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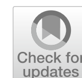
RESEARCH ARTICLE



Assessment of population structure and genetic diversity of wild and captive populations of *Ammotragus lervia* provide insights for conservation management

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Abstract

The aoudad (*Ammotragus lervia*) is a Vulnerable bovid endemic of North Africa. Although legally protected in almost every country of its native distribution, the aoudad continues to be hunted for meat and trophy in both North Africa and the countries where it has been introduced. The species was subject to past translocations planned irrespectively of the genetic diversity and local adaptations of source and receiving populations, and no management or conservation units have ever been designed. We aim to provide here important insights on the taxonomy of the aoudad subspecies and the genetic diversity of most of its wild and captive populations. We collected 127 invasive and non-invasive samples from five of the six subspecies of aoudad recognized to date. We could successfully retrieve genetic data for 74 samples. Of those, 36 provided both nuclear (11 microsatellites) and mitochondrial (*Cyt-b*) data, 31 just nuclear data, and six just mitochondrial data. We implement Bayesian approaches to infer the population structure and phylogenetic relationships between the different populations/subspecies and backtrack the maternal lineages of introduced individuals in European populations. Our results support the presence of four genetically different wild populations, corresponding to three distinct mitochondrial lineages plus a fourth group restricted to Egypt identified by the nuclear markers. We also provide genetic evidence on the affiliation of some introduced European populations with respect to the native ones. The genetic diversity instead of variation within all wild populations was low. This might be a consequence of small effective population size and/or high inbreeding degree, probably related to hunting, decline in habitat availability and quality (i.e. overgrazing, and frequent drought), and high inbreeding degree. Our results provide important information for the aoudad conservation, including reintroductions and reinforcement actions of wild populations, and the exchange of individuals among captive stocks.

Keywords Conservation genetics · Molecular systematic · Phylogeography · Sahara ungulates · Aoudad · Desert

Introduction

During the last century, the human impact on the planet's ecosystems and climate is leading wildlife through a sixth massive extinction (Estes et al. 2011). Human-mediated habitat depletion and overexploitation of natural resources (i.e., mining, logging, and overhunting), and climate change, are acting to threaten the persistence of wildlife and humans populations (Caro et al. 2022). Despite these worrying conditions, there is still inadequate or little information available for most species on which to base effective conservation decisions (Hortal et al. 2015). Within-species genetic diversity is one of the basic levels of biodiversity, which contributes to species persistence by providing adaptations to the environmental traits and changes, and by controlling

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biotic and abiotic variety (Frankham et al. 2010). The importance of genetic diversity in conservation strategies has been largely addressed already (Hoban et al. 2022, and references therein), being also included in the Convention on Biological Diversity targets (https://www.cbd.int/sp/; Hoban et al. 2020), which aims to prevent genetic erosion and safeguard diversity. The identification of intraspecific genetic patterns (or structures) and levels of population connectivity have also an important role in ensuring effective conservation and management of wildlife (Hoban et al. 2020). Such information is particularly relevant for species with fragmented distributions since it allows to design management and conservation units based on the partial reproductive isolation and/or outbreeding depression criterion (Frankham et al. 2017). Still, biodiversity management projects that routinely target the conservation of genetic diversity are few (Holderegger et al. 2019). Such a gap is usually due to financial restrictions, lack of knowledge, or limited accessibility to study areas (Brito et al. 2014, 2018; Holderegger et al. 2019).

North Africa is an example of how remoteness, low socioeconomic conditions, and political instability can limit scientific research and conservation projects (Brito et al. 2018; UNDP 2020; IEP 2022), resulting in knowledge gaps on local biodiversity (Brito and Pleguezuelos 2020). In particular, Saharan mammals are poorly investigated, and their conservation has attracted less attention in comparison to species in other regions (Brito et al. 2014; Brito et al. 2014). This is even more worrying considering that all large mammals historically inhabiting the Sahara Desert have been either locally extinct (e.g., *Panthera leo, Lycaon pictus, Oryx dammah*) or at the verge of extinction (IUCN 2019). Saharan ungulates are not an exception, ranking as Vulnerable (*Ammotragus lervia, Capra nubiana, Gazella cuvieri, G. dorcas*), Endangered (*G. leptoceros*,), Critically Endangered (*Addax nasomaculatus, Nanger dama*) or Extinct in the wild (*Alcelaphus buselaphus buselaphus, Oryx dammah*) (IUCN 2019). The range of most Saharan ungulates has drastically decreased (between the 66% and 99%) mostly due to habitat loss, overgrazing, and poaching (Brito et al. 2014; see Fig. S1 in Brito et al. 2018; Abáigar et al. 2019). The current critical status of Saharan ungulates has prompted several reintroduction projects, aiming to restore depleted or locally extinct ungulate populations (Ramzi et al. 2018; Abáigar et al. 2019; Mertes et al. 2019), but the use of genetic data in reintroduction decisions is still limited (Odgen et al. 2020).

The aoudad (*Ammotragus lervia*) is an endemic Saharan ungulate inhabiting rugged and mountainous areas of North Africa (Fig. 1; Cassinello et al. 2021). Until the mid-twentieth century, the audad was abundant and widespread across the Sahara (Cassinello 1998), but then it suffered a sharp decline in range and population size, ranking currently as a Vulnerable species (VU) with the total population estimated to be between 5000 and 10,000 individuals (Cassinello et al. 2021). Populations are presently highly fragmented and restricted to rocky mountain regions of limited accessibility, where they are butchered for trophy or bushmeat in poaching raids or where they must compete for natural resources with large domestic livestock (Manlius et al. 2003; Brito et al. 2018; Cassinello et al. 2021). Such factors probably have contributed to an increase in the genetic isolation of populations, which likely amplifies the demographic risks derived from excessive genetic drift and inbreeding. Despite the strong indication that this species is rapidly declining across all its original distribution, a lack of information persists about its genetic diversity and population status (Cassinello et al. 2021). Because of its cultural and economic relevance, the aoudad has been imported as a game species and livestock in several European countries, USA, Mexico, and South Africa (Cassinello et al. 2021); there have also been attempts of introducing the species into the wild, but only in Spain a free-ranging introduction was eventually successful (Cassinello 1998). However, some herds from fenced hunting grounds in the southwestern USA escaped, giving rise to free-ranging aoudad populations (Cugnasse and Tomeï 2016; Cassinello et al. 2021; Wright et al. 2022). Captive populations are also present inside its natural range (Algeria, Morocco and Tunisia; Fig. 2). Despite captive and ex-situ populations could represent a potential source of individuals for reinforcement of wild Sahara populations (Garzón-Machado et al. 2012; Pacioni et al. 2019), little is known about the genetic structure and diversity of wild populations (Derouiche et al. 2020), and about the geographic origin of most of the *ex-situ* captive populations (Cassinello et al. 2021; Stipoljev et al. 2021). In addition, the morphological variability observed in wild populations has been used to describe six subspecies, but this systematic arrangement has never been genetically assessed and there are uncertainties about the taxonomy and distribution of aoudad's subspecies (Cassinello et al. 2021).

