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Two new cryptic bat species within the *Myotis nattereri* species complex (Vespertilionidae, Chiroptera) from the Western Palaearctic

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The *Myotis nattereri* species complex consists of an entangled group of Western Palaearctic bats characterized by fringing hairs along the rear edge of their uropatagium. Some members are relatively common while others are rare but all forms are morphologically very similar and their taxonomy is unresolved. Recent studies based on different molecular markers have shown that several major and unexpected lineages exist within this group of forest-dwelling bats. All the mitochondrial and nuclear markers tested to date have shown that these major lineages evolved as fully independent and coherent units and therefore each qualifies as distinct species. In the absence of proper morphological diagnosis, these lineages are informally referred to in the literature under different names. We explore here the external and craniodental variation of these lineages. Although all morphological measurements were overlapping between these lineages, we show that lineages can be completely discriminated in a multivariate morphometric space. Consistent with previous molecular reconstructions, these four major lineages represent two pairs of related species, each represented by a named species (*Myotis nattereri* s. str. and *M. escalerai*, respectively) and by unnamed forms (*Myotis* sp. A and *Myotis* sp. B, respectively). Herein we describe formally these two unnamed forms to clarify the taxonomy within this species complex. This new taxonomic view has important implication for the protection of these species, as three of the four taxa must now be considered as range-restricted species in need of conservation actions.

Key words: cryptic species, DNA, systematics, speciation, taxonomy

INTRODUCTION

Molecular studies have unveiled an unexpectedly high diversity of cryptic lineages within bats worldwide, including areas such as Europe that are supposedly well-known taxonomically (Mayer and von Helversen, 2001; Bogdanowicz *et al.*, 2015). In fact, most recent surveys, particularly those based on mtDNA have shown that many species are subdivided in well-differentiated haplogroups (Çoraman *et al.*, 2013). For instance, and only in the Iberian Peninsula, major cryptic lineages were found in up to 20% of the traditional, morphologically-defined species (Ibáñez *et al.*, 2006).

Conversely, interspecific lineages with low mtDNA divergence among morphologically, well-isolated biological species were also evidenced (Artyushin *et al.*, 2009; Puechmaille *et al.*, 2014), raising the question whether mtDNA divergence alone is a good indicator for cryptic taxonomic diversity. At best, representatives of major cryptic mtDNA lineages should be considered as candidate species. As evidenced in recent taxonomic surveys, a combination of mitochondrial and nuclear (nDNA) markers is often necessary to provide a complete picture of the underlying evolutionary processes (Dool *et al.*, 2016; Freudenstein *et al.*, 2017) and allows us to confirm the presence of independent biological units. In bats, both extreme scenarios were evidenced, i.e. two deep lineages indeed representing cryptic biological species like in the soprano versus common pipistrelles (Barrat *et al.*, 1997; Racey *et al.*, 2007) or the reverse, where two deeply diverging lineages in *Pipistrellus kuhlii* were present in a single panmictic population (Andriollo *et al.*, 2015).

In this context, European and North African populations of Natterer's bat, *Myotis nattereri* (Kuhl, 1817) are particularly interesting since they were long considered to be part of a single, nominotypical species (Horáček and Hanák, 1984; Simmons, 2005). Based on an extensive morphological

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comparison of fossil and recent material, Horáček and Hanák (1984) further suggested that other parts of the Western Palaearctic were occupied by a larger subspecies, *M. n. tschuliensis* Kuzyakin, 1935, occurring in Transcaucasia to NE Iraq, or by the species *M. schaubi* Kormos, 1934. All other related forms living east of the Urals belonged to distinct species (e.g., *M. bombinus* Thomas, 1905; *M. pequinius* Thomas, 1908; *M. araxenus* Dahl, 1947). Horáček and Hanák (1984) also noticed size differences between the European populations, those from Central and Northern Europe being larger than those from Iberia, and North African populations being even smaller, but they provided no taxonomic clarity associated with these differences. Contrasting with this relative morphological conservatism within *M. nattereri* s. l., a series of genetic surveys focusing on Western European populations showed the existence of at least five major mitochondrial lineages, suggesting that *M. nattereri* is a species complex. Three of those lineages were located in continental Europe, one in Corsica and the last one in Northwestern Africa (Ibáñez *et al.*, 2006; Mayer *et al.*, 2007; García-Mudarra *et al.*, 2009; Salicini *et al.*, 2011; Puechmaille *et al.*, 2012).

The use of several nuclear markers (Ibáñez *et al.*, 2006; García-Mudarra *et al.*, 2009; Salicini *et al.*, 2011, 2013; Razgour *et al.*, 2015) confirmed that at least four of these lineages were evolving independently and thus represented true biological species. In a first attempt to clarify the taxonomic situation of this species complex in the light of these molecular findings, Ibáñez and coworkers (Ibáñez *et al*., 2006; García-Mudarra *et al.*, 2009; Salicini *et al.*, 2011) proposed to assign representatives of the major lineage occurring in Northern and Eastern Europe to *M. nattereri* s. str., and those restricted to Iberia and the Balearic Islands to *M. escalerai* Cabrera, 1904; the other lineages were left taxonomically unassigned. One of them is present across Iberia, parts of France, Italy and possibly Austria and was referred to as *Myotis* sp. A or *M*. sp. 1 (Ibáñez *et al.*, 2006; Salicini *et al.*, 2011, 2013; Puechmaille *et al.*, 2012; Bogdanowicz *et al.*, 2015), while the other unassigned lineage, restricted to the Mediterranean parts of western North Africa was called *M.* sp. B (Ibáñez *et al.*, 2006; García-Mudarra *et al.*, 2009; Puechmaille *et al.*, 2012). The last unnamed taxon, endemic to Corsica, was called *M.* sp. C (Puechmaille *et al.*, 2012). The phylogenetic reconstructions based on both mtDNA and nDNA markers (Fig. 1) strongly support a closer evolutionary relationship between the parapatric lineages *M. nattereri* s. str. and *M.* sp. A on one hand, and between the allopatric lineages *M. escalerai* and *M.* sp. B on the other. In this phylogenetic framework, the position of the Caucasian species, *M. schaubi* or of the lineage corresponding to *M*. sp. C are still unsettled, as either they lack nuclear data or their relationships are unsupported and ambiguous (Fig. 1).

In an attempt to further clarify the taxonomy within the *M. nattereri* species complex in Western Europe and North Africa, we analyse the morphological variation of representatives of the four continental lineages, and discuss its systematics implication. Because none of the older available names are appropriate to designate bats of the two unnamed lineages (see 'Available name' section below), we proceed to their formal description as new species. The last major European lineage in this species complex (*M*. sp. C from Corsica) is not considered in this revision, as no comparative material could be obtained and also because nuclear data showing its independent evolutionary trajectory are still lacking.

Material and Methods

The European and Moroccan specimens of the *M. nattereri* species complex deposited in the collections at the Natural History Museum of Geneva, Switzerland (MHNG), the Hungarian Natural History Museum of Budapest, Hungary (HNHM) and the Estación Biológica de Doñana Seville, Spain (EBD), were studied and measured following the respective Institutions' guidelines for collections. All 53 studied museum vouchers were assigned a priori to one of the four continental lineages according to genetic data, or to their geographical. In the only known region of co-occurrence between lineages (Northern Iberia and the Pyrenees), *M. escalerai* and *M*. sp. A were easily separated by external diagnostic criteria (Puechmaille *et al*., 2012; Agirre-Mendi and Ibáñez, 2012). Most of these data were published in a series of molecular surveys (Ibáñez *et al.*, 2006; García-Mudarra *et al.*, 2009; Salicini *et al.*, 2011, 2013) or when needed, new mitochondrial and nuclear DNA sequences were obtained following protocols and conditions described in these papers and they were uploaded in Genbank (https://www.ncbi.nlm.nih.gov).

