

Evolutionary ecology of hares (*Lepus* spp.) from northwest Africa: the existence of cryptic species and description of a new species (*Lepus saharae* sp. nov.)

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ABSTRACT

Objective: We examine the different pressures that lead to the occurrence of new species and thus to their phylogenies. From an evolutionary ecology perspective, we examine the phylogenetic relationships of different lineages, as well as the evolutionary pressures that lead to them and, in some cases, mask them.

Organisms and locales: Hares (*Lepus* spp.) in northwest Africa that are morphologically similar, which, superficially, makes it appear that there is only one species.

Methods: We describe different populations of hares in northwest Africa. We describe the morphology of a new species and provide photographs of specimens from across the study area. We analyse five mitochondrial DNA fragments.

Results: Mitochondrial DNA analysis revealed the presence of three *Lepus* species which are different from both *Lepus capensis* and *L. victoriae* and which have contiguous, partially overlapping geographical ranges. One of the species, found in the western Sahara (Morocco), is new (*Lepus saharae* sp. nov.). The other two species (*Lepus mediterraneus* Wagner, 1841 and *Lepus schlumbergeri* Remy-St. Loup, 1894) inhabit the northern and central regions of Morocco. The distributions of at least two of the three species extend to countries other than Morocco. Various eco-evolutionary pressures have masked this diversity until now. Certainly, the species have had to adapt to different habitats that include mountains and desert; and isolation of populations by distance, or because of climatic or geographical barriers has led – or at least facilitated – them to appear different. Yet some evolutionary pressures have made them converge morphologically, making it appear that there is only one species.

Keywords: Africa, DNA, holotype, *Lepus mediterraneus*, *Lepus saharae*, *Lepus schlumbergeri*, phylogeny.

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INTRODUCTION

In this paper, we show that Morocco has more hare species (genus *Lepus*) than previously thought. The failure to recognize this diversity is the result of close phenotypic similarity among species. We consider the evolutionary pressures that lead to such similarity.

Given their intrinsic characteristics, hares are not always easy to classify taxonomically. Indeed, hares from the same geographical area may be classified under different scientific names, and sometimes to the level of species by one author and to the level of subspecies by another. Morphological and phenotypic similarity between species (Flux, 1983; Palacios, 1989, 1996; Flux and Angemann, 1990; Suchentrunk *et al.*, 2008; Awadi *et al.*, 2016; Ben Slimen *et al.*, 2017) can make systematic classification difficult, despite various molecular analyses (Halanych and Robinson, 1999; Pierpaoli *et al.*, 1999; Wu *et al.*, 2000; Yamada *et al.*, 2002; Alves *et al.*, 2003, 2006; Waltari *et al.*, 2004; Kasapidis *et al.*, 2005; Melo-Ferreira *et al.*, 2005, 2009, 2011, 2014b; Waltari and Cook, 2005; Ben Slimen *et al.*, 2006, 2008a, 2008b; Kong *et al.*, 2014; Awadi *et al.*, 2016; Tolesa *et al.*, 2017). Therefore, relations between some groups remain unclear. The relevant literature reports introgressive hybridization among species (Melo-Ferreira *et al.*, 2014b; Tolesa *et al.*, 2017), signals of reticulate evolution, mainly in mitochondrial DNA (mtDNA) but also in the nuclear genome (Thulin *et al.*, 1997, 2006a, 2006b; Alves *et al.*, 2008; Melo-Ferreira *et al.*, 2009, 2012, 2014a, 2014b, 2014c; Liu *et al.*, 2011; Wu *et al.*, 2011; Tolesa, 2013), and evidence for shared ancestral polymorphisms (Pérez-Suárez *et al.*, 1994; Suchentrunk *et al.*, 2008; Melo-Ferreira *et al.*, 2012; Tolesa *et al.*, 2017), which together make interpretation of their phylogenetic relations less than straightforward.

In Africa, there are currently six acknowledged species, including the cape hare (*Lepus capensis* Linnaeus, 1758), first described in South Africa. *Lepus capensis* is found in large parts of Africa, the Arabian Peninsula, the Middle East, and East and West Himalayas (Wilson *et al.*, 2016). It is also believed to inhabit parts of the Far East and was once thought to be conspecific with the European brown hare (*Lepus europaeus* Pallas, 1778) (Petter, 1959, 1961; Ben Slimen *et al.*, 2005, 2006, 2008b; Wilson *et al.*, 2016). Recent publications consider the hares of North Africa to be *Lepus capensis*, *Lepus mediterraneus* Wagner, 1841, or *Lepus* sp. (Wilson *et al.*, 2016; Aulagnier *et al.*, 2017; Lado *et al.*, 2019). Based on molecular data, some studies (mtDNA PCR-RFLP, allozymes or microsatellites) support the notion that *L. capensis* is the only species of hare in North Africa (Ben Slimen *et al.*, 2005, 2006, 2008b; Ben Slimen, 2008). Other studies, based mainly on mtDNA or sometimes supported by nuclear DNA or microsatellite data, are congruent with the hypothesis that there are different species. Another common name often applied to the hares of North Africa is that of *Lepus mediterraneus* (Pierpaoli *et al.*, 1999; Scandura *et al.*, 1999; Alves *et al.*, 2003; Ben Slimen *et al.*, 2007; Ben Slimen, 2008). The related literature also highlights further taxonomic names for the hares of this area, though most of these studies provide very little morphological detail based on just one or at most a few specimens. Some of these descriptions of new species have often been made without recourse to previous research (de Winton, 1898; Cabrera, 1932). The various taxonomic names that have been proposed for the hares of North Africa include: *Lepus schlumbergeri* Remy-St. Loup, 1894; *Lepus atlanticus* de Winton, 1898; *Lepus kabylicus* de Winton, 1898; *Lepus pallidior* Barrett-Hamilton, 1898; *Lepus tunetae* de Winton, 1898; *Lepus harterti* Thomas, 1903; *Lepus sherif* Cabrera, 1906; *Lepus maroccanus* Cabrera, 1906; *Lepus sefranus* Thomas, 1913; *Lepus pediaeus* Cabrera, 1923; and *Lepus whitakeri* Thomas, 1902. A classification at the subspecies level has also been proposed that includes *Lepus schlumbergeri* ssp. and *Lepus capensis* ssp. (Cabrera, 1932; Aulagnier *et al.*, 2017). Finally, the African savanna hare (*Lepus victoriae* Thomas, 1893) is also reported to be present in the western Sahara (Morocco) (Wilson *et al.*, 2016).