With this study, we aim to answer the following questions: (i) What is the phylogeographic relationship between native and introduced populations in Europe? (ii) How populations are genetically structured in space? (iii) What is the level of genetic diversity of native and introduced populations? (iv) Does the distribution of genetic diversity geographically match the distribution of the current systematic arrangement? Overall, we aim to provide valuable information for the delineation of management and conservation units and for development of management actions targeting the conservation of both wild and captive populations.

Materials and methods

Study area, sampling, and DNA extraction

The study area encompasses the native distribution of aoudad in North Africa, and some Spanish localities where

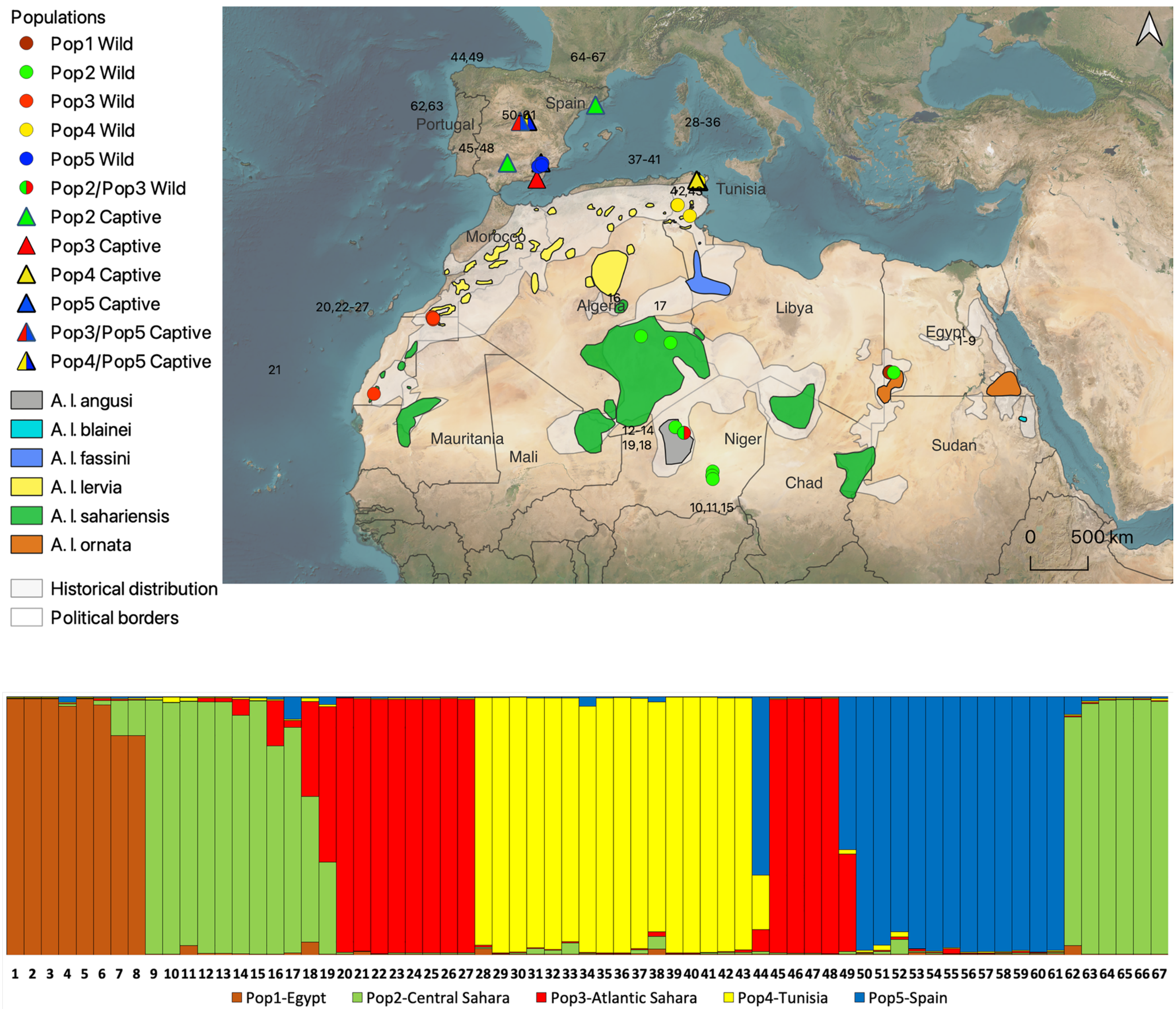
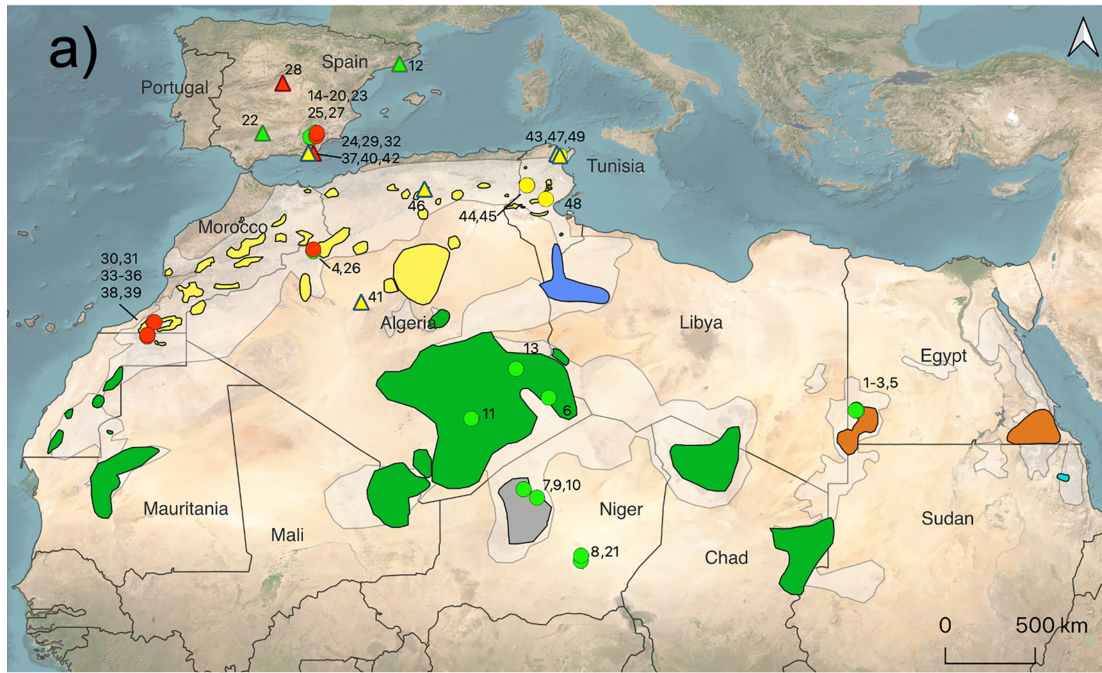
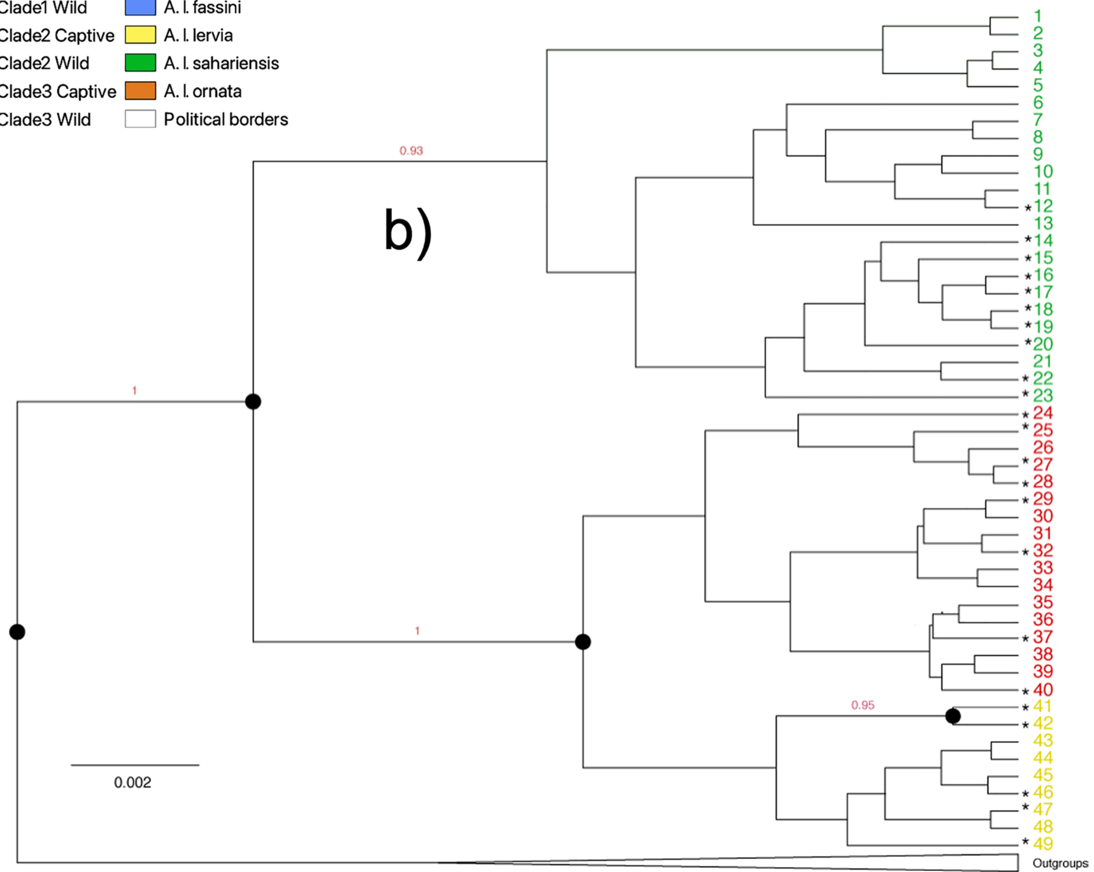