Qualitative and quantitative variation in skull and external morphology among lineages was inspected by direct comparison of preserved specimens. Morphometric variation was measured at eight external and 16 craniodental characters with a digital calliper (to the nearest 0.1 mm for external and 0.01 mm for skulls characters). These morphological traits and their abbreviation are: body weight (W, expressed in grams); head and body length (HB); tail length (TL); forearm length (FA); hindfoot length, including claws (HF); tibia length (TIB); ear length (EAR); tragus length (TRA); 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

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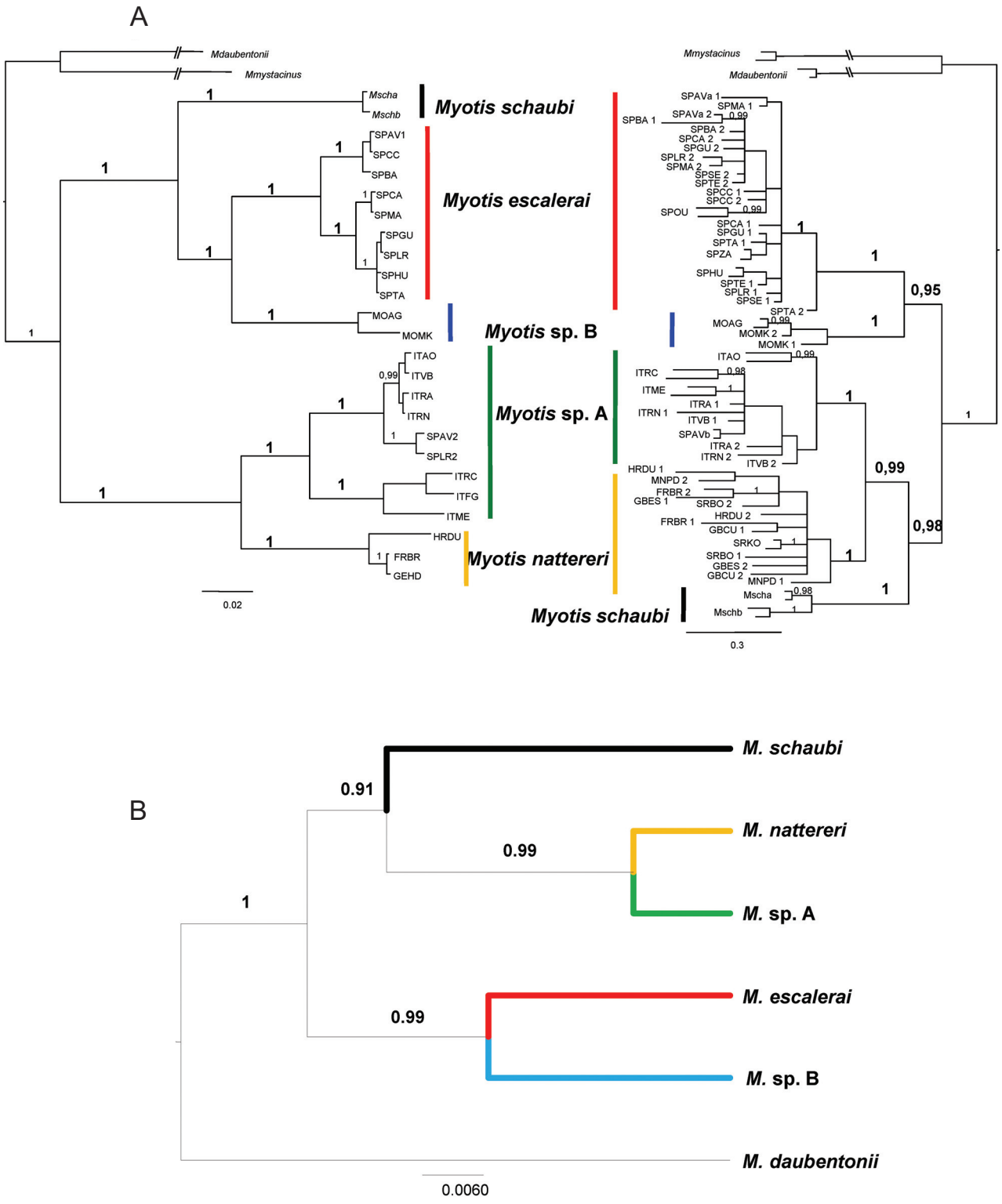


FIG. 1. A — Concatenated phylogenetic trees, based on Bayesian inference of two mitochondrial markers (*Cytochrome b* and *ND1* — left tree), and six nuclear introns (*SLC38A7-8, ABHD11-5, ACOX2-3, ACPT-4, COPS7A-4,* and *ROGDI-7* — right tree) within the *M. nattereri* species complex. Numbers on branches indicate posterior probabilities. The first two letters of the samples name refer to the countries of origin: Croatia (HR), France (FR), Germany (GE), Italy (IT), Montenegro (MN), Morocco (MO), Serbia (SR), Spain (SP), United Kingdom (UK). B — Species tree and posterior probabilities inferred using the nuclear and mitochondrial markers combined and modified from Salicini *et al.* (2011). Reproduced with authorization from Molecular Phylogenetics and Evolution, Elsevier

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(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 those measurements are presented in Ruedi *et al.* (2012).

Basic descriptive statistics of the morphological traits were obtained for each lineage and significance of mean differences between groups was tested by ANOVAs and associated *F*-tests. Skull variation of bats from the four different lineages within *M. nattereri* s. l. was further investigated through a multivariate discriminant function analysis (DFA) of the 16 craniodental characters. The discriminant functions were based on the covariance matrix of variables and maximized differences among groups. Two specimens with broken zygomatic arches could not be measured for ZB and hence, were excluded from the initial analysis but assigned a posteriori to the groups with their missing values replaced by the mean of the entire dataset. In order to maximize the discrimination among groups, we performed three separate DFA analyses: one including all four lineages, a second restricted to *M. nattereri* s. str. and *M.* sp. A. and a third comprising only *M. escalerai* and *M.* sp. B. All statistical analyses were performed with the SPSSv.16.0 package (SPSS Inc., Chicago).

Results

We examined the morphological variation for a total of 53 vouchered specimens belonging to the *M. nattereri* species complex and issued from Europe and Morocco (see Supplementary Table S1). Based on their geographic, external and/or molecular characteristics, they were assigned either to *M. nattereri* s. str. (14 specimens from Hungary, Greece, northern France and northern Germany), *M.* sp. A (21 specimens from Spain, Southern France, Italy and Western Switzerland), *M. escalerai* (12 specimens from Spain) or to *M.* sp. B (6 specimens from Morocco).

Morphometric Differentiation

Bats from the four lineages showed very similar external and skull morphology. Morphometric data were also very similar in all four lineages of *M. nattereri* s. l. (Table 1). In fact, not one of the external measurements showed significant differences based on the ANOVAs. However, mean values were in general slightly larger for *M. nattereri* s. str. and for *M. escalerai* despite a broad overlap. However, because only a limited number of specimens were examined for these external characters, and because measurements were taken from various sources (label records or taken from dry or spirit-preserved vouchers) these values should only be considered as indicative. Skull measurements also showed extensive overlap but the differences were significant for variables related to the broadness of the skull (e.g., M3M3W, CCW, ZB or MAB). As a general trend, they showed slightly larger values for *M. nattereri* s. str. with respect to either *M.* sp. A or *M. escalerai* (Table 2). When the closely related *M. escalerai* and *M.* sp. B were compared to each other, they differed both in external and skull characters, the former being slightly larger than the later, but overall, none of the external or craniodental measurements could discriminate them.

In the multivariate morphospace based on 16 craniodental measurements, the discrimination among lineages was more pronounced. When all four groups were considered in the DFA (Fig. 2A), specimens were mainly discriminated along the first axis (71% of the total variance) with positive values associated to *M. nattereri* s. str. and *M.* sp. A and negative ones associated to *M. escalerai* and *M.* sp. B. The second axis (23% of the total variance) maximised the differences between the lineages within each of those two species pairs.

