 0.08  | 0    | 0.51 | 0       | 0.08  | 0    |
| G30-Mluc | 0.92 | 0.63    | 0.83  | 0.83 | 0.83 | 0.72    | 0.65  | 0.82 |
| H23      | 0.69 | 0.37    | 0.75  | 0.67 | 0.7  | 0.45    | 0.63  | 0.62 |
| H29      | 0.85 | 0.48    | 0.75  | 0    | 0.75 | 0.51    | 0.53  | 0    |
| Mnatt8   | 0.73 | 0.74    | 0.58  | 0.83 | 0.88 | 0.74    | 0.66  | 0.88 |
| B22      | 0.77 | 0.59    | 0.58  | 0.67 | 0.97 | 0.6     | 0.53  | 0.47 |
| FV5AP    | 0.6  | 0.56    | 0.42  | 0    | 0.5  | 0.48    | 0.49  | 0    |
| Paur6    | 0.48 | 0.44    | 0.75  | 0.6  | 0.72 | 0.55    | 0.71  | 0.65 |

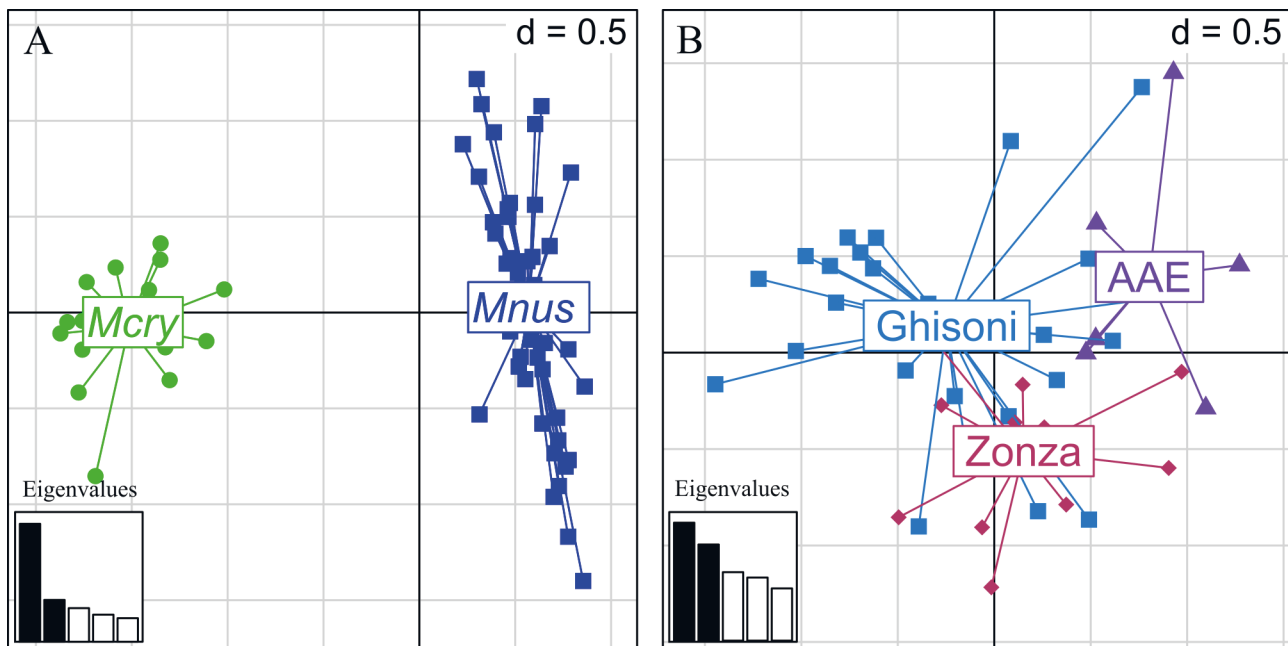


Fig. 3. PCA showing (A) the clear separation between *M. crypticus* (Mcry; green circles) and *M. nustrale* sp. nov. individuals (Mnus; blue squares), and (B), the differentiation between populations within *M. nustrale* sp. nov. Horizontal and vertical axis explain 23.9 and 8.5% of the variance, respectively, in (A) and 17.1 and 13.9% of the variance in (B).

when compared to Zonza and Ghisoni respectively, while the latter two had a pairwise  $F_{st}$  of 0.06. All pairwise  $F_{st}$  comparisons were significant (exact G test,  $p < 0.001$ ).