Fig. 1 **a** Distribution of the samples used in the Bayesian clustering analyses. Polygons in the map represent the current estimated distribution of the subspecies (from Cassinello et al. 2021), and the historical. **b** Boxplot resuming the results of the Bayesian clustering analyses implemented in STRUCTURE program (Evanno et al. 2005). Numbers in the map and STRUCTURE plot correspond to the genetic samples in Table 1 included in this analyse

aoudad populations have been introduced (Figs. 1 and 2). We collected 127 samples from 22 different populations distributed in North Africa and Spain (Fig. S1). Collected samples represent five of the six subspecies of aoudad recognized to date. Representative samples for the subspecies *angusi*, *lervia*, *ornata*, and *sahariensis* were collected from wild individuals in North Africa, while those for the subspecies *fassini* were collected by wild captured individuals from Libya kept at the zoos of Cordoba (sample NAG1059) and Barcelona (sample NAG1371). Due to the general remoteness of the area and political instability characterizing some countries (Brito et al. 2018), we were not able to obtain samples from the subspecies *blainei*, and from the populations inhabiting Mauritania, Mali, Chad and Libya. In total, we retrieved 82 samples from wild populations and 45 samples from captive and semi-captive populations (Table S1). Of the samples collected from captive and semi-captive populations, nine were from the Estación Experimental de Zonas Áridas (EEZA; Almeria, Spain), descendants of two wild individuals captured in the Atlantic Sahara; six from the Zoo of Cordoba, Spain (descendants of two individuals from south-east Lybia donated by the Libyan ambassador in Spain); four from the Zoo of Barcelona, Spain (descendants of the stock in the Zoo of Cordoba); six from the Zoo of Madrid, Spain (of unknown origin); three from the zoo of Tunis, Tunisia; and seven from three different semi-captive


a)
Spain
Portugal
Morocco
Algeria
Tunisia
Libya
Egypt
Mauritania
Mali
Niger
Chad
Sudan
28
12
14-20,23
25,27
22
24,29,32
37,40,42
43,47,49
46
44,45
48
30,31
33-36
38,39
4,26
41
13
11
6
1-3,5
7,9,10
8,21
0
500 km