Table 1. Descriptive statistics ($\bar{x} \pm$ SD, minimum and maximum values) of eight external measurements recorded in the four lineages studied within the *M. nattereri* species complex. See text for definitions of variables and acronyms. None of the external measurements differ significantly between lineages

External variable	*M. nattereri* s. str. *n* = 14		*M.* sp. A *n* = 21		*M. escalerai* *n* = 12		*M.* sp. B *n* = 6	
	$\bar{x} \pm$ SD	min–max	$\bar{x} \pm$ SD	min–max	$\bar{x} \pm$ SD	min–max	$\bar{x} \pm$ SD	min–max
FA	39.6 ± 1.1	38.5–41.2	39.3 ± 1.3	36.3–42.0	39.9 ± 1.3	37.1–42.0	39.3 ± 1.2	38.2–40.0
W	8.0		6.5		6.6 ± 1.4	4.5–6.3	–	–
HB	45.5 ± 2.1	43.0–48.0	45.2 ± 2.7	40–50	45.0 ± 3.0	41–50	46.0 ± 1.0	44–48
TL	34.9 ± 2.7	32.0–36.0	39.05 ± 4.1	32–46	39.1 ± 4.0	42–50	41.0 ± 2.0	38.0–42.0
EAR	16.2 ± 0.7	15.4–17.5	15.5 ± 1.0	14.1–17.1	16.8 ± 1.3	15.1–18.0	16.1 ± 1.0	15.3–17.3
TRA	10.1 ± 0.6	9.3–10.8	9.6 ± 0.6	8.2–10.6	9.8 ± 1.0	8.9–10.8	9.8 ± 1.0	8.8–10.2
HF	8.3 ± 0.8	7.1–9.3	8.2 ± 0.7	6.6–9.1	8.3 ± 1.0	6.7–10.0	8.3 ± 1.0	7.9–8.7
TIB	17.1 ± 0.4	16.6–17.8	16.9 ± 0.6	15.9–17.6	17.1 ± 1.0	15.9–19.0	17.1 ± 1.0	16.6–17.3

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TABLE 2. Descriptive statistics ($\bar{x}$ ± SD, minimum and maximum values) of 16 craniodental variables measured in the four lineages studied within the *M. nattereri* species complex. See text for definitions of variables and acronyms. The last column gives the *F*-values and significance of an ANOVA test of mean values among the four lineages (* — $P < 0.05$, ** — $P < 0.01$)

Craniodental variable	*M. nattereri* s. str. $n = 14$		*M.* sp. A $n = 21$		*M. escalerai* $n = 12$		*M.* sp. B $n = 6$		*F*-value
	$\bar{x}$ ± SD	min–max	$\bar{x}$ ± SD	min–max	$\bar{x}$ ± SD	min–max	$\bar{x}$ ± SD	min–max	
GTL	15.65 ± 0.27	15.09–16.03	15.53 ± 0.34	14.99–16.44	15.59 ± 0.66	15.21–15.95	15.37 ± 0.28	15.06–15.94	1.41
CBL	14.70 ± 0.32	14.00–15.12	14.46 ± 0.31	13.94–15.20	14.52 ± 0.28	14.17–14.91	14.34 ± 0.19	14.12–14.63	3.01
CCL	13.72 ± 0.30	13.05–14.14	13.52 ± 0.30	12.90–14.12	13.58 ± 0.20	13.27–13.96	13.38 ± 28	13.21–13.66	2.69
CM3	6.13 ± 0.14	5.86–6.38	6.00 ± 0.03	5.64–6.33	5.99 ± 0.04	5.77–6.27	5.79 ± 0.03	5.72–5.91	7.67**
M3M3W	6.47 ± 0.15	6.22–6.73	6.22 ± 0.03	5.92–6.44	6.23 ± 0.05	5.93–6.53	6.08 ± 0.13	5.94–6.30	11.46**
CCW	4.03 ± 0.08	3.88–4.14	3.90 ± 0.03	3.57–4.19	4.09 ± 0.03	3.95–4.33	3.84 ± 0.03	3.75–3.94	12.39**
ZB	9.90 ± 0.23	9.54–10.37	9.60 ± 0.05	9.26–10.06	9.70 ± 0.05	9.40–10.13	9.67 ± 0.06	9.42–9.82	5.36**
BCW	7.89 ± 0.15	7.64–8.23	7.77 ± 0.16	7.47–8.10	7.60 ± 0.09	7.41–7.78	7.80 ± 0.12	7.66–7.94	9.93**
MAB	7.94 ± 0.16	7.66–8.31	7.73 ± 0.13	7.54–7.98	7.77 ± 0.12	7.49–7.90	7.80 ± 0.16	7.64–7.94	7.23**
POC	3.95 ± 0.13	3.69–4.16	3.77 ± 0.07	3.62–3.89	3.60 ± 0.08	3.47–3.71	3.71 ± 0.81	3.55–3.78	29.78**
ROL	4.69 ± 0.25	4.08–4.99	4.76 ± 0.20	4.46–5.35	4.63 ± 0.09	4.50–4.76	4.49 ± 0.20	4.25–4.76	3.44*
M1M3	3.54 ± 0.086	3.35–3.67	3.48 ± 0.12	3.30–3.71	3.53 ± 0.08	3.40–3.68	3.49 ± 0.10	3.35–3.61	0.99
ml	11.38 ± 0.31	10.55–11.73	11.26 ± 0.30	10.76–11.92	11.36 ± 0.20	11.04–11.84	11.11 ± 0.16	10.91–11.30	1.80
cm3L	6.43 ± 0.12	6.13–6.61	6.40 ± 0.20	6.00–6.87	6.38 ± 0.16	6.17–6.64	6.15 ± 0.10	6.07–6.34	4.40**
m1m3L	3.89 ± 0.12	3.66–4.08	3.88 ± 0.10	3.64–4.05	3.90 ± 0.08	3.81–4.06	3.85 ± 0.08	3.75–3.97	0.42
coh	3.31 ± 0.10	3.12–3.47	3.21 ± 0.10	2.98–3.44	3.45 ± 0.10	3.29–3.57	3.30 ± 0.05	3.21–3.35	15.60**

Finally, the third axis (6% of the total variance), essentially isolated *M.* sp. B from the other species (not shown). According to the classification functions of this DFA only four skulls were misclassified. Three of the misclassified specimens were mixed between European *M.* sp. A and North African *M.* sp. B, the last one being a Hungarian specimen of *M. nattereri* s. str. that was set, albeit with low posterior probability (0.68), close to the *M.* sp. A cluster (Fig. 2A). When each of the related species pairs (i.e., *M. nattereri* s. str. versus *M.* sp. A and *M. escalerai* versus *M.* sp. B) was analysed separately, the resulting DFAs completely separated the distinct lineages, each specimens falling in its respective cluster (Fig. 2B –C).

The first five most correlated craniodental characters entered in the DFA differentiating *M. nattereri* s. str. and *M.* sp. A (Fig. 2B), were related to measures of the broadness of the skull (POC, M3M3W, MAB, ZB and CCW). For the DFA, contrasting *M. escalerai* with *M.* sp. B (Fig. 2C), the highest correlations were related to the broadness of rostrum (CCW) and braincase (BCW), to the height of the coronoid process (coh), to the toothrow length (CM3) and to the least interorbital constriction (POC) (see details in Supplementary Table S2).

Globally, the multivariate analyses of craniodental characters strongly support the separation of European and North African representatives of the *M. nattereri* s. l. species complex into four distinct and morphologically diagnosable groups. This result is fully concordant with the corresponding four major lineages (Fig. 1) evidenced in the previous molecular analyses (Salicini *et al.*, 2011, 2013; Puechmaille *et al.*, 2012). Hence, and according to most accepted species concepts including the biological (Mayr, 1999), genetic (Baker and Bradley, 2006) or evolutionary species concepts (de Queiroz, 2007), they deserve full species status. Since two of these lineages are unnamed, we herein proceed to their formal description.

SYSTEMATICS

Available names

The name '*Vespertilio nattereri*' was originally used by Kuhl (1817) to describe a bat species from Hanau, Hessen, Germany. This location is close to the location of origin of the samples used by Ibáñez *et al.* (2006) from Germany (Heidelberg and surroundings), which therefore represent *Myotis nattereri* s. str. in their molecular survey. One of the specimens representing *M. nattereri* s. str. in the current discriminant analyses comes from Baden-Würtenberg, which is less than 200 km away from the type locality. This, again, supports our a priori hypothesis that the corresponding morphogroup in our analyses represent the nominal species.