### Morphometric differentiation

According to Courtois *et al.* (2011), the Corsican *Myotis* has a weight (W 5.5-9.5 g) and a forearm length (FA: 38.4-41.3 mm; n = 22 individuals) that are very similar to those of continental representatives of the *M. nattereri*

species complex (Table 2). The single individual from Corsica measured for the ear length had a slightly larger value for this character, but this difference should be confirmed with more specimens. Its skull measurements are also very similar to those of *M. crypticus*. On a projection of the first two dimensions of the morphospace discriminating skulls of *M. escaleraei*, *M. zenatius*, *M. nattereri* s. str. and *M. crypticus* (Juste *et al.*, 2018), the Corsican sample (Fig. 4) falls close to the centroid



Table 2. External measurements (mean  $\pm$  SD, minimum and maximum values) recorded in the four compared species within the *M. nattereri* species complex. See text for definitions of the variables and acronyms. Besides ears which appear to be slightly longer in *Myotis nustrale* sp. nov., none of the external characters differ from those measured in the other species.

| External character | <i>Myotis nustrale</i> sp. nov.<br>holotype | <i>M. crypticus</i><br>(n = 21) | <i>M. nattereri</i> s. str.<br>(n = 14) | <i>M. escaleraei</i><br>(n = 12) |
|--------------------|---|---------------------------------|---|----------------------------------|
| W                  | --  | 6.5                             | 8.0                                     | 6.6 $\pm$ 1.4<br>(4.5 – 6.3)     |
| FA                 | 39.5  | 39.3 $\pm$ 1.3<br>(36.3 – 42.0) | 39.6 $\pm$ 1.1<br>(38.5 – 41.2)         | 39.9 $\pm$ 1.3<br>(37.1 – 42.0)  |
| HB                 | 42  | 45.2 $\pm$ 2.7<br>(40 – 50)     | 45.5 $\pm$ 2.1<br>(43 – 48)             | 45.0 $\pm$ 3.0<br>(41 – 50)      |
| TL                 | 40  | 39.1 $\pm$ 4.1<br>(32 – 46)     | 34.9 $\pm$ 2.7<br>(32 – 36)             | 39.1 $\pm$ 4.0<br>(42 – 50)      |
| HF                 | 7.8   | 8.2 $\pm$ 0.7<br>(6.6 – 9.1)    | 8.3 $\pm$ 0.8<br>(7.1 – 9.3)            | 8.3 $\pm$ 1.0<br>(6.7 – 10.0)    |
| TIB                | 17.0  | 16.9 $\pm$ 0.6<br>(15.9 – 17.6) | 17.1 $\pm$ 0.4<br>(16.6 – 17.8)         | 17.1 $\pm$ 1.0<br>(15.9 – 19.0)  |
| EAR                | 18.5  | 15.5 $\pm$ 1.0<br>(14.1 – 17.1) | 16.2 $\pm$ 0.7<br>(15.4 – 17.5)         | 16.8 $\pm$ 1.3<br>(15.1 – 18.0)  |
| TRA                | 10.9  | 9.6 $\pm$ 0.6<br>(8.2 – 10.6)   | 10.1 $\pm$ 0.6<br>(9.3 – 10.8)          | 9.8 $\pm$ 1.0<br>(8.9 – 10.8)    |

of the latter species, which suggests very close overall phenetic resemblance between those two taxa (results not shown). A single reliable external character distinguishes Corsican *Myotis* from related continental taxa. It is the dark, pigmented spot on the lower lip which persists beyond adulthood in all examined Corsican individuals (see Figs 5 and 6). We never noticed such a blackish spot in adult individuals from other species in the *Myotis nattereri* species complex. At most did we observe a very narrow black line running along the edge of the lower lip in some adult *M. crypticus* (Fig. 6), but never forming a distinctive spot.

## SYSTEMATICS

To our knowledge, no taxonomic name has been assigned previously to Corsican representatives of the *M. nattereri* species complex. Because the taxon informally referred to as *M. sp. C* is genetically clearly distinct from other lineages found elsewhere, we describe it here as a species new to science.