Clades
Clade1 Captive
Clade1 Wild
Clade2 Captive
Clade2 Wild
Clade3 Captive
Clade3 Wild
A. l. angusi
A. l. blainei
A. l. fassini
A. l. lervia
A. l. sahariensis
A. l. ornata
Political borders
Historical distribution


b)
0.93
1
1
0.95
0.002
Outgroups

◂**Fig. 2** **a** Distribution of the samples used in the phylogenetic analyses. Polygons in the map represent the current estimated distribution of the subspecies (from Cassinello et al. 2021), and the historical distribution. **b** Bayesian inferred reconstruction of the evolutionary history of sampled populations. Black dots represent well-supported nodes (PP > 0.95). Colours of numbers represent the clade subdivision of the samples: Clade 1 in green; Clade 2 in red and Clade 3 in yellow. Numbers in the map and phylogenetic tree correspond to the genetic samples in Table 2 included in this analyses

populations in Tunisia. We also collected samples from a wild population in Sierra Maria-Los Velez mountains (Almería, Spain) originated by vagrant aoudads coming from the nearby mountains of Murcia, where the species was introduced back in 1970 (Cassinello et al. 2004).

Samples included scats, hair, bones, and tissue, and were stored in ethanol (96%) until DNA extraction. Our extraction process followed Maudet et al. (2004), which we adapted with the use of a commercial Kit (E.Z.N.A.® Tissue DNA Kit). We extracted hair and bone samples with a commercial kit (Quiagen® QIAamp DNA Micro Kit). We processed all non-invasive samples (scats, hair, and bones) in dedicated low-quality DNA facilities equipped with positive air pressure and UV lights. We extracted total DNA from tissue samples using the Genomic DNA Minipreps Tissue Kit (EasySpin).

DNA amplification and genetic identification

All genetic identifications relied on the mitochondrial (mtDNA) fragment (450 bp of the *Cytochrome-b, Cyt-b*) used also for the phylogeographic analysis. Standard protocols for Saharan ungulates were used (Silva et al. 2015). We generated strand sequences using the amplification primers following Tiedemann et al. (2012), allowing the confirmation of sequence consistency and quality. Cycle sequencing reactions were carried out using BIGDYE TERMINATOR v3.1 Cycle Sequencing Kit (Applied Biosystems, Carlsbad, CA, USA). We subsequently separated sequencing products on a 3130xl Genetic Analyser (Applied Biosystems). Sequence alignment was performed using Clustal W (Thompson et al. 1994), and manually checked and reassessed for any discrepancy in BIOEDIT software (Hall 1999). We excluded all the sequences with large amount of missing data.

We amplified eleven nuclear dinucleotide species-transferred microsatellite loci: AMEL, INRA63, OarFCB20, ILST87, INRA172, INRA023, SPS113, MAF065, McM527, INRA005 and INRA006 (all primers recommended for Caprinidae characterization by the International Society of Animal Genetics—ISAG). Forward primers of the 11 markers were fluorescently labelled with VIC, FAM, PET and NED. We arranged the primer pairs into three separate multiplex reactions. PCR amplification was as follows: initial denaturation at 95 °C for 15 min; 30 cycles of denaturation at 94 °C for 30 s, annealing at 58 °C for 90 s, and elongation at 72 °C for 60 s, and a final elongation at 60 °C for 30 min, according to Glowatzki-Mullis et al. (2007). We conducted a minimum of four replicates polymerase chain reactions, per non-invasive sample per locus to minimize genotyping errors resulting from potential degraded DNA from scats. Fragment analysis was carried out using a 3130xl Genetic Analyser (Applied Biosystems) under standard run conditions with LIZ500 as the internal size standard. We scored alleles and binned using GENEMARKER 1.7.

Population structure and nuclear genetic diversity

GenAlEx 6.5b3 (Peakall et al. 2012) and GENEPOP (Raymond 1995) were used to test for Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium (LD) in the microsatellite data, respectively. Genotyping data were screened with MICRO-CHECKER 2.2.3 (Van Oosterhout et al. 2004) to identify allele dropout, scoring errors due to stuttering, and the presence of null alleles. We also used GenAlEx 6.5b3 to calculate genetic diversity measures, including the number of alleles, effective number of alleles, observed (H_o) and expected (H_e) heterozygosity, and fixation index (F). We estimated the overall and pairwise genetic differentiation with the θ estimator of F_{st} (Weir and Cockerham 1984) in FSTAT (Goudad 1995). Statistical significance was determined by 1000 permutations. We used STRUCTURE 2.3.4 (Pritchard et al. 2000) to estimate the nuclear genetic partitioning by inferring the most probable number of genetic clusters. We assumed a number of clusters (K) between one and 20 (*i.e.*, $K=1$ to $K=20$) for five runs, repeating each run 10 times. We used a burn-in period of 10,000 followed by 1,000,000 Markov chain Monte Carlo (MCMC) steps for each run, choosing the admixture model with the option of correlated allele frequencies. We conducted the same analyses without a priori definition of populations and with sampling locations as prior information (LOCPRIOR option) and lambda parameter was maintained fixed ($\lambda=1$) as default. To verify if the results were not affected by null alleles, we used the recessive alleles option provided in STRUCTURE. We then used Harvester (Earl and VonHoldt 2012) to implement the Evanno method (Evanno et al. 2005) and infer the best-supported clusters.

Phylogeographic reconstruction

We used a Bayesian approach to infer the phylogenetic relationships between the different populations/subspecies and backtrack the maternal lineages of introduced individuals.