As the taxonomic position of northwest African hares requires further clarification, this paper investigates the evolutionary pressures that have contributed to their formation.

MATERIAL AND METHODS

Samples

Hair and tissue were obtained from 18 hares collected in Morocco between 2013 and 2018 (see Table S1 at: evolutionary-ecology.com/data/3207Appendix.pdf). All samples were taken from hares found dead after being hit by motor vehicles (i.e. roadkill). Photographs of specimens retrieved from throughout the study area were taken by one of the authors or using camera traps.

DNA extraction, amplification and sequencing

DNA was extracted from hair and tissue samples using the protocols and materials for DNA isolation in accordance with the Invisorb® Spin Forensic Kit (STRATEC Biomedical AG, Berlin). Five mtDNA fragments were amplified by polymerase chain reaction (PCR). The fragments selected for the mtDNA analyses were 12S and 16S ribosomal DNA (rDNA), control region (CR), cytochrome b (CYTB), and cytochrome c oxidase subunit I (COI). For one specimen, only the control region was obtained because the sample was in poor condition. Information about primers, PCR conditions and references is provided in Appendix Table S2. PCR products were obtained from Research Technical Services (RTS) at the University of Alicante, Spain (<https://ssti.ua.es/en/scientific-instrumentation/genomics-and-proteomics-unitt.html>). The PCR products were purified and sent to MacroGen (<http://www.macrogen.com/eng/>) for sequencing. Composite sequences of both strands were obtained for each sample and the necessary repetitions were run until the results could be verified.

Mitochondrial DNA analyses

Sequences were checked by eye, aligned using BioEdit 7.2.5 and ClustalW (Hall, 1999), and deposited in GenBank (Benson *et al.*, 2013) (accession numbers available in Appendix Table S3). In the present study, 2947–2954 base pairs (bp) were obtained (Table 1). Our sequences were aligned with others from GenBank (Appendix Table S3) and the European rabbit (*Oryctolagus cuniculus* Linnaeus, 1758) was considered to be an outgroup. The size of the final dataset for analysis was reduced after alignment with the other sequences and removal of any positions containing gaps or missing data.

Haplotypes (h) were calculated using the DnaSP 5.10 software (Librado and Rozas, 2009). Pairwise genetic distances were calculated by MEGA 7 (Kumar *et al.*, 2016) with 2000 bootstrap replications. The best models of nucleotide substitution were determined by jModelTest 2.1.7 (Darriba *et al.*,

Table 1. Consensus size of the sequences obtained in this study

Gene	Consensus size (base pairs)
12S ribosomal DNA (rDNA)	390–392
16S ribosomal DNA (rDNA)	527–569
Cytochrome c oxidase subunit I (COI)	659
Control region (CR)	445–448
Cytochrome b (CYTB)	926
All mitochondrial DNA (mtDNA)	2947–2954

2012) using 24 models of evolution based on both the Akaike Information Criterion (AIC) and the Bayesian Information Criterion (BIC).

Bayesian phylogenetic trees were constructed using BEAUti and the StarBEAST2 package available in BEAST 2.4.8 (Ogilvie *et al.*, 2017). The DNA mutation rate was estimated assuming a divergence of 11.8 million years (Myr) between *Lepus* sp. and *O. cuniculus* (Mathee *et al.*, 2004), and using the molecular clock equation $T = K/(2r)$ (Li, 1997), where T = divergence time in years, K = genetic distance, and r = rate of nucleotide substitutions. To construct the phylogenetic trees using the combined mtDNA, the specific mutation rate of each fragment was entered into BEAUti. To calculate the specific rates, the genetic distances (K) between hares and the outgroup were also calculated for each fragment using MEGA 7. We used an uncorrelated lognormal clock (Drummond *et al.*, 2006) and the Yule tree prior, and performed two independent replicate runs of 250 million generations each. The results were checked using Tracer v1.7.1 (Rambaut *et al.*, 2018) and consensus trees were generated with TreeAnnotator v2.4.8 (Ogilvie *et al.*, 2017). The first 10% of sampling trees were considered burn-in and discarded, and the resulting trees were visualized in FigTree v1.4.2 (<http://tree.bio.ed.ac.uk/software/figtree/>).

The parameters used to construct the Bayesian phylogenetic trees are shown in Appendix Table S4. Trees from the control region (CR) and cytochrome b (CYTB) were constructed to increase the number of sequences of hares available in GenBank from the study area. For the CR, the resulting tree was constructed with 137 haplotypes (382 bp) representing 153 hares while for CYTB, the resulting tree had 168 haplotypes (409 bp) representing 327 hares. For both CR and CYTB, *O. cuniculus* was also included as an outgroup.