### *Myotis nustrale* sp. nov. Ruedi, Beuneux & Puechmaille

Figs 4-6

ZooBank Life Science Identifier (LSID): urn:lsid:zoobank.org:pub:6804EDB9-2337-4253-9381-D1DDBFD239FE

**Holotype:** One adult female (MNHN-ZM-2023-12,

with field number SPC.37 and sample number MNA008\_CO\_ALB) found dead by Julien Barataud on the 25th of July 2006. The whole carcass has been preserved in alcohol but is currently in a poor state, with large portions of the dorsal and ventral fur missing and the patagium of the left wing torn apart. Epiphyses are fully fused. The skull has been removed and preserved separately; it is intact, except for the right zygomatic arch that is broken (Fig. 4); teeth are only slightly worn. External measurements (in mm; Table 2) were taken on the fluid-preserved carcass: FA 39.5, HB 42, TL 40; HF 7.8, TIB 17.0, EAR 18.5, TRA 10.9. Skull measurements (in mm; Table 3) are: GTL 15.62; CBL 14.49; CCL 13.47; CM3 5.77; M3M3W 6.26; CCW 3.92; ZB 9.64; BCW 7.86; MAB 7.94; POC 3.93; ROL 4.79; M1M3 3.50; ml 11.14; cm3L 6.04; m1m3L 3.81; coh 3.29. Tissue samples from this specimen are stored in alcohol at  $-20^{\circ}\text{C}$ . DNA sequences from this specimen are deposited in the European Nucleotide Archive and include regions of the nuclear introns SLC38A7 (OX958065), ABHD11 (OX958096), ACOX2 (OX958129) and ROGDI (OX958185).

**Type locality:** Albertacce, Haute-Corse, France ( $42^{\circ}16'42''\text{N}$ ,  $8^{\circ}54'43''\text{E}$ ), ca. 1100 m a.s.l.

**Paratypes:** No paratypes are designated.

**Distribution:** This species is endemic to the island of Corsica (France), in the Mediterranean Sea (Fig. 1).

**Diagnosis:** Medium sized *Myotis* (W 5.5-9.5 g; FA 38.4-41.3 mm) with relatively long, unnotched ears;



Fig. 4. Lateral and occlusal views of the skull and mandible of the holotype female *Myotis nustrale* sp. nov. (MNHN-ZM-2023-12). The scale is 5 mm.

tragus straight, reaching beyond half the length of the conch (Fig. 5). The long, unkeeled and S-shaped calcar running along the proximal half of the uropatagium, as well as the stiff hairs visible on the trailing edge of the uropatagium are typical features of all taxa in the *Myotis nattereri* species complex. Stiff hairs of the uropatagium occur in two rows, with only few hairs being reflected backwards (Fig. 5).

Wing membrane (plagiopatagium) attaching to the base of the toe, as in *M. nattereri* s. str. or *M. crypticus* (Fig. 6), but unlike in *M. escaleraei* or *M. zenatius* which attaches to the base of metatarsus (Puechmaillé *et al.*, 2012). Pelage greyish-brown above and whitish below, with a sharp demarcation line running along the sides of the body. Feet relatively small, half or less the length of tibia. Wing and tail membranes essentially naked and ochraceous-brown. Face hairy, except around eyes. Muzzle relatively flat and pointed. A prominent dark-pigmented chin spot is visible on the lower lip in adult individuals (Figs 5 and 6) which distinguish them from all other European *Myotis* in this group.

Skull and dentition (Fig. 4) like that of other members of the *M. nattereri* species complex (GLS 15.62 mm; CM3 5.77 mm; ml 11.14 mm). Neurocranium globose without visible crests. The frontal part raises sharply above the orbits and becomes relatively flat near apex. Rostrum nearly flat. Teeth strong, uncrowded, with long canines, third premolars and molars. All premolars in-line with the tooth row; the second premolar (both upper and lower

jaw) much smaller than the first (Fig. 4). Lower molars myotodont.

As the only examined specimen is a female, the shape of the baculum is unknown.