Table 1 List of samples used in the population structure analyses in Fig. 1

Number	Sample	Locality	Country	Status	Population
1	NAG1495	Gilf plateau upper hamra	Egypt	Wild	Pop1
2	NAG1497	Wadi hamra	Egypt	Wild	Pop1
3	NAG1498	Wadi hamra	Egypt	Wild	Pop1
4	NAG1502	Gilf plateau upper hamra	Egypt	Wild	Pop1
5	NAG1504	Gilf plateau	Egypt	Wild	Pop1
6	NAG1507	Gilf plateau upper hamra	Egypt	Wild	Pop1
7	NAG1509	Karkur thal	Egypt	Wild	Pop1
8	NAG1510	Karkur thal	Egypt	Wild	Pop1
9	NAG1513		Egypt	Wild	Pop2
10	NAG1162	Zinder	Niger	Wild	Pop2
11	NAG1324		Niger	Wild	Pop2
12	NAG1379		Niger	Wild	Pop2
13	NAG1384		Niger	Wild	Pop2
14	NAG1386		Niger	Wild	Pop2
15	NAG393		Niger	Wild	Pop2
16	NAG1029	Tassili-n'Ahelakane	Algeria	Wild	Pop2
17	NAG1370	Tassili Ahelakane	Algeria	Wild	Pop2
18	NAG1378		Niger	Wild	Pop2/Pop3
19	NAG1380		Niger	Wild	Pop2/Pop3
20	NAG1083		Morocco	Wild	Pop3
21	NAG1199	Koudyat Laghnam	Western Sahara	Wild	Pop3
22	NAG228	Bajo Oued Draa-Aidar	Morocco	Wild	Pop3
23	NAG229	Bajo Oued Draa-Aidar	Morocco	Wild	Pop3
24	NAG230	Bajo Oued Draa-Aidar	Morocco	Wild	Pop3
25	NAG231	Bajo Oued Draa-Aidar	Morocco	Wild	Pop3
26	NAG232	Bajo Oued Draa-Aidar	Morocco	Wild	Pop3
27	NAG271	Bajo Oued Draa-Aidar	Morocco	Wild	Pop3
28	NAG437	Boukornine	Tunisia	Captive	Pop4
29	NAG438	Boukornine	Tunisia	Captive	Pop4
30	NAG442	Boukornine	Tunisia	Wild	Pop4
31	NAG443	Boukornine	Tunisia	Wild	Pop4
32	NAG444	Boukornine	Tunisia	Wild	Pop4
33	NAG446	Boukornine	Tunisia	Wild	Pop4
34	NAG448	Tunis Zoo	Tunisia	Wild	Pop4
35	NAG449	Tunis Zoo	Tunisia	Captive	Pop4
36	NAG461	Chaambi	Tunisia	Captive	Pop4
37	NAG479	Chaambi	Tunisia	Wild	Pop4
38	NAG485	Chaambi	Tunisia	Wild	Pop4
39	NAG490	Chaambi	Tunisia	Wild	Pop4
40	NAG494	Chaambi	Tunisia	Wild	Pop4
41	NAG504	Chaambi	Tunisia	Wild	Pop4
42	NAG601	Bou-Hedma	Tunisia	Wild	Pop4
43	NAG604	Bou-Hedma	Tunisia	Wild	Pop4
44	NAG903	Madrid Zoo	Spain	Captive	Pop4/Pop5
45	NAG100	EEZA-Almeria	Spain	Captive	Pop3
46	NAG713	EEZA-Almeria	Spain	Captive	Pop3
47	NAG714	EEZA-Almeria	Spain	Captive	Pop3
48	NAG715	EEZA-Almeria	Spain	Captive	Pop3
49	NAG904	Madrid Zoo	Spain	Captive	Pop3/Pop5
50	NAG1314		Spain	Captive	Pop5
51	NAG1316		Spain	Wild	Pop5

Table 1 (continued)

Number	Sample	Locality	Country	Status	Population
52	NAG1319		Spain	Wild	Pop5
53	NAG1193		Spain	Wild	Pop5
54	NAG1194		Spain	Wild	Pop5
55	NAG1195		Spain	Wild	Pop5
56	NAG1196		Spain	Wild	Pop5
57	NAG1197		Spain	Wild	Pop5
58	NAG1311		Spain	Wild	Pop5
59	NAG1312		Spain	Wild	Pop5
60	NAG1313		Spain	Wild	Pop5
61	NAG1315		Spain	Wild	Pop5
62	NAG1371	Córdoba Zoo	Spain	Captive	Pop2
63	NAG1372	Córdoba Zoo	Spain	Captive	Pop2
64	NAG1058	Barcelona Zoo	Spain	Captive	Pop2
65	NAG1059	Barcelona Zoo	Spain	Captive	Pop2
66	NAG1060	Barcelona Zoo	Spain	Captive	Pop2
67	NAG1062	Barcelona Zoo	Spain	Captive	Pop2

Number, sample ID, country of provenience, locality, status, and population (K) of the samples included in the population structure analyses (Fig. 1). The numbers correspond to the numbers in the map and structure analyses in Fig. 1 (please see Table 2 for the numbers used in the phylogenetic reconstruction)

We aligned the mtDNA sequences with four previously published sequences of the aoudad *Cyt-b* gene from Derouiche et al. (2020) (GenBank accession numbers MN641980, MN641981, MN641983, and MN641985) using MAFFT v.7 (Katoh et al. 2019) with default parameters (Auto strategy, Gap opening penalty: 1.53, Offset value: 0.0). To root our phylogenetic tree, we used as outgroups published sequences of *Arabitragus jayakari* (GeneBank access code AY846791.1) and *Rupicapra rupicapra* (GeneBank access code FJ207539). We deposited all sequences produced for the present study in GenBank. We created a first alignment including 40 sequences of 647 bp (Alignment 1). We also generated a shorter alignment based on 373 bp and including 51 individuals (Alignment 2). We analysed the alignments using BEAST 1.10.4 (Suchard et al. 2018). We determined the best-fit model of sequence evolution using PartitionFinder 2.1.1 (Lanfear et al. 2017) under the Bayesian Information Criterion (BIC). To test whether the genes studied evolve in a clock-like manner (strict clock) we run a preliminary analysis in BEAST 1.10.4 using a relaxed clock. We verified the results of this preliminary run using TRACER 1.6 (http://tree.bio.ed.ac.uk/software/tracer). We rejected the strict clock model when the standard deviation of the uncorrelated lognormal relaxed clock parameter (ucld.stdev) and the coefficient of variation were greater than one. We used a Speciation Yule Process model to assume a constant lineage birth rate for each branch in the tree. We run three separated Markov Chain Monte Carlo (MCMC) analyses for 100 million generations. After a burn-in phase of 10 million generations, trees were sampled every 10,000 generations. We checked chain stationarity and run parameter convergence using TRACER 1.6. Independent runs were combined using LogCombiner v.1.10.0. We assessed tree topologies using TreeAnnotator v.1.10.0 and FigTree v.1.4.4.