Two phylogenetic trees based on combined mtDNA were also constructed, one with and one without the addition of a sequence belonging to an African savanna hare (*L. victoriae*). In the latter case, it was constructed with 55 sequences including the outgroup (2890 bp). Each sequence includes the 12S and 16S rDNA, COI, CR, and CYTB fragments. When adding the sequence belonging to *L. victoriae*, the resulting tree had 56 sequences including the external group (1807 bp). The combined fragments were 12S and 16S rDNA, COI, and CYTB, but not CR since there are no CR sequences for *L. victoriae* in GenBank. This second analysis, although shorter, was performed to clarify the phylogenetic relationship of *L. victoriae* to the other hares found in Morocco.

The relations between haplotypes were estimated by a parsimonious median-joining (MJ) method (Bandelt *et al.*, 1999) with default parameter settings using Network 5.0.1.1 (<http://www.fluxus-engineering.com>). The maximum parsimony (MP) calculation was run in all the files produced by the MJ network calculations. The median-joining networks were constructed to better visualize the relationships among hares.

The networks for CYTB (with 131 haplotypes representing 264 sequences of 409 bp) and COI (with 139 haplotypes representing 248 sequences of 626 bp) were constructed to increase the number of sequences of hares available in GenBank from the study area. We included the European brown hare (*L. europaeus*) in this network. Another network with 50 haplotypes (representing 54 sequences of 2899 bp) was constructed by combining the 12S and 16S rDNA, COI, CR, and CYTB fragments, and a final one in which *L. victoriae* was included, resulting in a network with 43 haplotypes representing 55 sequences (1810 bp) without the CR fragment. Both were constructed from the same data as the combined mtDNA phylogenetic trees.

Morphological assignment of a new species

One hare (isolate LsahMoroc3) obtained for this work has been described morphologically. This specimen is the holotype of the species and is deposited in the Museum at the Higher School of

Technology in Laâyoune, Morocco (#REF 021/2019 Awserd and 022/2019 Awserd for the skin and skull respectively) for its conservation.

RESULTS

Phylogenetic analyses

Based on molecular and some morphological data, the results of our analyses suggest the existence of three hare species in Morocco (including the western Sahara area). These species are found in three geographical areas: northern (NM), central (CM), and southern Morocco (SM) (Fig. 1). The hares in northern Morocco (NM, green in Fig. 1) have been grouped with hares from North Africa (Tunisia, Libya, and Egypt) and Sardinia (Italy). The central zone (CM, red in Fig. 1) runs south from the NM zone and continues into the northern half of the western Sahara. (Fig. 1) runs south from the NM zone and continues into the northern half of the western Sahara.

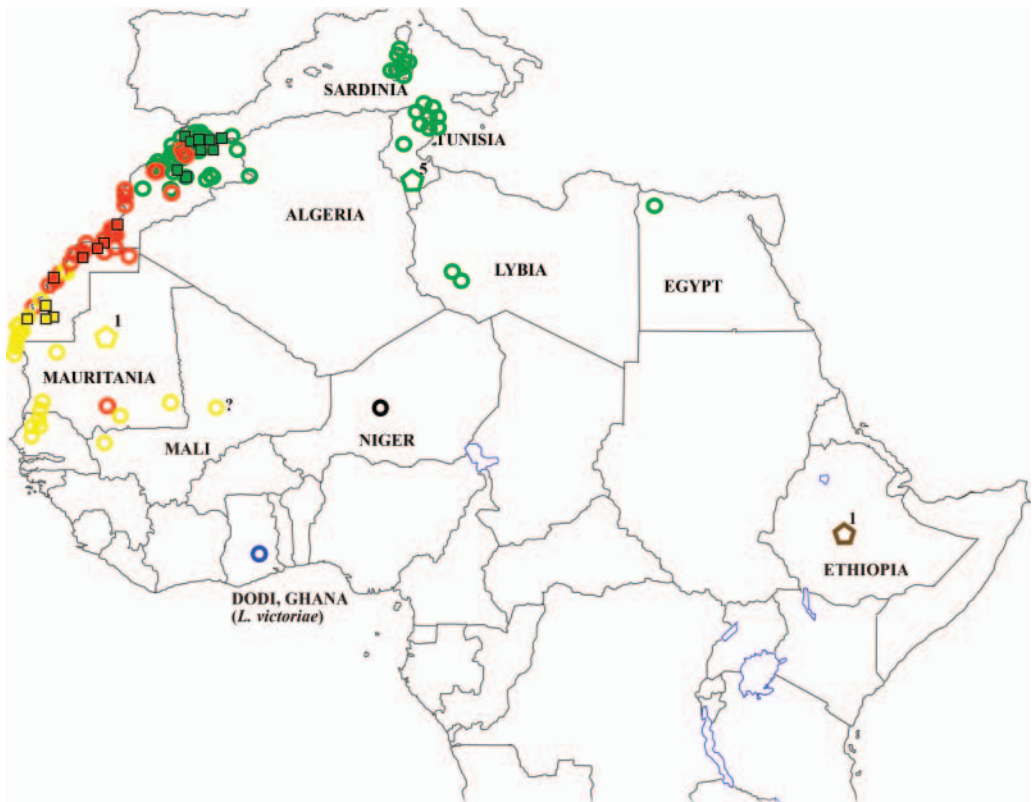


Fig. 1. Geographical distributions of Moroccan hares and other hares closely related to them based on the present study. The map was compiled according to the groupings obtained from the phylogenetic trees and networks. Squares represent hares from the present study, while circles and pentagons denote hares from other studies; circles represent hares from a specific geographical area, while pentagons denote hares from a country with unspecified geographic area. Green = hares related to those from northern Morocco (NM), red = hares related to those from the central zone (CM), and yellow = hares related to those from the southwestern Sahara (SM).