**Etymology:** The specific epithet *nustrale* is a pronoun in apposition meaning “ours - le notre” in the Corsican language.

**Description:** The new species of *Myotis* from Corsica shares external morphological characters with all other members of the *Myotis nattereri* species complex, including a long straight tragus, unnotched ears (Fig. 5), a long, S-curved, unkeeled calcar and the presence of stiff hairs along the trailing edge of the uropatagium. However, its wing insertion at the base of the toe (Fig. 6) and double row of stiff hairs bordering the uropatagium on the ventral side classify the Corsican species into the morpho-group comprising *M. nattereri* s. str. and *M. crypticus*. Morphological comparisons will therefore concentrate on the distinction of *M. nustrale* from *M. nattereri* s. str. and *M. crypticus*, all three being distributed exclusively in Europe and western Turkey (Çoraman *et al.*, 2019; Uvizl & Benda, 2021). Except for the genetically very distinct but similar-sized *M. hovei* from the Levant, Asian members of this species complex are indeed larger in most respects (Uvizl & Benda, 2021). Externally, *M. nustrale* is very similar to *M. nattereri* s. str. and *M. crypticus* and probably cannot be reliably distinguished from

Table 3. Descriptive statistics (mean  $\pm$  SD, minimum and maximum values) of 12 cranial and four mandibular variables measured in four species within the *M. nattereri* species complex. See text for definitions of variables and acronyms. Measurements of the single known skull of *Myotis nustrale* sp. nov. are very much like those measured in other species from the continent.

| Craniodental variable | <i>Myotis nustrale</i><br>sp. nov. holotype | <i>M. crypticus</i><br>(n = 21)     | <i>M. nattereri</i> s. str.<br>(n = 14) | <i>M. escalerai</i><br>(n = 12)     |
|-----------------------|---|-------------------------------------|---|-------------------------------------|
| GTL                   | 15.62                                       | 15.53 $\pm$ 0.34<br>(14.99 – 16.44) | 15.65 $\pm$ 0.27<br>(15.09 – 16.03)     | 15.59 $\pm$ 0.66<br>(15.21 – 15.95) |
| CBL                   | 14.49                                       | 14.46 $\pm$ 0.31<br>(13.94 – 15.20) | 14.70 $\pm$ 0.32<br>(14.00 – 15.12)     | 14.52 $\pm$ 0.28<br>(14.17 – 14.91) |
| CCL                   | 13.47                                       | 13.52 $\pm$ 0.30<br>(12.90 – 14.12) | 13.72 $\pm$ 0.30<br>(13.05 – 14.14)     | 13.58 $\pm$ 0.20<br>(13.27 – 13.96) |
| CM3                   | 5.77  | 6.00 $\pm$ 0.03<br>(5.64 – 6.33)    | 6.13 $\pm$ 0.14<br>(5.86 – 6.38)        | 5.99 $\pm$ 0.04<br>(5.77 – 6.27)    |
| M3M3W                 | 6.26  | 6.22 $\pm$ 0.03<br>(5.92 – 6.44)    | 6.47 $\pm$ 0.15<br>(6.22 – 6.73)        | 6.23 $\pm$ 0.05<br>(5.93 – 6.53)    |
| CCW                   | 3.92  | 3.90 $\pm$ 0.03<br>(3.57 – 4.19)    | 4.03 $\pm$ 0.08<br>(3.88 – 4.14)        | 4.09 $\pm$ 0.03<br>(3.95 – 4.33)    |
| ZB                    | 9.64  | 9.60 $\pm$ 0.05<br>(9.26 – 10.06)   | 9.90 $\pm$ 0.23<br>(9.54 – 10.37)       | 9.70 $\pm$ 0.05<br>(9.40 – 10.13)   |
| BCW                   | 7.86  | 7.77 $\pm$ 0.16<br>(7.47 – 8.10)    | 7.89 $\pm$ 0.15<br>(7.64 – 8.23)        | 7.60 $\pm$ 0.09<br>(7.41 – 7.78)    |
| MAB                   | 7.94  | 7.73 $\pm$ 0.13<br>(7.54 – 7.98)    | 7.94 $\pm$ 0.16<br>(7.66 – 8.31)        | 7.77 $\pm$ 0.12<br>(7.49 – 7.90)    |
| POC                   | 3.93  | 3.77 $\pm$ 0.07<br>(3.62 – 3.89)    | 3.95 $\pm$ 0.13<br>(3.69 – 4.16)        | 3.60 $\pm$ 0.08<br>(3.47 – 3.71)    |
| ROL                   | 4.79  | 4.76 $\pm$ 0.20<br>(4.46 – 5.35)    | 4.69 $\pm$ 0.25<br>(4.08 – 4.99)        | 4.63 $\pm$ 0.09<br>(4.50 – 4.76)    |
| M1M3                  | 3.50  | 3.48 $\pm$ 0.12<br>(3.30 – 3.71)    | 3.54 $\pm$ 0.086<br>(3.35 – 3.67)       | 3.53 $\pm$ 0.08<br>(3.40 – 3.68)    |
| ml                    | 11.14                                       | 11.26 $\pm$ 0.30<br>(10.76 – 11.92) | 11.38 $\pm$ 0.31<br>(10.55 – 11.73)     | 11.36 $\pm$ 0.20<br>(11.04 – 11.84) |
| cm3L                  | 6.04  | 6.40 $\pm$ 0.20<br>(6.00 – 6.87)    | 6.43 $\pm$ 0.12<br>(6.13 – 6.61)        | 6.38 $\pm$ 0.16<br>(6.17 – 6.64)    |
| m1m3L                 | 3.81  | 3.88 $\pm$ 0.10<br>(3.64 – 4.05)    | 3.89 $\pm$ 0.12<br>(3.66 – 4.08)        | 3.90 $\pm$ 0.08<br>(3.81 – 4.06)    |
| coh                   | 3.29  | 3.21 $\pm$ 0.10<br>(2.98 – 3.44)    | 3.31 $\pm$ 0.10<br>(3.12 – 3.47)        | 3.45 $\pm$ 0.10<br>(3.29 – 3.57)    |