Results

From the 127 samples collected for this study (Fig. S1), we could successfully retrieve genetic data for 74 of them. Of those, 36 provided both nuclear and mitochondrial data, 31 just nuclear data, and 6 just mitochondrial data.

Population structure

Our analyses in STRUCTURE suggested the presence of five different clusters (K = 5) as the best ΔK value (results for larger K range ($2 < K < 7$) can be found in the supplementary material Fig. S2). These clusters match the geographical distribution of our samples, corresponding to populations from: (i) Egypt (Pop1 in brown; Fig. 1); (ii) the Central Sahara, namely from south-east Algeria, Niger and Egypt (Pop2, green; Fig. 1); (iii) the Atlantic Sahara, namely the Atlantic Sahara and southern Morocco (Pop3, red; Fig. 1); (iv) Tunisia (Pop4, yellow; Fig. 1); and (v) Sierra María-Los Vélez in Spain (Pop5, blue; Fig. 1). The individuals kept at the Zoos of Barcelona and Cordoba (Spain) clustered with the Central Sahara populations (Pop2, green; Fig. 1) and the one kept at the Estación Experimental de Zonas Áridas (EEZA, Spain) clustered with the Atlantic Sahara populations (Pop3,

Table 2 List of samples used in the phylogenetic analyses in Fig. 2

Number	Sample	Locality	Country	Status	Clade mtDNA
1	NAG1497	Wadi Hamra	Egypt	Wild	Clade1
2	NAG1507	Gilf plateau-Hamra	Egypt	Wild	Clade1
3	NAG1510	Karkur Thal	Egypt	Wild	Clade1
4	MN641981	Béchar Province	Algeria	Wild/Introduced	Clade1
5	NAG1504	Gilf plateau-Hamra	Egypt	Wild	Clade1
6	MN641985	Madak, Djanet District	Algeria	Wild	Clade1
7	NAG1380		Niger	Wild	Clade1
8	NAG1324		Niger	Wild	Clade1
9	NAG1384		Niger	Wild	Clade1
10	NAG1379		Niger	Wild	Clade1
11	AlerviaNC009510		Algeria	Wild	Clade1
12	NAG1059	Barcelona Zoo	Spain	Captive	Clade1
13	NAG1370	Tassili Ahelakane	Algeria	Wild	Clade1
14	NAG1194	Almeria	Spain	Wild	Clade1
15	NAG1315	Almeria	Spain	Wild	Clade1
16	NAG1312	Almeria	Spain	Wild	Clade1
17	NAG1195	Almeria	Spain	Wild	Clade1
18	NAG1319	Almeria	Spain	Wild	Clade1
19	NAG1197	Almeria	Spain	Wild	Clade1
20	NAG1193	Almeria	Spain	Wild	Clade1
21	NAG392	Termit, Fargassane	Niger	Wild	Clade1
22	NAG1371	Cordoba Zoo	Spain	Captive	Clade1
23	NAG1318	Almeria	Spain	Wild	Clade1
24	NAG714	EEZA	Spain	Captive	Clade2
25	NAG1313	Almeria	Spain	Wild	Clade2
26	MN641983	Béchar Province	Algeria	Wild/Introduced	Clade2
27	NAG1311	Almeria	Spain	Wild	Clade2
28	NAG903	Madrid Zoo	Spain	Captive	Clade2
29	gi|3,417,578		Spain	Captive	Clade2
30	NAG232	Bajo Oued Draa-Aidar	Morocco	Wild	Clade2
31	NAG271	Bajo Oued Draa-Aidar	Morocco	Wild	Clade2
32	NAG713	EEZA	Spain	Captive	Clade2
33	NAG73	P. N. Bas Draa	Morocco	Wild	Clade2
34	NAG228	Bajo Oued Draa-Aidar	Morocco	Wild	Clade2
35	NAG1568	Djebel Ouarziz	Morocco	Wild	Clade2
36	NAG231	Bajo Oued Draa-Aidar	Morocco	Wild	Clade2
37	NAG715	EEZA	Spain	Captive	Clade2
38	NAG230	Bajo Oued Draa-Aidar	Morocco	Wild	Clade2
39	NAG229	Bajo Oued Draa-Aidar	Morocco	Wild	Clade2
40	NAG100	Almeria	Spain	Captive	Clade2
41	NAG848	Timimmoun	Algeria	SemiCaptive	Clade3
42	NAG95	EEZA	Spain	Captive	Clade3
43	NAG443	Boukornine	Tunisia	Wild	Clade3
44	NAG504	Chaambi	Tunisia	Wild	Clade3
45	NAG430		Tunisia	Wild	Clade3
46	MN641980	Moutas Reserve	Algeria	Wild/Introduced	Clade3
47	NAG461	Chaambi	Tunisia	Captive	Clade3
48	NAG604	Bou-Hedma	Tunisia	Wild	Clade3
49	NAG437	Boukornine	Tunisia	Captive	Clade3

Number, sample ID, country of provenience, locality, status, and mtDNA clade of the samples included in the phylogenetic analyses (Fig. 2). The numbers correspond to the numbers in the map and phylogenetic tree in Fig. 2 (please see Table 1 for the numbers used in the population structure analyses)

red; Fig. 1). Some wild Egyptian individuals (7, 8 and 9 in Fig. 1) displayed affinities from 13% up to 98% with the wild Algeria-Niger population. Signs of admixture were found between populations Pop2 and Pop3 in Niger (individuals 16, 18 and 19; Fig. 1), and in the introduced populations kept at the Zoo of Madrid (Spain) displaying admixed genotypes belonging to Pop3, Pop4, and Pop5.