The hares in the southern zone (SM, yellow in Fig. 1), which includes the southwestern Sahara, have been grouped with hares from Morocco, Mauritania, Mali, and Senegal. The hares in the central zone have slightly overlapping geographical ranges both to the north and to the south.

The phylogenetic trees of CR and CYTB mtDNA (Figs. 2 and 3) reveal different sequences for African hares. In the CR tree, one Egyptian hare is grouped more closely with the hares from

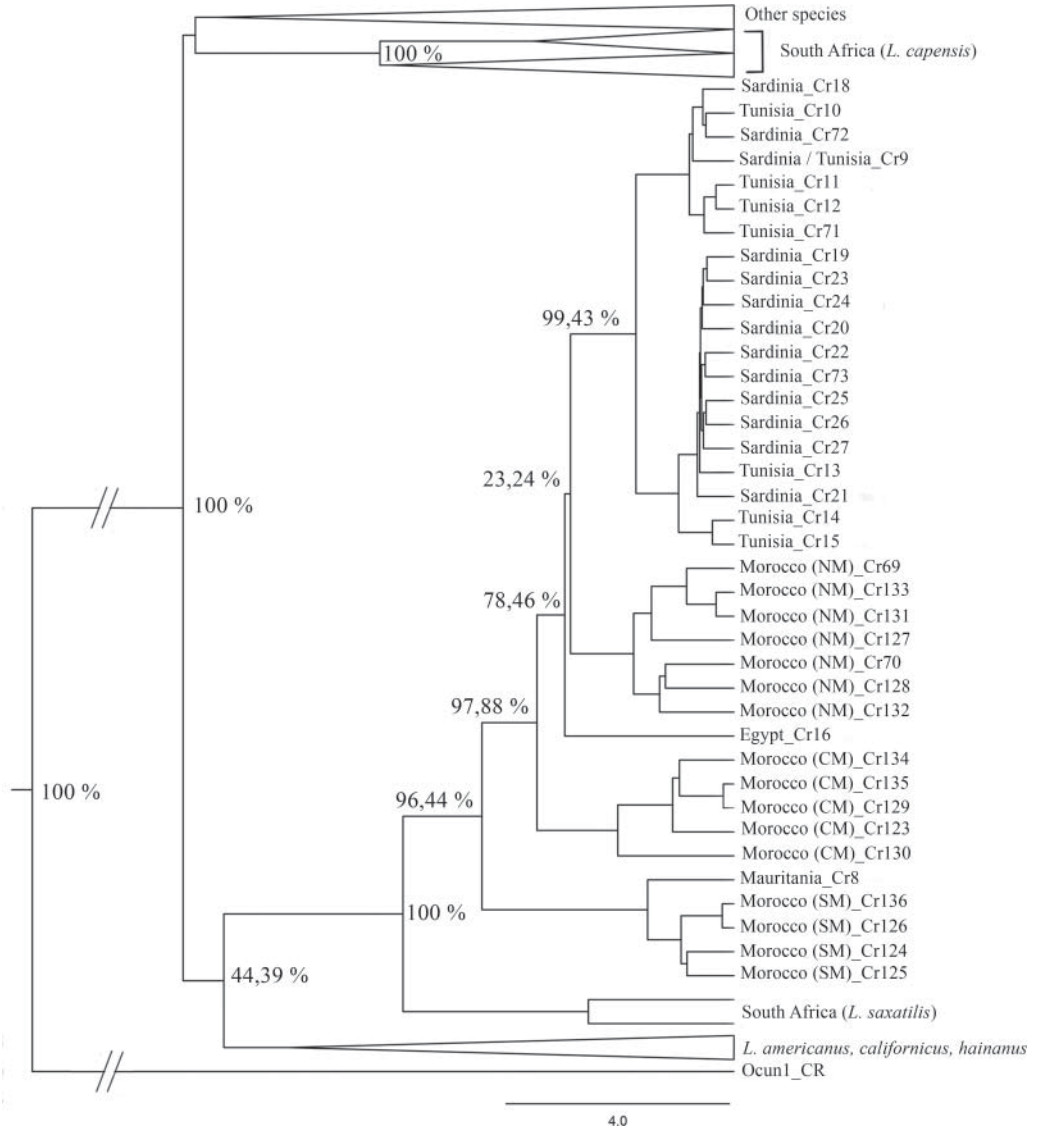


Fig. 2. Bayesian phylogenetic tree based on CR mtDNA. The phylogenetic tree was constructed with 137 haplotypes (382 bp), representing 153 hares and with the outgroup (*Oryctolagus cuniculus*) included. Numbers in nodes indicate posterior values as a percentage (%). Names of sequences correspond to those of the sequences used to represent each haplotype. NM: northern Morocco; CM: central Morocco; SM: southwestern Sahara.