them, except by the presence of a conspicuous blackish spot present on the lower lip of all adult individuals examined in Corsica (Figs 5 and 6). Such a pigmented spot is present in the lower lip of young individuals of a few other *Myotis* species (e.g. in *M. daubentonii*, *M. dasycneme*) but vanishes when they attain adulthood (Richardson, 1994; Haarsma & Van Alphen, 2009). *M. nustrale* has relatively longer ears and tragus than *M. nattereri* s. str. or *M. crypticus* (Table 2), but because a single individual has been measured for these characters so far, individual variation may blur these apparent differences. The same applies to the shape of the tragus, the margins of which appear slightly more linear in the Corsican species than

in the other continental taxa; tragus shape in these taxa, however, are subject to substantial individual variation. Fur colouration, greyish-brown above and whitish below, is also similar in all three species. Although craniodental dimensions of all three species are again largely overlapping (Table 3), the general shape of the skull of *M. nustrale* is more similar to that of *M. crypticus* than to the slightly larger and more robust skull of *M. nattereri* s. str. Sequences of the mitochondrial *cytochrome b* gene, forming a strongly supported monophyletic group, are unique in the Corsican species and diverge by an average 7.5 % Kimura-2-parameter distance from its closest relative *M. crypticus* (Puechmaille *et al.*, 2012). Nuclear genes



Fig. 5. External characters of *Myotis nustrale* sp. nov. Panels are close-ups of the female holotype (MNHN-ZM-2023-12), except the lower right one which is a picture from a released adult individual (Photo © Yann Le Bris). The upper left panel illustrates the stiff hairs running along the uropatagium, viewed from below. The upper right panel is the right foot with wing insertion to the base of the outer toe, in ventral view. The lower left panel illustrates the long and straight tragus and unnotched ear of the female holotype. On the portrait of the live individual, notice the black chin spot on the lower lip.

and microsatellite data for *M. nustrale* also differ considerably from other taxa in the *M. nattereri* species complex (Figs 2 and 3) and suggest that *M. nustrale* may be basal to other members of this clade (Fig. 2). Recordings of echolocation calls of *M. nustrale* in Corsica show the typical acoustic signature of members of the *M. nattereri* species complex (Russo & Jones, 2002), i.e. short and strongly frequency modulated calls dominated by sweeps ranging from >105 kHz down to ca. 25 kHz frequencies, but again these calls are too plastic to serve for species identification.