We did not record stuttering or significant allelic dropout. MICRO-CHECKER suggested the presence of null alleles in populations Pop2 (INRA63, MAF065, OarFCB20, INRA172, and McM527), Pop3 (MAF065 and INRA23), Pop4 (McM527), and Pop5 (INRA06). We found a departure from the Hardy-Weinberg equilibrium for these loci in populations Pop2 for MAF065, OarFCB20, INRA172, and McM527, Pop3 for MAF065, and Pop4 for McM527.

Genetic diversity in wild and captive populations

Given that the Egyptian individuals were identified as a monophyletic group by the mtDNA analyses (although with low support, PP < 93%) and as distinct cluster by the SRUCTURE analyses, we analysed the genetic diversity for the Egyptian population separately from the Algerian and Nigerian ones. The overall F_{ST} value was 0.284. The lowest pairwise F_{ST} value ($F_{ST} = 0.179$) was found between the Atlantic Sahara (Pop3) and Central Sahara (Pop2) populations, while the highest value ($F_{ST} = 0.520$) was found between the Egyptian (Pop1) and Tunisian (Pop4) populations (Table 2). Global and all pairwise F_{ST} values were significantly different from zero ($P < 0.01$). The eleven microsatellite loci presented a total of 80 alleles, ranging from 2 (*Amelogenina*) to 11 (*INRA23*), with a mean number of alleles per locus of 3.61. The Polymorphism Information Contentent (PIC) values ranged from 0.531 to 0.836, with an average of 0.715. We recorded 20 private alleles distributed between the studied populations. We observed the highest average number of private alleles (2.661) in the Atlantic Sahara (Pop3). The observed heterozygosity (H_o) in wild populations ranged from 0.374 (Egyptian, Pop1) to 0.532 (Atlantic Sahara, Pop3). The H_o was always lower than the expected heterozygosity (H_e), but not for the Atlantic Sahara populations ($H_o = 0.532$; $H_e = 0.472$; $x^2 = 0.999$) (Table 3).

Phylogeographic relationships of native and introduced populations

Partitionfinder 2.1.1 highlighted as best substitution model HKY with gamma model for rate variation. The phylogenetic tree recovered three deep and well supported (posterior probability > 95%) lineages (Fig. 2), corresponding to: (i) Central Sahara lineage including the wild individuals from the mountains of south-eastern Algeria, Niger, and south-western Egypt, some individuals from Sierra María-Los Vélez (Spain), and the captive individuals at the Zoos of Barcelona and Cordoba (Spain) (Clade 1 in green; Fig. 2); (ii) Atlantic Sahara lineage including the wild individuals from the Atlantic Sahara, southern Morocco and north-western Algeria (province of Bechar), some other individuals from Sierra Maria-Los Velez (Spain), and the captive individuals at the Zoo of Madrid and some at EEZA (Spain) (Clade 2 in red; Fig. 2); and (iii) Tunisian lineage including the wild individuals from the mountains of central-west Tunisia, and some other captive individuals at EEZA (Spain), Timimmoun (Algeria), and Chaambi and Boukornine National Parks (Tunisia) (Clade 3 in yellow; Fig. 2).

Nucleotide distance between clades ranged between 0.23% between the Atlantic Sahara lineage (Clade 2) and the Tunisian lineage (Clade 3), and 0.49% between the Egyptian lineage (Clade 1) and the Tunisian lineage (Clade 3) (Table 4).

Implications for taxonomy and systematics

The phylogenetic reconstructions and population structure analyses do not match the current sub-specific taxonomic classification of the aoudad. Our results on the mitochondrial DNA show that: (i) individuals from the subspecies *angusi, fassini, ornata* and *sahariensis* cluster in the same monophyletic clade from the Central Sahara Desert distributed from Algeria (province of Bechar), Niger, Chad, Libya, Egypt, and Sudan (Clade 1, in green in Fig. 2); (ii) the clade including the wild individuals inhabiting West Africa, from the Atlantic Sahara and eastern Algeria, is monophyletic and sister to the clade of the subspecies *lervia* (Clade 2, in red in Fig. 2); (iii) the clade of the subspecies *lervia* is monophyletic and sister of the above-mentioned clade from Atlantic Sahara, the geographical distribution of the wild populations of *lervia* clade is restricted to Tunisia (Clade 3, in yellow in Fig. 2). The genetic structure analyses on the nuclear DNA agree with our phylogenetic reconstruction, if not for the Egyptian population forming its own cluster (in brown in Fig. 1).

Discussion

Here we provide the first distribution-wide overview of the mtDNA phylogeography, the nuclear genetic diversity and the genetic structure of the aoudad wild populations. At least three distinct mitochondrial clades were found, and four genetically and geographically distinct populations of aoudad in North Africa were identified. Of these four genetic groups, three were sources of individuals for the introductions in Spain. Additionally, our data do not support the current subspecies classification.

Population structure and signs of gene flow

Our results on the population structure highlighted the presence of four distinct wild populations: Egypt (Pop1), Central Sahara (Algeria, Niger, and Egypt; Pop2), Atlantic Sahara (Pop3), and Tunisia (Pop4). We recorded the highest F_{ST} value ($F_{ST} = 0.520$; Table 3) between Egyptian and Tunisian populations, suggesting a long history of divergence and reduced gene flow between the populations living in these areas. On the contrary, signs of admixture were found between the Atlantic and Central Sahara populations (individuals 16, 18, and 19; Fig. 1) and between the Central Sahara and Egyptian populations (individuals 7, 8, and 9; Fig. 1). Likewise, the lowest pairwise F_{ST} value ($F_{ST} = 0.179$) was observed between Atlantic and Central Sahara populations. These findings open the hypotheses that gene flow may presently occur between these two populations. The aoudad is known to be able to make small migratory movements in relation to food availability, being capable to survive far from water sources for long periods (even years), relying mostly on water obtained from plants (Cassinello et al. 2021). Natural gene flow may occur between the Atlantic and Central Sahara populations through the Tademait plateau (Algeria), which may potentially connect the populations in the province of Bechar with those in southern regions. Populations in southwestern Egypt have been recorded to conduct long migrations along the Egypt-Libya bordering area (Manlius et al. 2003, and references herein). Local grasslands may ensure ecological connectivity between populations in Egypt and those inhabiting the Tibesti mountains in Chad, which in turn may also be connected with those in Niger. However, individual 4 (Clade 1, in green in Fig. 2, from Algeria), and individual 9 (Pop2, in green in Fig. 1, from Egypt), show different haplotypes and genotypes from the other individuals of the populations where the samples were collected (Egyptian and Nigerian populations, respectively). Game species are often subject to translocations of individuals to re-establish or strengthen overhunted populations (Silva et al. 2015; Cassinello et al. 2021; Stipoljev et al. 2021). For instance, in Naâma (Algeria) thousands of ungulates of captive breeding origins are systematically released since 2016 on the scope of a reintroduction programme of the Algerian government in partnership with the United Arabic Emirates (https://www.djazairess.com/fr/lesoirdalgerie/1019815). These translocations might have been made irrespectively of the genetic diversity and local adaptations of source and receiving populations (Silva et al. 2015; Cassinello et al. 2021; Stipoljev et al. 2021). For this reason, it is unclear if the genetic admixture we recorded is caused by natural gene flow or if it is the result of human-mediated translocation events. Additional sampling and landscape genetics studies are needed to test for the presence and levels of gene flow between these populations, as well as the occurrence of functional population connectivity.