Koch (1863) further described two colour variants from Germany: var. '*typus*' and var. '*spealeus*' but both were considered as junior synonyms of *M. nattereri* s. str. by Miller (1912). As all these

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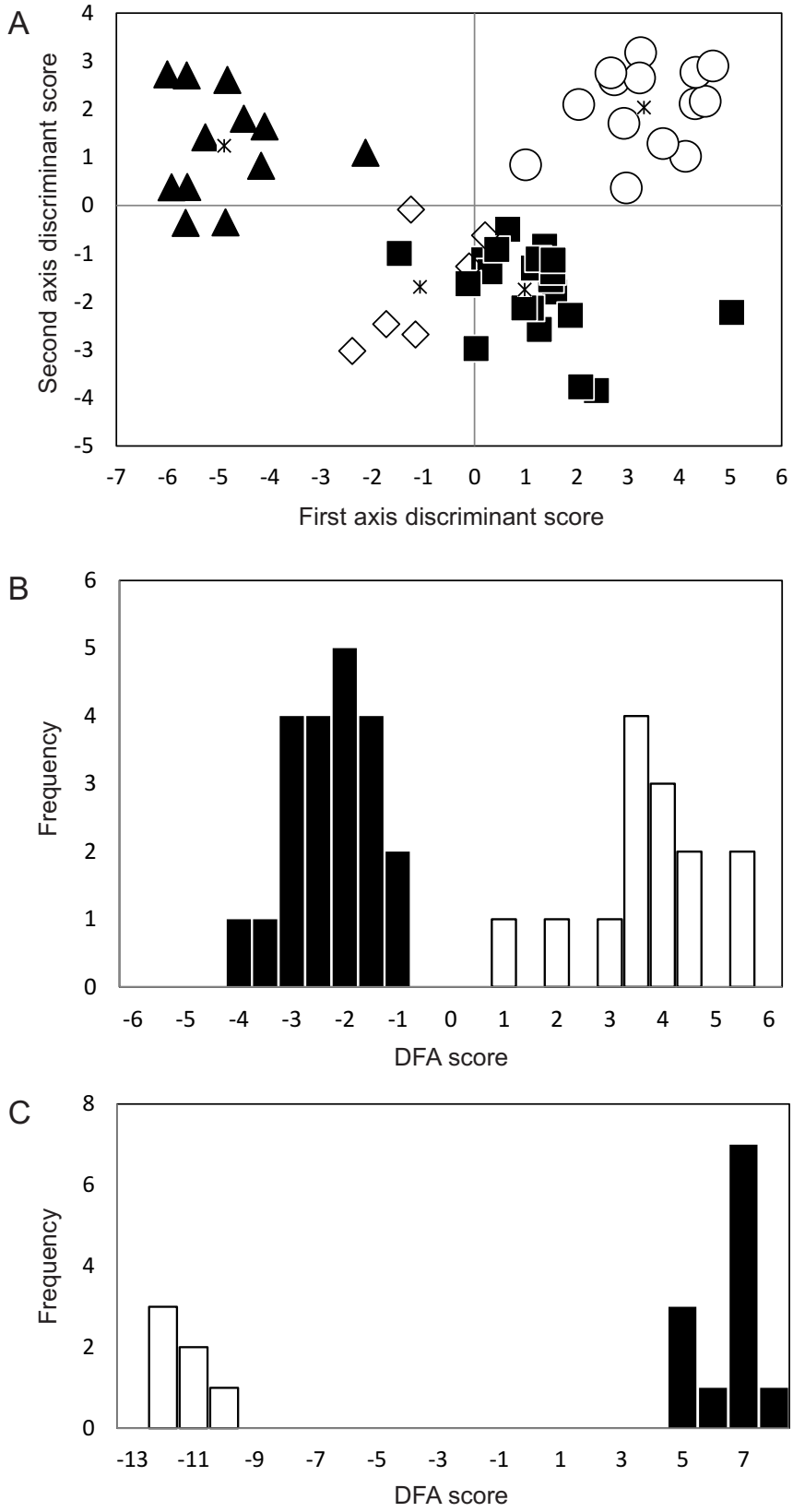


FIG. 2. Representation of the multivariate discriminant function analyses (DFAs) based on 16 craniodental variables measured for 53 skulls of the *M. nattereri* species complex: A — first and second discriminant functions for all the four lineages together: *M. nattereri* s. str. (white circles), *M*. sp. A (black squares), *M. escalerai* (black triangles), and *M*. sp. B. (white diamonds); stars represent the centroids for each group on this morphospace. B — Discriminant function contrasting *M. nattereri* s. str. (white bars) versus *M*. sp. A (black bars). C — Discriminant function contrasting *M. escalerai* (black bars) versus *M*. sp. B (white bars)

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forms were described from areas far from the known range of both *M.* sp. A or *M.* sp. B, they cannot be considered as potential names for them. Regarding the Iberian lineages, Cabrera (1904) first described the taxon *M. escalerai* from four syntypes without designating a holotype (Cabrera, 1912). Two of the syntypes originated from Foyos (Valencia) and two from Bellver (Lérida). This taxon was considered as junior synonym of *M. nattereri* by Miller (1912) and by Cabrera himself (Cabrera, 1914). Ibáñez and Fernández (1989) designated as a lectotype of *escalerai* the voucher from Valencia labelled MNCN 863, which is the only voucher from the original series still housed in the collections at the Natural Sciences Museum of Madrid (MNCN). The type locality of this taxon is therefore now restricted to Foyos, Valencia, which falls within the range of the molecular lineage ascribed to *escalerai* (Ibáñez *et al.*, 2006; Salicini *et al.*, 2011). Morphologically, specimens examined here and used in previous molecular studies also perfectly fit the original description of *M. escalerai*, notably concerning the wing membrane insertion on the base of the metacarpus (Puechmaille *et al.*, 2012) and the characteristics of the fringing hairs of the uropatagium (Agirre-Mendi and Ibáñez, 2012).

Recently, Allegrini and Puechmaille (2013) suggested that one of the names, *Myotis latipennis*, assigned by Crespon (1844) to a series of bats collected in southern France could be related to *M. emarginatus* or to *M. nattereri* s. l., and hence be a potential name for *M.* sp. A living currently in this area. However, it is not possible to identify objectively the species name corresponding to *latipennis* based on the ambiguous morphological description by Crespon. Furthermore, the vouchered bat bearing a label with "*latipennis*" and held in the collection housing Crespon's specimens (the Natural History Museum of Nîmes, France) is actually an immature *M. myotis* that does not correspond to Crespon's description (see Allegrini and Puechmaille, 2012 for details). Hence, the real type specimen corresponding to *M. latipennis* could not be located and has most likely been lost. For these reasons, we consider *latipennis* as a nomen dubium.

Likewise, there is currently no available name corresponding to the *M.* sp. B lineage living in north-western Africa, as bats from this region were always assigned to the nominal *M. nattereri* (e.g., Horáček and Hanák, 1984). Hence, as both *M.* sp. A and *M.* sp. B cannot be assigned objectively to any existing named taxon, we describe herein both as new to science.

Myotis crypticus sp. nov.
Ruedi, Ibáñez, Salicini, Juste and Puechmaille

Synonyms

Myotis nattereri (Kuhl, 1817): Miller (1912) (partim).
Myotis nattereri (Kuhl, 1817): Simmons (2005) (partim).
Myotis nattereri North Iberia (Kuhl, 1817): Ibáñez *et al.* (2006)
Myotis sp. (Mayer *et al.*, 2007).
Myotis sp. A (García-Mudarra *et al.*, 2009).
Myotis sp. A and Clade A (Salicini *et al.*, 2011).
Myotis sp. A and *M.* sp A (Salicini *et al.*, 2013).
Myotis sp. A and *Myotis* sp. C (Galimberti *et al.*, 2012).
Myotis sp. A (Puechmaille *et al.*, 2012).
Myotis sp. A (Allegrini and Puechmaille, 2013).
M. nattereri 2 (Kuhl, 1817): Ruedi *et al.* (2013).
Myotis nattereri (Kuhl, 1817): Bogdanowicz *et al.* (2015).

Holotype

One adult male (EBD 15974, with field number CI633) collected by C. Ibáñez on 7 August 1987 (Fig. 3A). It is prepared as a dry skin with skull removed. External measurements (in mm except for weight in g) are: W: 6.3; HB: 50; TL: 41; FA: 36.3; HF: 9; TIB: 16.8; EAR: 15.2; TRA: 8.4. Skull measurements are: GTL: 15.33; CBL: 14.35; CCL: 13.44; CM3: 5.85; M3M3W: 6.28; CCW: 4.07; ZB: 9.79; BCW: 7.83; MAB: 7.70; POC: 3.88; ROL: 4.78; M1M3: 3.62; ml: 11.08; cm3L: 6.22; m1m3L: 3.83; coh: 3.17. Tissue samples from this specimen are stored at -20°C. Sequences issued from extracted DNA are deposited in GenBank and include partial *Cytochrome b* (MK214766) and *COI* genes (MK214775), and the nuclear introns *ACOX2-3* (MK214786) and *COPS7A-4* (MK214795).