**Proposed vernacular names:** Korsican Mausohr (German), Corsican myotis (English), murin de Corse (French), murin nustrale (Corsican), murciélago ratonero de Córcega (Spanish).

**Natural history:** Several females equipped with transmitters were radio tracked in spring and summer in two mountainous areas of central Corsica: Ghisoni

and Bavella (Groupe Chiroptères Corse, 2019). Most records were gathered from forested areas above 500 m a.s.l., although a few observations have been recorded near sea level (Fig. 1). These animals were hunting in various habitats located within 8 km of their day roosts. Hunting territories were used regularly by single individuals and were either located in dense maquis populated with evergreen oak (*Quercus ilex*), ash (*Fraxinus* spp.) and abundant undergrowth, or in more open forests with Corsican pines (*Pinus nigra*) surrounded by ferns. The bats were hunting both high in the canopy and closer to the ground. All known day roosts (Fig. 1) were in cliffs, rocky outcrops, stone bridges or in an artificial tunnel (where the only recorded nursery colony of ca. 60 individuals was established), denoting a strong rupicolous roosting behaviour for this Corsican myotis. This clear affinity for rocky habitats is similar to the roosting behaviour





Fig. 6. Portraits and hind feet (dorsal view) of an adult female *M. nustrale* sp. nov. caught near Bavella, in southern Corsica (left) and an adult female *M. crypticus* from the Jura mountains in Switzerland (right). Notice that the latter individual has a faint line bordering the lower lip, while the former has a much more conspicuous chin spot. Wing insertion to the outer toe is, however, identical in both species. For the left individual, the wing was not stretched while it was stretched for the right individual.

of *M. escaleraei*, but is distinct from the typical tree-roosting behaviour of *M. nattereri* or *M. crypticus* (Agirre-Mendi & Ibáñez, 2012).

Other bat species recorded in the same hunting habitats as those frequented by the Corsican myotis included *Eptesicus serotinus*, *Hypsugo savii*, *Nyctalus leisleri*, *Pipistrellus pipistrellus*, *Rhinolophus ferrumequinum* and *Plecotus austriacus*, but not other *Myotis* species (Groupe Chiroptères Corse, 2019).

**Conservation:** This endemic species is apparently very rare (Courtois *et al.*, 2011) and owing to its limited distribution range in the mountains of Corsica (Fig. 1), should be considered as globally endangered. Based on our current knowledge, the species has an area of occupancy of 1,300 km<sup>2</sup> and an extent of occurrence of 2,400 km<sup>2</sup>. For the present and given its broad tolerance for diverse forested habitats as hunting grounds, human-induced habitat change is not foreseen as a threat for the species. Forest fires might, however, temporarily limit hunting habitats. Rocky outcrops used as day roosts do

not appear to be limiting factors in Corsica, but they could be subject to disturbance from rock climbing or abseiling and associated activities.

## DISCUSSION

To resolve the phylogenetic relationships between all currently recognised species of the *M. nattereri* species complex from the Western Palaearctic, we used several other *Myotis* species and a common pipistrelle as outgroups (Fig. 2). In agreement with Morales *et al.* (2019) who used 1,610 ultra-conserved elements, our reconstructions based on five nuclear introns recovered *M. alcaethoe* and *M. mystacinus* as the most basal clade in the *Myotis* radiation. Also consistent with previous reconstructions, we recovered a sister-group relationship between the New World clade (here only represented by *M. brandtii*) and members of the Old World clade, including the so-called ‘large-Myotis’ clade (sensu Ruedi *et al.*, 2013). This ‘large-Myotis’ clade includes

all species of the *M. nattereri* species complex from the Western Palaearctic and four large *Myotis* species (*M. myotis*, *M. blythii*, *M. punicus* and *M. chinensis*). In this phylogenetic tree (Fig. 2), however, we support for the first time the reciprocal monophyly of both groups whereas all previous molecular reconstructions recovered the *M. nattereri* species complex as paraphyletic (e.g. Ruedi *et al.*, 2013; Morales *et al.*, 2019). This paraphyly is contradicted by the striking morphological resemblance amongst members in this species complex (e.g. Horáček & Hanák, 1984; Juste *et al.*, 2018), a resemblance that was the primary reason for considering these taxa initially as a single species (Razgour *et al.*, in press).