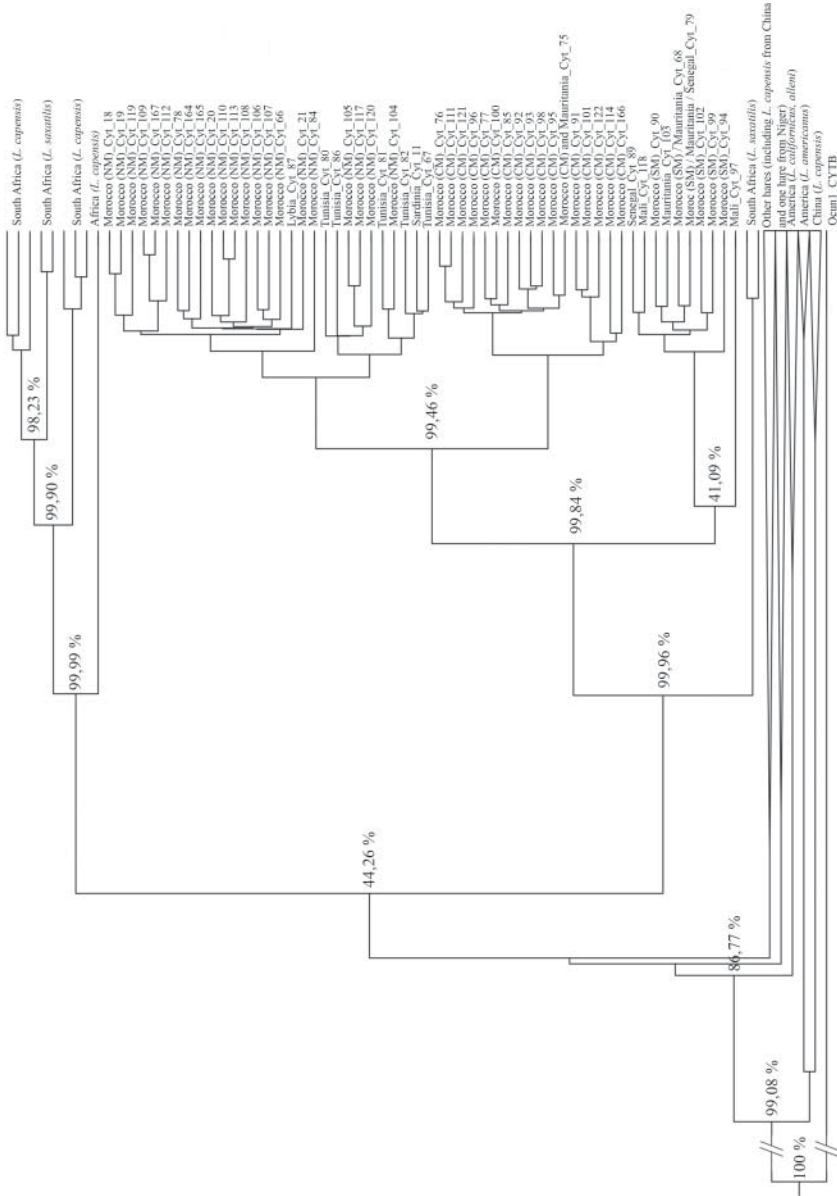


Fig. 3. Bayesian phylogenetic tree based on CYTB mtDNA. The phylogenetic tree was constructed with 168 haplotypes (409 bp), representing 327 hares and with the outgroup (*Oryctolagus cuniculus*) included. Numbers in nodes indicate posterior values as a percentage (%). Names of sequences correspond to those of the sequences used to represent each haplotype. NM: northern Morocco; CM: central Morocco; SM: southwestern Sahara.

northern Morocco, Tunisia, and Sardinia (NM). The Mauritanian hare is grouped with those from the southwestern Sahara (SM). Cape hares and bush hares appear in different clades. The CYTB tree is more complex and includes hares from other countries, such as Senegal, Mali, or Libya. The scrub hare (*Lepus saxatilis*) and cape hare (*Lepus capensis*) are separated into different groups. This analysis reinforces the results of the previous one by showing an identical structure. The hares from Morocco are separated into three distinct groups. Only in areas of geographical overlap do we find hares from different groups sharing a territory (Fig. 1). Phylogenetic trees based on combined DNA (evolutionary-ecology.com/data/3207Appendix.pdf Fig. S1) were constructed to determine whether the size of the analysed sequences would provide different results. However, these trees were congruent with those for CR and CYTB. Thus all the phylogenetic trees reveal that the same three groups of hares are associated with the zones shown in Fig. 1: NM, CM, and SM.

The results of the networks (Fig. 4 and Appendix S2) were in line with those for the phylogenetic trees. In all cases, the analyses suggested three groups of Moroccan hares. When the African savanna hare (*L. victoriae*) was added to the COI and combined mtDNA networks (Figs. 4b and S2b), it was distinct from the three groups, although closer to the hares from the southwestern Sahara (SM).

The pairwise distances between species varied according to the fragment under consideration, but showed a common patterns (Appendix Table S5). The genetic distances between northwest African hares and those from South Africa (scrub hares, *L. saxatilis* and cape hares, *L. capensis*) were significant, as were those between the African hares and hares from elsewhere in the world. The distances among the three lineages of hares from northwest Africa and the African savanna (*L. victoriae*) were less significant. We found similar distances between species from other locations, some distributed more closely together and others with geographical ranges very distant from one another, highlighting the complexity of the relations within the genus *Lepus*.

Morphological analysis of a new species

Below, we describe the morphology of a new species, which corresponds to the lineage found in the southwestern Sahara: Morocco, Mauritania, Mali, and Senegal (SM, yellow in Fig. 1).