Type locality

Cueva Cerraúco, El Rasillo de Cameros, La Rioja, Spain (ca. 42°11'0"N, 2°44'20"W), 1400 m a.s.l.

Paratypes

Four paratypes, all from the same area near El Rasillo de Cameros, La Rioja, Spain, were captured by the same collector. They are housed in the EBD collections under the identification numbers: 1) EBD 15976: an adult female caught on 13 August 1987, with field number CI639 and conserved as dry skin with skull prepared separately, 2) EBD 16005: subadult male (based on skull ossification) caught on same date, with field number CI640 and conserved as dry skin with skull prepared separately), 3) EBD 17802: an adult female caught on 24 August 1988 with field number CI658 and conserved in alcohol with skull prepared separately, and 4) EBD 26174: an adult male captured on 12 August 2003

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without field number and conserved in alcohol with skull prepared separately. Sequences issued from DNA extracts of these specimens are deposited in GenBank and include, respectively, part of the *Cytochrome b* (MK214767–MK214770), *COI* (MK214776–MK214779), *ACOX2-3* (MK 214787–MK214790) and *COPS7A-4* (MK214796–MK214799).

Distribution

Animals identified as *M. crypticus* sp. nov. based on molecular characters were recorded in mountain areas of provinces of central and northern Spain (Salicini *et al.*, 2011), southern France (Salicini *et al.*, 2011; Puechmaille *et al.*, 2012), across the Italian Peninsula (Salicini *et al.*, 2011; Galimberti *et al.*, 2012) and probably to the adjacent southwestern parts of Austria (Mayer *et al.*, 2007). Based on nuclear genetic markers (unpublished data), marginal areas to the north and west of the Alps, e.g. in western Switzerland or Rhône-Alpes (France), are also occupied by *M. crypticus* sp. nov. However, the northern and eastern limits of the distribution of *M. crypticus* sp. nov. notably in relation to the occurrence of *M. nattereri* s. str., are unknown. Furthermore, as the populations from Sicily and southern Italy show important genetic discontinuities (Salicini *et al.*, 2013; Bogdanowicz *et al.*, 2015), they deserve further scrutiny as they might present further taxonomic complexity.

Diagnosis

Externally, the combination of a long, S-shaped calcar without epiblema, very long and pointed tragus, smooth and unnotched rear edge of ears and presence of stiff hairs along the uropatagium margin distinguish *M. crypticus* sp. nov. and other members of the *M. nattereri* species complex from all remaining Eurasian *Myotis* taxa. The wing membrane insertion at the base of the toe (Fig. 4) as described by Puechmaille *et al.* (2012) and the pattern of curved nature of the stiff uropatagial hairs (Fig. 5) further distinguish *M. crypticus* sp. nov. from species related to the *M. escalerai* clade. The skull shape is very similar to that of *M. nattereri* s. str. but is relatively more slender in *M. crypticus* sp. nov., particularly the rostral and occipital regions, which seem narrower in the latter species (Fig. 3). Finally, numerous diagnostic mutations in both mitochondrial and nuclear sequences (see Supplementary Fig. S1) clearly support the uniqueness of *M. crypticus* sp. nov. compared to any other species in this group.

Etymology

The epithet *crypticus* is derived from the Greek 'kryptos', which means hidden or concealed, in reference to this species' long history of remaining undetected.

Description

As the geographic limits and morphological distinction of the new species and *M. nattereri* s. str. are unclear in the eastern parts of their range, we base our description exclusively on specimens from Spain, where *M. crypticus* sp. nov. only co-occurs with *M. escalerai*, both of which can be told apart morphologically (see below). The general appearance of *M. crypticus* sp. nov. is of a medium-sized *Myotis* (forearm length 36–40 mm; body mass 5–12 g) characterized by the following features that are in common with other species of the *M. nattereri* species complex: relatively long, unnotched ears reaching slightly (3–4 mm) beyond the nose tip when laid forward; long, narrow and nearly straight tragus that is higher than half the conch height; pointed muzzle and areas around the eyes devoid of fur; uropatagium bordered by two parallel rows of stiff, slightly curled hairs (Fig. 5); calcar is long, slightly S-shaped and runs from the ankle to two-thirds the length of uropatagium border; relatively small feet, shorter than half of tibia length. Ears, wings and tail membranes are essentially naked, except close to the body. The wing membranes are joining the feet to the basis of the outer toe (Fig. 4), unlike in species related to *M. escalerai*, which have the membrane joining to the metatarsus (see Puechmaille *et al.*, 2012). The pelage is long and dense, but not wholly, clover-brown on the dorsum, and whitish ventrally with a sharp demarcation line running from the ear basis to the flanks. Individual hairs are bicolored, slaty-black along the shift from the basis to 2/3 of their length and the apical 1/3 of the shift pale brown (dorsal hairs) or whitish (ventral hairs). Although probably no single external character may distinguish both species with confidence, when compared to typical *M. nattereri* s. str., *M. crypticus* sp. nov. has slightly smaller dimensions and longer ears. Asian taxa morphologically related to the *M. nattereri* species complex, like *M. tschuliensis* or *M. schaubi*, are larger (forearm larger than 40 mm), while the Far Eastern *M. bombinus* is smaller (see Horáček and Hanák, 1984); all are also genetically very divergent (Puechmaille *et al.*, 2012; Ruedi *et al.*, 2013).

The skull is medium-sized for a *Myotis* (GLS 15.53 ± 0.34 mm); with sharply raising frontals and

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FIG. 3. Dorsal, ventral and lateral views of the cranium and lateral view of the mandible of A — the holotype of *Myotis crypticus* sp. nov. (EBD 15974) from Spain, B — *M. nattereri* s. str. (MHNG 1714.044) from Germany, C — the holotype of *M. zenatius* sp. nov. (EBD 29831) from Morocco, and D — *M. escalerai* (EBD 19877) from Spain. Scale bar = 5 mm. Photographs by M. Ruedi

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a globose braincase, devoid of sagittal or occipital crests; the summit of the skull is in front of the braincase, whereas it is more flat or the summit located further backwards in *M. nattereri* s. str. (Fig. 3). When viewed from below, the skull is relatively narrower, as expressed (see Table 2) by its breadth measured across molars (M3M3W), or mastoids (MAB) respect to the braincase (BCW). The rostrum is relatively longer (ROL 4.76 ± 0.2 mm) and narrower in *M. crypticus* sp. nov. No single craniodental character can unambiguously distinguish *M. crypticus* sp. nov. from *M. nattereri* s. str., but both species can be separated in multivariate space based on a combination of skull measurements (Fig. 2B). Dental formula (2/3, 1/1, 3/3, 3/3 = 38) and myotodont lower molars are typical for the genus.

Phylogenetically and based on the *Cytochrome b*, *M. crypticus* sp. nov. (= *M. nattereri* 2 in supplementary figure 2 of Ruedi *et al.*, 2013) is part of Clade II within the Old World *Myotis* radiation, and is the sister species of *M. nattereri* s. str. (= *M. nattereri* 1 in figure 1 of Ruedi *et al.*, 2013). Other mitochondrial and nuclear makers also support this sister-group relationship (Salicini *et al.*, 2011, 2013). Based only on mtDNA data, the genetically distinct *M.* sp. C from Corsica seems to be even more closely related to *M. crypticus* sp. nov. (Puechmaille *et al.*, 2012), but exact relationships of this allopatric lineage has not been investigated in any details so far. Likewise, no bioacoustic characters are known to differentiate *M. crypticus* sp. nov. from other congeners in the *M. nattereri* species complex (Puechmaille *et al.*, 2012).

Proposed vernacular names

Kryptisches Mausohr (German), cryptic myotis (English), murin cryptique (French), murciélago ratonero críptico (Spanish).