Within the *M. nattereri* species complex, each of the seven recognised species were recovered as monophyletic with one exception. Two *M. crypticus* individuals from northern Italy appeared distant from conspecifics, and instead were basal to *M. nattereri s. str.* (Fig. 2). Nuclear sequences of each of these two individuals have been obtained by two research teams independently (Salicini *et al.*, 2011; this study) and are similar to each other. We interpret the inconsistent placement of those two individuals as a possible sign of introgression between *M. nattereri* and *M. crypticus*. Indeed, Salicini *et al.* (2011) and Çoraman *et al.* (2019) detected mito-nuclear discordance that was best explained by past introgression between those two species, whereby *M. nattereri* genes introgressed the genome of *M. crypticus*.

Our molecular reconstruction places *M. nustrale* from Corsica in a basal position relative to other species in the *M. nattereri* species complex (Fig. 2), further supporting its clear distinction from all seven recognised species in this group. Global relationships are similar to what has been recovered previously (Salicini *et al.*, 2011; Çoraman *et al.*, 2019), including the early divergence of *M. escaleraei* and *M. zenatius* from the remaining species in this complex. Both species share the morphological key characteristic of the wing membrane being inserted in the mid-metatarsus rather than at the base of the toe for the other members of this group (figs 2-3 in Puechmaile *et al.*, 2012; fig. S3.2 in Çoraman *et al.*, 2019; fig. 3 in Smirnov *et al.*, 2020). Other phylogenetic relationships suggested by our reconstructions included *M. schaubi* and *M. tschuliensis* as sister species, both together being sister to a clade containing *M. nattereri s. str.*, *M. crypticus* and *M. hovei*. Relationships were only moderately supported and should therefore be interpreted with caution.

According to all genetic evidence, *M. nustrale* is genetically well differentiated both for mitochondrial (Puechmaile *et al.*, 2012) and for nuclear DNA markers from all currently recognised species in the *M. nattereri* species complex. These differences are key to defining *M. nustrale* as a species new to science. The uniqueness of this taxon suggests that it has been genetically isolated from other species for a long time. This isolation could be partly due to its isolated geographic position on an island (50 km off Elba Island, itself 10 km off mainland Europe)

but this situation alone is unlikely to explain the genetic distinctiveness of the species (Fig. 2) and lack of gene flow with other related species (Fig. 3). Indeed, for other species in this complex, there is evidence for multiple sea crossing events in recent history (i.e. post-glaciation), as demonstrated by shared mitochondrial and/or nuclear lineages between islands and the continent. For example, the populations of *M. crypticus* from Sicily and southern Italy recently exchanged genes despite being separated by a sea channel of 3 km. A similar situation prevails between the mainland European, British and Irish populations of *M. nattereri s. str.* (33 and 78 km; Salicini *et al.*, 2011; Puechmaile *et al.*, 2012; Salicini *et al.*, 2013; Çoraman *et al.*, 2019), or between Cyprus and southern Turkey for *M. hovei* (69 km; Uvizl & Benda, 2021), and also between populations of *M. escaleraei* from the Iberian Peninsula and the Balearic islands (86, 81 and 36 km; Ibiza, Mallorca and Menorca) (Salicini *et al.*, 2013; Razgour *et al.*, 2015). Besides, based on ringing data, it has been shown that *M. nattereri s. l.* can travel more than 60 km to mate at swarming sites (Rivers *et al.*, 2006) or can undertake seasonal movements up to 327 km (Hutterer *et al.*, 2005), providing evidence that the species is vagile. Other species within the species complex were rarely part of ringing programs and therefore information on their movements is lacking; however, it seems plausible that they should have similar flight capabilities to *M. nattereri*. For example, using microsatellites, Razgour *et al.* (2015) showed that the population of *M. escaleraei* from Mallorca (200 km off Iberia but 81 km from Ibiza, itself 86 km off Iberia) was only weakly differentiated (average  $F_{st}$  0.10) from the mainland populations sampled across Iberia. Although it is challenging to directly compare  $F_{st}$  measures, this value is 2.5-3 times lower than the difference we measured between Corsican *M. nustrale* and *M. crypticus* from southern France ( $F_{st}$  0.25-0.28). Hence, considering information on the biology of the different taxa in the species complex, their respective distribution and molecular differentiation, there is clear support that *M. nustrale* represents a distinct biological species.