Lepus saharae sp. nov.: *Lepus saharae* Urios, Soria-Boix, Rguibi & Donat-Torres, 2019 [urn:lsid:zoobank.org:act: BE7B1B1B-F456-4132-BA23-EF3A99870CE0].

Etymology and name: Both the specific and common names have been chosen based on the habitat and geographic area where this hare is found: Sahara hare (*Lepus saharae* sp. nov.).

Holotype: The specimen forms part of the collection in the Museum of the Higher School of Technology in Laâyoune, Morocco (#REF 021/2019 Awserd and 022/2019 Awserd for the skin and skull respectively) (Fig. 5). The specimen, which was found dead by two of the authors (V.U. and H.R.I.), was collected from the northwest of Aoussard Province, western Sahara, Morocco.

Differentiation: *Lepus saharae* sp. nov. can be differentiated from its congeners by the following characteristics: its ears are larger than in other hares from Africa; the black spot on the tip of its ears is not as obvious as in *L. capensis* and other African hares; the white pelage extends further along the underparts of *L. saharae* sp. nov. than in *L. capensis*; *L. saharae* does not present the grey and cinnamon shades on the back and rump as in *L. capensis* and other African hares; its body is both narrower and longer than that of other African species, with very long hind legs in proportion to its body.

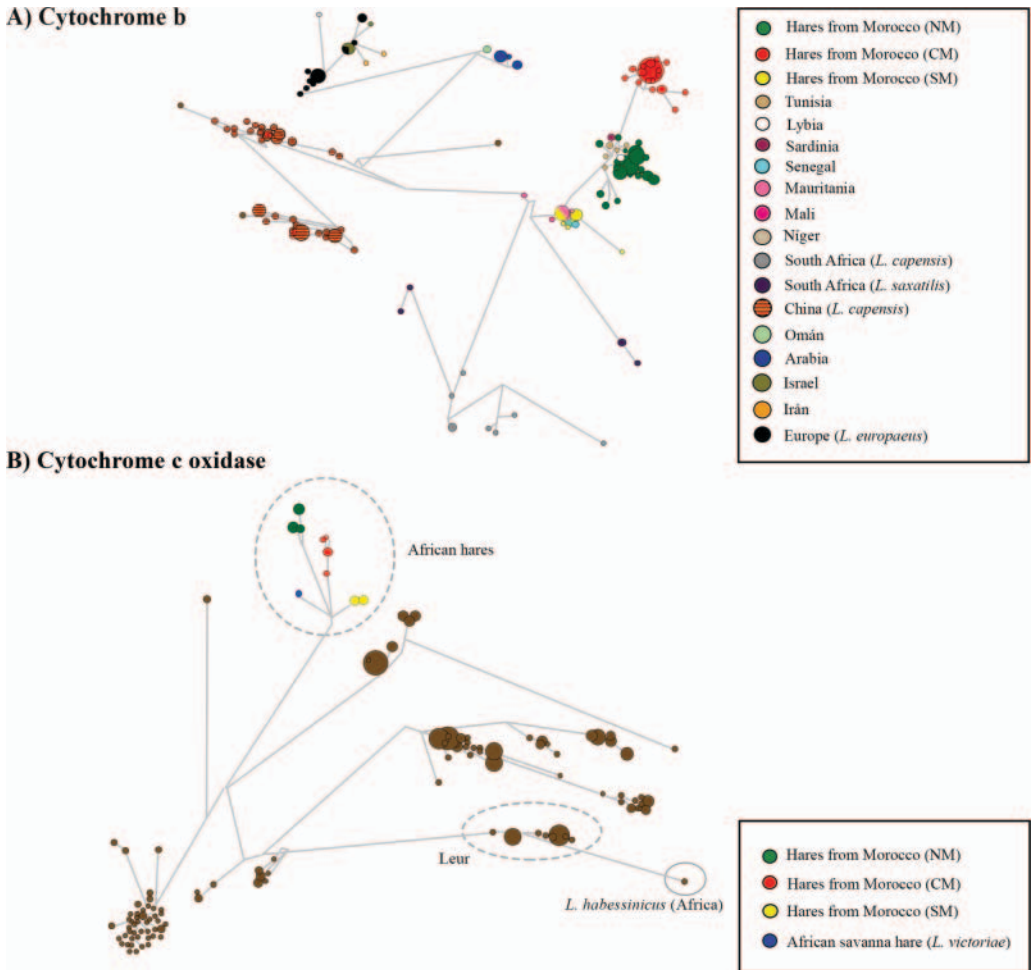


Fig. 4. Median-joining networks based on CYTB and COI mtDNA. (A) Network constructed with CYTB mtDNA including 131 haplotypes (409 bp) representing 264 sequences. (B) Network constructed with COI mtDNA including 139 haplotypes (626 bp) representing 248 sequences. In both cases the outgroup was excluded. NM: northern Morocco; CM: central Morocco; SM: southwestern Sahara.

Description: *Lepus saharae* sp. nov. is small in size (holotype weight: 1.76 kg). Its ears are longer than those of other species, with a white line along the edge. The underparts are pure white, with no clear separation between the colours of the underparts and back. The back is generally of a very light sandy colour, with some white and dark hairs interspersed. The nape is suede, and the front and rear legs are reddish in colour on the outside. The tail is sandy coloured with some black hairs on the top and is pure white beneath. The colour of the head is similar to that of the back, with both white on the chin and white cheeks. The snout is of a slightly darker sandy colour. The colouring both around and over the eyes is white. The hairs of the pad are slightly darker and very long (see Fig. 5).



Fig. 5. Sahara hare (*Lepus saharae* sp. nov.) (holotype).