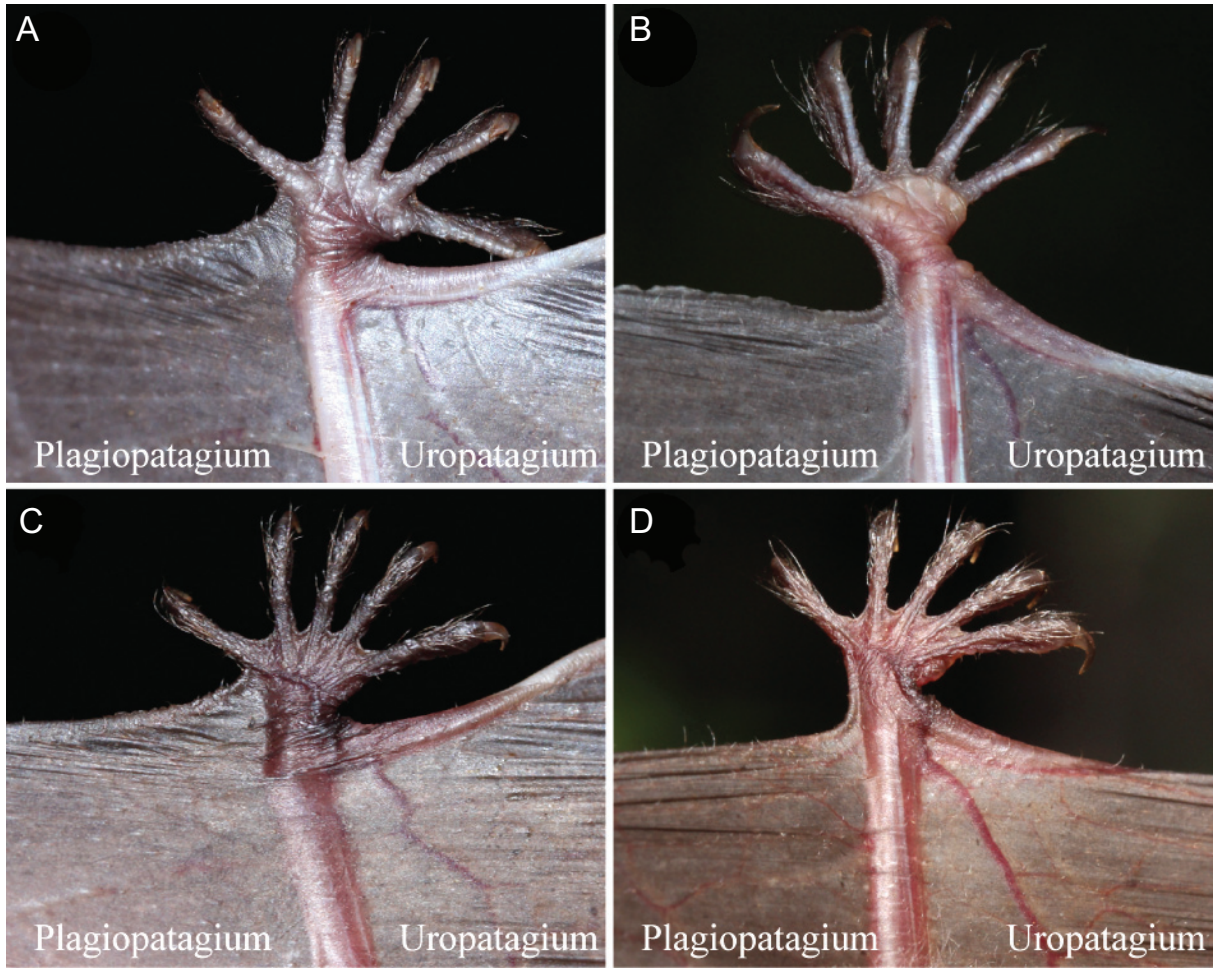


FIG. 4. Ventral (A, B) and dorsal views (C, D) of the different types of insertion of the plagiopatagium at the foot in the *M. nattereri* species complex. This insertion is at the base of the toe (A, C) in *M. crypticus* sp. nov., or at the mid-metatarsus (B, D) for *M. escalerai*. Only two species are represented as the insertion is similar between *M. zenatius* sp. nov. and *M. escalerai* and between *M. nattereri* s. str. and *M. crypticus* sp. nov. Photographs by S. J. Puechmaille

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Natural history

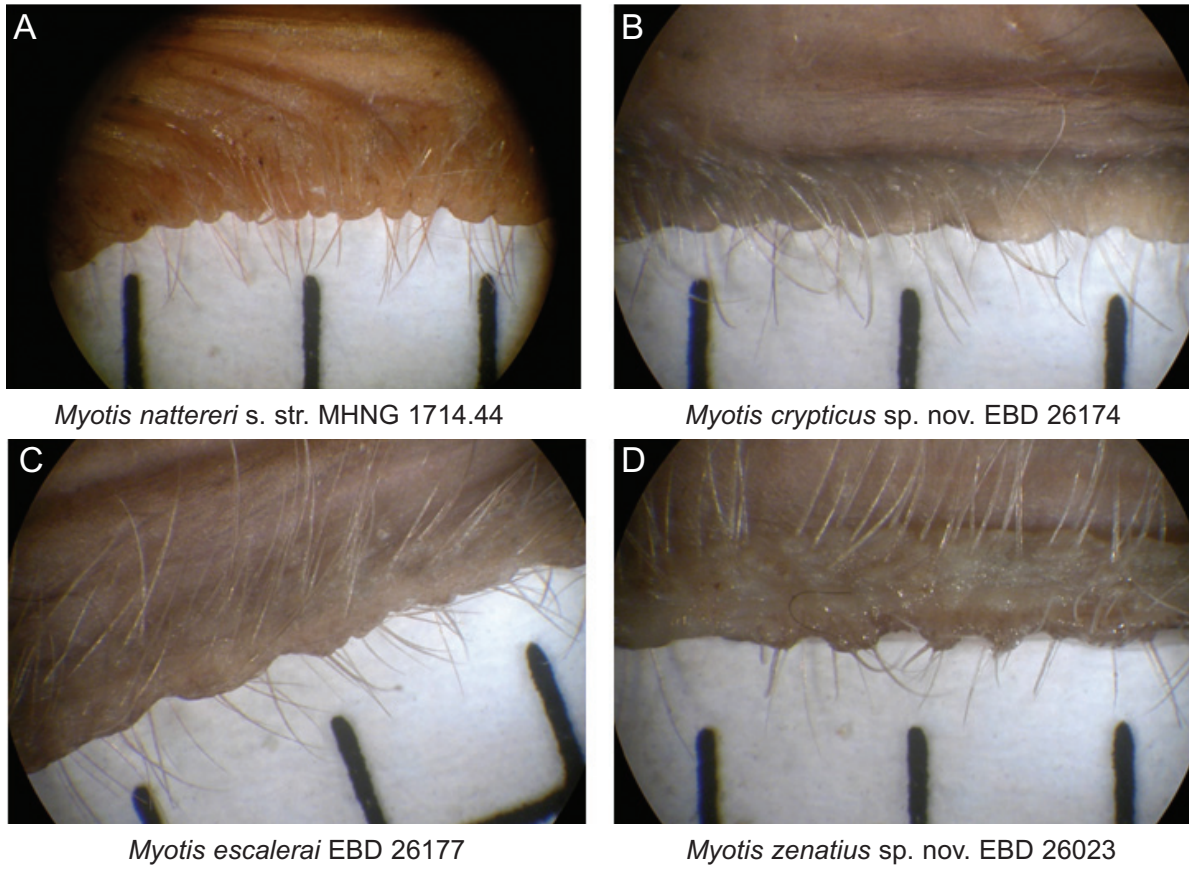
Little is known about the behaviour and habits of *M. crypticus* sp. nov. as it has not previously been distinguished from its sister species *M. nattereri* s. str. and specific studies on its ecology are yet to be conducted. However, and given the high degree of morphological similitude to it sister species, the new species most likely hunts in cluttered environments close to the substrate (Siemers and Schnitzler, 2000). In Western Switzerland, where *M. crypticus* sp. nov. might be the only representative of the species complex, it lives in forests at all altitudes and is considered a gleaner bat, feeding on various invertebrates, including spiders and caterpillars (Beck, 1991; Arlettaz, 1996). Similarly, in France and Italy, it is found in a broad altitudinal range from sea level to above 1,000 m a.s.l. (Puechmaille *et al.*, 2012; Salicini *et al.*, 2013). Around the type locality in Spain, *M. crypticus* sp. nov. is commonly found in dense forests of Pyrenean oak (*Quercus pyrenaica*) and beech (*Fagus sylvatica*) or in forests cleared for pasture but with still scattered old-growth trees, but also in subalpine prairies up to 2,000 m a.s.l. As far as we know, the new species roosts in tree hollows but breeding colonies may also occupy man-made structures such as unoccupied buildings in Western Switzerland (Gilliéron *et al.*, 2015).