The single available specimen did not allow us to extensively compare morphological traits of *M. nustrale* with other species of the *M. nattereri* species complex. However, the general pattern of high morphological similarity between species reported in the species complex (e.g. Juste *et al.*, 2018; Smirnov *et al.*, 2020; Kruskop & Solovyeva, 2021; Uvizl & Benda, 2021) corroborates our observations suggesting that morphologically, *M. nustrale* is virtually indistinguishable from *M. crypticus* or *M. nattereri s. str.* Interestingly, such high morphological similarities have also been noted in the large *Myotis* clade, for which authors have long disagreed on the taxonomic assignment of several forms in *M. myotis* and *M. blythii* (e.g. Beuneux, 2004; Evin *et al.*, 2008). Prevalent morphological stasis seems to be widespread



in several other bats, such as between *P. pipistrellus* and *P. pygmaeus* that cannot be confidentially assigned to species based on multiple craniodental measurements or mandible shape (Barlow *et al.*, 1997; Sztencel-Jablonka *et al.*, 2009). Such surprisingly high cryptic species diversity is encountered across different genera (e.g. *Miniopterus*, *Myotis*, *Rhinolophus*, *Hipposideros*, *Plecotus*; Spitzenberger *et al.*, 2006; Larsen *et al.*, 2012; Thong *et al.*, 2012; Šrámek *et al.*, 2013; Puechmaille *et al.*, 2014; Dool *et al.*, 2016; Andriollo & Ruedi, 2018; Patterson *et al.*, 2020; Yusefovich *et al.*, 2020; Chornelia *et al.*, 2022).

The distribution of *M. nustrale* is restricted to the island of Corsica where the species is globally rare (Fig. 1). Despite specific searches for the species throughout the island, it appears to be relatively localised to a small region within the “Parc Naturel Régional de Corse”. Considering IUCN red list criteria, the species should be classified as Endangered given that it has an extent of occurrence and an area of occupancy of less than 5,000 km<sup>2</sup>. According to our estimates of population genetic structure showing significant *F*<sub>st</sub> over short distances, its populations also seem to be fragmented. The observed high and significant *F*<sub>st</sub> values observed for *M. nustrale* contrast with small and non-significant values classically observed over such short distance in *M. nattereri* *s. str.* (Rivers *et al.*, 2005; Halczok *et al.*, 2017). Furthermore, Razgour *et al.* (2019) investigated the vulnerability to climate change of *M. escaleraei* and *M. crypticus* by combining information on adaptive genetic variation and ecological niche modelling. They revealed that the future of both species depends on their adaptive capacity, their connectivity and whether they will be able to colonise new areas towards the North. Although ecological niche modelling has not been specifically carried out for *M. nustrale*, nor estimates of population densities in Corsica, it seems likely that it has a very small overall population size which might compromise its capability to adapt to climate change. Furthermore, the high *F*<sub>st</sub> values between sampled locations suggest reduced connectivity between existing populations. Last, unlike on the continent where northward range shifts could theoretically occur, *M. nustrale* might not have such an option as there is no indication that this species is able to leave the island to colonise continental Europe. Even an altitudinal range shift within Corsica seems limited because the species already reaches 2,000 m a.s.l., i.e., close to the highest altitudes found on the island. It seems therefore likely that the area suitable for the species will decline in the near future as a result of climate change. More studies are critically needed to better evaluate the conservation status of *M. nustrale*, the only wild mammal endemic to metropolitan France.

## DATA ACCESSIBILITY

Supplementary information is available at Zenodo: 10.5281/zenodo.8023121 including primer and alignment details, figures of gene trees and trees in nexus format, and detailed sample information (locality, voucher, ENA/GenBank accessions). Sequences generated in the current study are available in ENA OX958063-OX958213, project PRJEB63130.

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