Distribution: *Lepus saharae* sp. nov. is found in the southwestern Sahara (i.e. Morocco, Mauritania, Mali, and Senegal) and may be present in other areas with similar habitat.

DISCUSSION

Selective pressures that influence the evolutionary ecology of the genus *Lepus*

There are groups of species in the world among which cryptic species are unusually frequent. The phenotypes of cryptic species do not differ much because of the evolutionary convergence that comes from having similar lifestyles. Cryptic species have been described in mammals, including bats (Barratt *et al.*, 1997; Goodman and Ramasindrazana, 2015; López-Wilchis *et al.*, 2016; Soisook *et al.*, 2016; Juste *et al.*, 2019; Srinivasulu *et al.*, 2019), pikas (Koju *et al.*, 2017), rodents (Riddle *et al.*, 2014; Hulme-Beaman *et al.*, 2018), and primates (Hotaling *et al.*, 2016). A recent study detected cryptic divergence in the snowshoe hare (*Lepus americanus*) (Melo-Ferreira *et al.*, 2014b). In northwest Africa, the identification of three different species, where previously only a single species, *Lepus capensis*, had been recognized, suggests that the existence of cryptic species in *Lepus* might be considered the result of common selection factors. It is to these common characteristics we now turn.

Hares can reach speeds of 60 km/h when running in a straight line. The ability to run this fast requires a number of morphological characteristics, including long legs and a superior cardiac capacity, i.e. a larger heart than might be expected for an animal of its size (compare with rabbits). Myoglobin is another important property because it concentrates in muscle tissue, resulting in much improved muscle oxygenation. These characteristics appear to be present in the different species. Variation, however, can result in a larger or smaller body size, as well as

differences in body shape, which can make them the target of predators. It would appear that any departure from the optimum characteristics for running fast will be eliminated by morphological convergence (Wilson *et al.*, 2016).

Regarding fur colour, it is important to consider that hares crouch outdoors, whereas rabbits occupy burrows. *Lepus* spp. are mainly nocturnal and do not move around in vegetation during the day, like rabbits. Their fur pattern should thus provide the best possible camouflage. In fact, the coat of individual *Lepus* can change according to the season of the year – for instance, it turns white when the landscape is covered with snow. And they mimic their environment as much as possible the rest of the year. In hares from northwest Africa and other parts of the world, earthy colours predominate so that individuals go unnoticed in the daytime. This camouflage is so effective that we observed how a golden eagle (*Aquila chrysaetos*) was unable to spot a hare, one of its favourite prey, from a distance of only 10 m – the hare remained completely still, however. Thus any minor variation in colour will be quickly eliminated to avoid detection by predators. This will result in the convergence of several hare species towards a common colour pattern (Wilson *et al.*, 2016) if those hares live in similar habitats. Therefore, several hare species that might originally have been easy to distinguish would become difficult to differentiate phenotypically and would be considered cryptic species.

In northwest Africa, two species of *Lepus* were described more than a century ago. Our work on the mitochondrial DNA of these hares suggests the existence of a third species: *Lepus saharae* sp. nov. The fact that these three species have gone unnoticed is the result of phenotypic resemblance.

The divergence times (Appendix Table S6) derived from the phylogenetic trees indicate a separation of the lineages from Morocco during the Pleistocene, a period when one-quarter of the land's surface was covered in ice. We believe that the succession of glaciations resulted in reproductive isolation, leading to the appearance of the different hare species. In addition, about 90,000 years ago, North Africa and the Iberian Peninsula were joined owing to low sea levels (Soria-Boix *et al.*, 2017). This variation in the isolation of the Iberian Peninsula and North Africa could have also contributed to the formation of today's hare species. Based on our analyses, *Lepus victoriae* diverged from the hares of the southwestern Sahara (SM) around one million years ago. This indicates a relatively recent speciation time that would be consistent with the fast radiation of the genus *Lepus* in the Pleistocene. Perhaps that explains the closeness in the phylogenetic tree of the lineages present in Morocco, which had shorter genetic distances compared with other species. This is a similar scenario to that observed among other African species (Matthee *et al.*, 2004; Tolesa *et al.*, 2017). Outside Africa, we also find cases with similar genetic distances between species (Appendix Table S5).

Three hare species in northwest Africa

Our analyses suggest the presence of three hare species in northwest Morocco. Based on their geographical distributions and their morphological characteristics (Fig. 6), we propose: *Lepus mediterraneus* to be the scientific name for the hares found in northern Morocco (NM); *Lepus schlumbergeri* to be that for the hares from the central zone (CM); and *Lepus saharae* sp. nov. for the new species described here for the first time, and which is found in southern Morocco (SM).

The genetic distances (Appendix Table S5) indicate separation of the three groups of hares based on the phylogenetic trees and networks. We find similar pairwise distances between *Lepus victoriae* and the three Moroccan lineages for both cytochrome c oxidase subunit I (COI) and the combined mtDNA (Tables S5a and S5d). For the control region (CR) (Table S5b), the distances between

A)



Lepus schlumbergeri



Lepus schlumbergeri

B)



Lepus saharae sp. nov.



Lepus saharae sp. nov.



Lepus saharae sp. nov.

Fig. 6. Photographs of individuals from northwest Africa. (A) *Lepus schlumbergeri*: a live specimen (on the left) photographed south of Aghbala, Morocco and a specimen (on the right) found dead 70 km from Smara. (B) *Lepus saharae* sp. nov.: live specimen (on the left) and specimen found dead (on the right). Both hares were photographed in the southwestern Sahara.