In Switzerland, France, Italy, and Spain, individuals genetically identified as *M. crypticus* sp. nov. were observed gathering in large numbers with other species of *Myotis* at swarming sites in autumn, at altitudes ranging from 200 to 1,500 m a.s.l. They apparently overwinter in underground sites, hiding in crevices.

Myotis zenatius sp. nov.
Ibáñez, Juste, Salicini, Puechmaille and Ruedi

Synonyms

Myotis nattereri (Kuhl, 1817): Brosset (1963).
Myotis nattereri (Kuhl, 1817): Gaisler (1983).



Myotis nattereri s. str. MHNG 1714.44 | *Myotis crypticus* sp. nov. EBD 26174

Myotis escalerai EBD 26177 | *Myotis zenatius* sp. nov. EBD 26023

FIG. 5. Diagnostic patterns of fringing, stiff hairs present along the tail membrane in members of the *M. nattereri* species complex. In the related species A — *M. nattereri* s. str. and B — *M. crypticus* sp. nov. the hairs are simple, while in the other species pair, C — *M. escalerai* and D — *M. zenatius* sp. nov. an extra row of stiff and long hairs pointing inwards is also present. Scale bar = 1 mm. Photographs by C. Ibáñez

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Myotis nattereri (Kuhl, 1817): Gaisler (1983–1984).
Myotis nattereri (Kuhl, 1817): Horáček and Hanák (1984) (partim).
Myotis nattereri (Kuhl, 1817): Kowalski *et al.* (1986).
Myotis nattereri (Kuhl, 1817): Aulagnier and Thevenot (1986).
Myotis nattereri (Kuhl, 1817): Kowalski and Rzebik-Kowalska (1991).
Myotis nattereri (Kuhl, 1817): Horáček *et al.* (2000) (partim).
Myotis nattereri (Kuhl, 1817): Benda *et al.* (2004).
Myotis nattereri (Kuhl, 1817): Simmons (2005) (partim).
Myotis sp. B (García-Mudarra *et al.*, 2009).
Myotis nattereri (Kuhl, 1817): Dieuleveut *et al.* (2010).
Myotis sp. B and Clade B (Salicini *et al.*, 2011).
Myotis sp. B and Clade B (Salicini *et al.*, 2013).
Myotis sp. B (Puechmaille *et al.*, 2012).

Holotype

One adult male (EBD 29831, with field number 110718MspB12) collected by I. Salicini, C. Ibáñez and J. Juste on 18 July 2011 (Fig. 3C). External measurements are: HB: 46; TL: 42; FA: 40.0; HF: 8.2; TIB: 16.6; EAR: 16.5; TRA: 10.2. Skull measurements are: GTL: 15.21; CBL: 14.12; CCL: 13.21; CM3: 5.75; M3M3: 6.00; CCW: 3.78; ZB: 9.42; BCW: 7.77; MAB: 7.68; POC: 3.55; ROL: 4.37; M1M3: 3.46; ml: 10.91; cml: 11.03; cm3L: 6.14; coh: 3.27; m1m3L: 3.86. It is conserved in alcohol with skull removed. Tissue samples from this specimen are stored at -20°C. Sequences issued from extracted DNA are deposited in GenBank and include partial *Cytochrome b* (MK214774) and *COI* genes (MK214785), and the nuclear introns *ACOX2-3* (MK214794) and *COPS7A-4* (MK214803).

Type locality

Mizou Cave, Tetouan, Morocco (ca. 35°30’42”N, 5°19’53”W), at an altitude of 330 m a.s.l.

Paratypes

A total of five paratypes were collected, three of them are from the same cave, date and collectors as the holotype and include: EBD 29826, an adult female, dry skin and skull removed; EBD 29829, an adult male in alcohol with skull removed; and EBD 29830, an adult female in alcohol with skull removed. The other two paratypes are EBD 26023, a male in alcohol with skull removed, collected on 11 July 2002 from Kef Aïssa, Bir-Reggada Forest House, 12 km W Imouzzer Kandar (33º42’05”N, 5º06’41”W), 1250 m a.s.l. and EBD 26020, a female in alcohol with skull removed, collected on 10 June 2002 in Wintimdouine Cave, Agadir (30º40’50”N, 9º20’42”W), 1350 m a.s.l. Sequences issued from extracts of these specimens are deposited in GenBank and include, respectively, part of the *Cytochrome b* (MK214771–MK214773, JN591502, JN591503), *COI* (MK214780–MK214784), and the nuclear introns *ACOX2-3* (MK214791–MK214793, JN601559, JN601560) and *COPS7A-4* (MK214800–MK214802, JN601627, JN601628).

Distribution

The species *Myotis zenatius* sp. nov. is probably endemic from the Mediterranean region of Morocco and Algeria, and possibly Tunisia. In Morocco it is very rare, known only from three localities in the central part and western coast (Benda *et al.*, 2004), and from one locality in the hills of Rekkam, in the eastern part of the country (Dieuleveut *et al.*, 2010). We add in this study three new localities (see Supplementary Table S1) plus another one in Azrou (Ait-Sebaa) where six additional individuals were biopsied and released. In the Appendix of Salicini *et al.* (2013), the species is wrongly mentioned from Errachidia. The mistake stems from the switching between the similar names of two caves, Kef Azigza (near Errachidia) where *M. zenatius* sp. nov. does not occur, and Kef Aïssa (the correct locality). The species is thus rare but widely distributed across Morocco from the northern slopes of the Riff (near Tetouan) to the dry mountains of the Great Atlas (e.g. Wintimdouine Cave). The Atlas Mountains apparently delineate two distinct haplogroups (Salicini *et al.*, 2013) that may represent distinct subpopulations. We can assume that the Algerian populations previously classified as *M. nattereri* (see e.g., Kowalski and Rzebik-Kowalska, 1991) represent *M. zenatius* sp. nov., given that the distance between the easternmost known locality from Morocco (Rekkam Hills — Dieuleveut *et al.*, 2010) is only around 200 km far from the westernmost known locality from Algeria (near Tlemcen — Kowalski *et al*., 1986). The new species occurs in the northern parts of Algeria, where it is known only from three localities (Kowalski and Rzebik-Kowalska, 1991; Ahmim, 2017). As no specimen from this country has been analysed genetically, it is however unknown to which extent these represent interconnected or isolated subpopulations.

Diagnosis

Externally, *M. zenatius* sp. nov. shares all the characters previously referred to for *M. crypticus* sp. nov. that distinguish this and the other members of the *M. nattereri* complex from all remaining European *Myotis* taxa. The wing membrane is inserted in the mid-metatarsus (Fig. 4) as in *M. escalerai* but contrary to *M. crypticus* sp. nov. and *M. nattereri* s. str. that have the wing membrane inserted

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at the base of the toe (Puechmaille *et al.*, 2012). This character was first used by Cabrera (1904) in the original description of *M. escalerai* and later validated by Puechmaille *et al.* (2012). Similarly, the characteristic stiff fringing hairs bordering the tail membrane show the same distinct pattern described for *M. escalerai* by Agirre-Mendi and Ibáñez (2012); accordingly, the hairy edge of this membrane looks thicker than in *M. nattereri* and *M. crypticus* sp. nov. due to the presence of an additional line of relatively long and conspicuous stiff hairs facing inwards (Fig. 5). The sharing of these two morphological characters between *M. escalerai* and *M. zenatius* sp. nov. is in agreement with their phylogenetic relationships, which place them as sister species, while they are distinct from *M. nattereri* s. str. and *M. crypticus* sp. nov. Nevertheless, the darker and more greyish dorsal fur colour in adult *M. zenatius* sp. nov. (resembling a juvenile coloration in other *Myotis* species) distinguishes the new species from adult *M. escalerai*. Again, the skull morphology is very similar between *M. zenatius* sp. nov. and *M. escalerai*, but is in general more delicate in the new species and slightly smaller in all dimensions (as previously described by Benda *et al.*, 2006), except for the postorbital constriction which is wider in *M. zenatius* sp. nov. (Table 2). The braincase is also relatively broader and more globose in the new species than in *M. escalerai* (Fig. 3 and Supplementary Fig. S2).

Etymology

The epithet *zenatius* is derived from the word 'Zanatah' which refers to a little known Berber tribe that lived in the Maghreb region of North Africa in the Middle Ages. The Zanatah people were famous for their horse riding skills and mobility.

Description

Myotis zenatius sp. nov. is a medium-sized member of the genus (forearm length 38–40 mm), very similar to the other species of the *M. nattereri* species complex. As in the other species within this complex, *M. zenatius* sp. nov. has relatively long, unnotched ears reaching slightly extending beyond the nose tip when laid forward. The tragus is long, narrow and nearly straight or slightly curved; the muzzle is pointed and the face shows furless the areas around the eyes. The edge of the uropatagium shows two rows of stiff, slightly curled hairs facing outward and a distinctive row of long hair facing inward; the calcar is long and S-shaped running from the ankle to two-thirds the length of uropatagium; relatively small feet, shorter than half of tibia length. Ears, wings and tail membranes are essentially naked, except close to the body. The wing membrane is joining the feet to the mid-metatarsus as in the related species *M. escalerai*. The dorsal fur is relatively long and particularly dark-greyish brown (as in immature animals of other species), this coloration contrasts sharply with the whitish colour ventrally on the flanks. The individual hairs are tricolored slate-black basally, brown medially and whitish at the tips. Probably the most useful single external character that distinguishes *M. zenatius* sp. nov. from *M. escalerai* is its darker dorsal fur.

The skull is delicate and medium-sized for a *Myotis* (GLS 15.37 ± 0.28 mm); the cranium has a globose braincase and is devoid of sagittal or occipital crest (Fig. 3C); the frontal bone is raising to the braincase relatively sharply to the summit, which is located in the front part, whereas the braincase appears more flattened in its close relative *M. escalerai* (Fig. 3C–D); when viewed from above, the braincase is also relatively wider in *M. zenatius* sp. nov. than in *M. escalerai*, despite that most skull dimensions are larger in the later. In occlusal view, the cranium is relatively narrower and the rostrum relatively longer. Again, no single craniodental character may distinguish clearly *M. zenatius* sp. nov. from *M. escalerai*, but both species can be easily separated in multivariate space based on a combination of skull measurements (Fig. 2A and 2C, and Supplementary Fig. S2). Dental formula (2/3, 1/1, 3/3, 3/3 = 38) and myotodont lower molars are typical for the genus. Finally, numerous diagnostic mutations in both mitochondrial and nuclear sequences (see Supplementary Fig. S1) clearly support the species identity as opposed to the close relative species. Phylogenetically *M. zenatius* sp. nov. shows sister-group relationship with *M. escalerai* supported by mitochondrial and nuclear makers (Salicini *et al.*, 2011, 2013; Puechmaille *et al.*, 2012) and appears more closely related to *M. schaubi* than to the 'nattereri' group (Salicini *et al.*, 2011).

Proposed vernacular names

Zenati Mausohr (German), Zenati myotis (English), murin Zenati (French), murcielago ratonero Zenate (Spanish).

Natural history

Very little is known about this species, which is one of the rarest bats in the Mediterranean. As most identified individuals were captured at cave roosts, including breeding females from nursery colonies,

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it shares with *M. escalerai* troglophilous habits throughout the year. Such strong cave-dwelling habits mark a significant ecological contrast with the other two species (*M. nattereri* s. str. and *M. crypticus* sp. nov.), which roost preferably in tree holes during the summer. Furthermore, the maternity colonies of *M. zenatius* sp. nov. can reach up to 300 individuals (Kowalski *et al*., 1986; authors' unpublished data), which are distinctly larger than the tree-dwelling species.

Evolutionary perspective

From a biogeographic point of view, the deep genetic differentiation and current distribution of members of the *M. nattereri* species complex mirror the situation found in several other organisms living in temperate areas (Hewitt, 2004, 2011). Given the phylogeographic patterns evidenced in previous molecular reconstructions (Puechmaille *et al.*, 2012; Salicini *et al.*, 2013; Bogdanowicz *et al.*, 2015; Razgour *et al.*, 2015), the following scenario of diversification can be proposed: a first, deep split occurred between ancestors of the two species pairs in the late Pliocene (ca. 2.5 MYA — Salicini *et al.*, 2013), which is supported by the fossil record reporting the existence of two *nattereri*-type forms differing in size in European deposits of this epoch (Horáček and Hanák, 1984). For each lineage, further speciation events (that were not necessarily synchronous) occurred during the intense glacial-interglacial cycles along the Pleistocene (Klotz *et al.*, 2006; Razgour *et al.*, 2015), when the new forms survived in distinct refugia (i.e. *M. nattereri* s. str. in the Balkans or Anatolia, *M. crypticus* sp. nov. and *M. escalerai* in the Appennine and Iberian Peninsula respectively, and *M. zenatius* sp. nov. in the Maghreb). They accumulated mutations and retained their independent evolution even during the interglacial period, when some of those lineages recolonized the northern parts of Europe. Currently, the only known area of sympatry between members of this species complex is Iberia and parts of southwestern France, where the distributions of *M. escalerai* and *M. crypticus* sp. nov. overlap (Puechmaille *et al.*, 2012; Salicini *et al.*, 2013). Although externally these two species are similar, they are not sister species (Fig. 1) and have distinct ecologies including different roosting and altitudinal preferences (Razgour *et al.*, 2015).

Conservation

The recognition of *M. crypticus* sp. nov. and *M. zenatius* sp. nov. as full and independent biological species within the *M. nattereri* species complex has two important implications for conservation. Firstly, considering that a substantial portion of Western and Southern Europe, the Balearic Islands, and North Africa are now known to be occupied by other species (i.e. *M. escalerai*, *M. crypticus* sp. nov. or *M. zenatius* sp. nov.) and a possibly further still undescribed species (*M.* sp. C) exists in Corsica (Puechmaille *et al*., 2012), the current range of *M. nattereri* s. str. is now restricted to a fraction of its previously known range; its status as 'least concern' under the UICN criteria should therefore be revised. Conversely, the second implication for conservation is that the recognition of *M. crypticus* sp. nov. and *M. zenatius* sp. nov. as species on their own implies the urgent need to evaluate their populations status under the criteria of the IUCN in order to receive appropriate attention for protection. Regarding *M. zenatius* sp. nov., the situation may be already worrisome given its general scarcity (considered one of the rarest bats in the whole of Africa, Kowalski *et al.*, 1986) and vulnerability as a strict cave-dweller species. For instance, the type locality of the species in Morocco is at the edge of an intensively exploited quarry with an unknown effect on the bats population while the taxonomic assignation of the Algerian populations still needs to be ascertained with appropriate methods. In the case of *M. crypticus* sp. nov., as the oriental and northern limits in Europe are still unexplored, it is a priority to delineate its exact distribution, and examine the nature of biological and ecological interactions it may have with the allopatric (or possibly parapatric) *M. nattereri* s. str. Furthermore, potential diagnostic morphological or echolocation characters are still lacking to differentiate those species in the field, which poses serious challenges for their protection (see Ashrafi *et al.*, 2010). Finally, the divergent mitochondrial lineages found in *M. crypticus* sp. nov. from Italy (Salicini *et al*., 2013; Bogdanowicz *et al*., 2015) need to be examined in further details across the Appenine Peninsula to clarify their taxonomic status.

Supplementary Information

Contents: Table S1. External and cranio-dental measurements of specimens used in the present study; Table S2: Unstandardized coefficients and correlations of 16 craniodental variables in the discriminant functions aimed to maximize the differences in the four groups of the *M. nattereri* species complex defined by molecular characters. The discriminant functions for each axis were obtained in a discriminant function analysis (DFA) based on the 16 skull variables measured for 53 *Myotis* previously assigned to the four European lineages;

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Table S3. Unstandardized coefficients for the 16 craniodental variables used in discriminant functions aimed to maximize differences between the species. Fig. S1. DNA sequences alignment of partial *Cytochrome b*, partial *COI*, and nuclear markers (*ACOX2-3*, *COPS7A-4*). Only variable positions are shown to highlight differences between individuals/species; Fig. S2. Bivariate representation of the width of the cranium measured (mm) across the canines (CCW) and the breadth of the braincase (BBW) of *M. escalerai* (black triangles) and of *M. zenatius* sp. nov. (white circles). Supplementary Information is available exclusively on BioOne.

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