Lepus saxatilis and the hares from Morocco were similar to those between the three Moroccan lineages. So, they are likely all different species.

The divergence between the brown hare (*L. europaeus*) and hares from the Near East and Niger (Africa) was below 3% (Table S5c) for cytochrome b (CYTB). For the near eastern hares and *L. europaeus*, one possible explanation could be contact between them in some areas, and the consequent genetic introgression. For the hares from Niger, dispersal corridors must have allowed contact between different species to be made (Lado *et al.*, 2019). This would have resulted in shared ancestral polymorphisms. We can also deduce that the hares from Niger must belong to another lineage present in Africa (Lado *et al.*, 2019). Although the possibility of contact between the hares from Morocco are more likely given their geographical proximity and the territory's continuity, the genetic distances were greater (>3%) than those between the aforementioned species (<3%). This suggests that, in the larger context, there are no signs of introgression, even though the genus *Lepus* has experienced allopatric speciation. Even so, we have to consider that a certain degree of genetic introgression may have occurred in the contact areas between the lineages from Morocco. This indicates a clear evolutionary separation at the mtDNA level, even in those cases in which there are no obvious geographical barriers to prevent contact. However, the hares of northern Morocco (NM), which can be grouped with the hares from Tunisia, Libya and Egypt, have the Atlas Mountains to overcome. This would support the notion that the presence or absence of such barriers would not have affected hares' behaviour when dispersing.

The results also show that the hares from North Africa are quite distant from most other species at the mtDNA level, including other African species. It has been suggested that the Sahara Desert shouldn't be viewed as a significant barrier because since its emergence, corridors have emerged over time. So one possibility is that the relative isolation of their populations during speciation could have caused relative genetic isolation (Drake *et al.*, 2011; Brito *et al.*, 2014; Gonçalves *et al.*, 2018).

In this study, we relied mainly on molecular analyses to clarify the number of species of hare in Morocco. Nuclear DNA analysis was unable to conclusively differentiate among species (see Appendix Tables S3e and S5e, Fig. S3). As in our nuclear DNA analyses of the genus *Lepus*, there are other cases in which it is observed that mitonuclear discordance may be caused by different factors, homogenizing the nuclear but not the mitochondrial genome (Hinojosa *et al.*, 2019). A description of the new species was included and photos of some specimens were added to support the obtained results. In upcoming research, a more thorough morphological analysis (including measures) will be carried out. This work has yet to be performed simply because obtaining sufficient specimens under the proper conditions is a difficult task.

Detecting new species or the presence of important variations between populations is important to prevent their disappearance before we know of their existence. By knowing them, the status of these species can be studied and protective measures implemented as necessary (Bickford *et al.*, 2007; Sheets *et al.*, 2018; Huntley *et al.*, 2019). Hence the importance of describing new taxa before they are endangered or even disappear.

Assignment of names to species

***Lepus mediterraneus* Wagner, 1841:** The hares of northern Morocco are grouped with the hares of Tunisia, Libya, Egypt and Sardinia (Italy), and differ markedly from *Lepus capensis* from South Africa. This is consistent with the results of previous studies. We also propose the name *Lepus mediterraneus* Wagner, 1841 (de Winton, 1898) for the hares occupying this geographical area (Pierpaoli *et al.*, 1999; Scandura *et al.*, 1999; Alves *et al.*, 2003; Ben Slimen *et al.*, 2007; Ben Slimen, 2008).



Fig. 7. Photographs of a single specimen of *Lepus schlumbergeri* sampled for this study. There is a characteristic white spot between the ears.

***Lepus schlumbergeri* Remy-St. Loup, 1894:** In one hare collected from central Morocco (Fig. 7), there is a differentiating characteristic. It is a white spot on the top of the head. This spot has been described for hares where the distributions of *Lepus schlumbergeri* and *Lepus mediterraneus* overlap (Cabrera, 1932). Due to the characteristics of these hares and their geographical distribution, we propose the name *Lepus schlumbergeri* Remy-St. Loup, 1894 for the species found in central Morocco (CM).

***Lepus saharae* sp. nov.:** In molecular terms, this hare differs from the other species in North Africa, as does *L. victoriae*. As the morphological evidence supports the results of the molecular analyses, we conclude that this hare is a different species to those described above, and we name it *Lepus saharae* sp. nov. This hare is present in the southwestern Sahara: Morocco, Mauritania, Mali and Senegal, and may also be present in areas of similar habitat.

CONCLUSIONS

We identified a group of three cryptic species among the hares (*Lepus*) of Morocco. Mitochondrial DNA revealed sufficient genetic distances to define the three lineages. The three species' geographical ranges do overlap but to a large extent they are separate. From northern Morocco to southern Senegal and Mali, the distribution of the species is as follows (Fig. 1): *L. mediterraneus*,

L. schlumbergeri, and then *L. saharae* sp. nov. For the new species, *L. saharae* sp. nov., we describe its morphology and have deposited a type specimen for conservation in the Museum of the Higher School of Technology in Laâyoune, Morocco. We believe the significant evolutionary pressures that have made these hares appear so similar include the need for speed and fur colouring that provides good camouflage.

ACKNOWLEDGEMENTS

We would like to thank Le Haut Commissariat aux Eaux et Forêts et à la Lutte Contre la Désertification du Maroc for authorizations and the Emirates Centre for Wildlife Propagation (Morocco) for help with fieldwork. This paper is part of the PhD thesis of Carmen Soria at the University of Alicante.

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