Table 3 Pairwise values of genetic differentiation (F_{ST}) between African and Spanish aoudad populations based on 11 microsatellite loci

Population	Country	Pop1	Pop2	Pop3	Pop4
Pop1	Egypt				
Pop2	Central Sahara	0.390			
Pop3	Atlantic Sahara	0.407	0.179		
Pop4	Tunisia	0.520	0.206	0.287	
Pop5	Spain	0.449	0.269	0.221	0.210

All pairwise F_{ST} values between populations were significant at $P < 0.01$

Genetic diversity in wild and captive populations

Despite the relatively limited sample size of our study, our preliminary results highlight the worrisome low genetic diversity of the native populations of this species. We recorded low genetic diversity in both, native and introduced populations (Table 4). Except for the Atlantic Sahara ones, all sampled populations displayed lower observed heterozygosity (H_o) than expected (H_e). The observed low genetic diversity is likely the result of high levels of inbreeding (Slate et al. 2004), probably caused by the continuous decline in range and population size recorded for the native populations of this species over the last century (Kowalski and Rzebik-Kowalska 1991; Smet 1997; Bounaceur et al. 2016; Derouiche et al. 2020; Cassinello et al. 2021). Reports from scientific expeditions in Algeria, Niger, and Tunisia during the 19th century testify that the aoudad was widely distributed in these regions at the time (e.g. Gervais 1848; Aucapitaine 1856; Loche 1867; Colomb 1858; Tristram 1860). However, a decline caused by poaching and habitat loss started at the beginning of the 20th century (Joleaud 1927), and continued until today, leading the Central Sahara populations on the verge of extinction (Bounaceur et al. 2016; Derouiche et al. 2020; Gašparová et al. 2020). The Egyptian population is also suffering from low genetic diversity, displaying the lowest levels of genetic diversity compared to both African and Spanish populations (Pop1; $H_o = 0.304$ $H_e = 0.427$) (Table 4). As in other areas of its distribution, in less than two centuries the Egyptian aoudad went from being spread all over Egypt (until the Arabian Peninsula) to being present in just three remote locations on the border with Libya and Sudan (Amer 1997). Further genetic assessments of aoudad's wild populations are needed to validate the current genetic patterns found and deepen

Table 4 Estimates of genetic diversity for the five wild populations of *Ammotragus lervia* highlighted by our phylogenetic and STRUCTURE results

Microsatellites						
Populations	N	Nav	Npr	Ho	He	F
Pop1-Egypt	9	2.909	1.622	0.374	0.403	0.163
Pop2-Central Sahara	10	4.182	2.618	0.409	0.558	0.237
Pop3-Atlantic Sahara	8	2.909	2.661	0.532	0.472	−0.097
Pop4-Tunisia	16	2.636	2.143	0.344	0.437	0.169
Pop5-Spain	24	3.727	2.547	0.490	0.569	0.123

Based on 11 microsatellite loci

N number of samples; *Nav* average number of different alleles per locus; *Npr* average number of alleles unique to a single population; *Ho* observed heterozygosity; *F* fixation index [(He-Ho)/He = 1-(Ho/He)]

the determination of intraspecific genetic diversity of each specific population.

Spanish populations have the highest difference between expected (H_e = 0.616) and observed (H_o = 0.403) heterozygosity among all the populations analysed. Low levels of mitochondrial and nuclear genetic diversity were already recorded in European populations by Stipoljev et al. (2021). The study also highlighted that the populations from Sierra María-Los Velez might be the ones with the highest genetic diversity among all the populations introduced in Europe, which is in line with our phylogenetic results showing the presence of at least two maternal clades from Atlantic and Central Sahara (red and green in Fig. 2). Overall, our preliminary results suggest that before including individuals from Spain in future reintroduction projects, the genetic diversity of these individuals should be carefully evaluated to avoid interbreeding in wild populations.

Null alleles and allelic dropout are particularly frequent when analysing non-invasive samples, due to the low quality (and quantity) of DNA found in these sample types (Pompanon et al. 2005). The null alleles and Hardy-Weinberg disequilibrium recorded for some of the loci targeted in this study likely derive from the un-specificity of the markers used, originally developed for the genus *Ovis* (ISAG). In future studies, primers specifically designed for the aoudad should be implemented to counteract for the presence of genotyping errors and for giving more precise information on population genetic structure and diversity of this species.

Phylogeographic relationship of native and introduced populations

Our phylogenetic reconstruction groups the North African aoudad populations in three distinct mitochondrial lineages from the Atlantic Sahara, Tunisia, and Central Sahara. This classification is discordant with the current systematic of the species (discussed below) based on morphological characters (see Cassinello 1998), but sheds light on the likely African origin of the introduced populations in Spain: (i) individuals sampled in the Zoo of Madrid display mitochondrial affinities to the lineage from the Atlantic Sahara (Clade 2 in red; Fig. 2), but admixed genotypes belonging to populations from Tunisia, and Sierra María-Los Velez (Spain); (ii) individuals sampled in the Zoos of Cordoba and Barcelona display similar genotypes and mitochondrial affinities to the Central Sahara populations (Clade 1 and Pop2 in green in Figs. 1 and 2); (iii) individuals at the EEZA research centre in Almeria (Spain) clustered with individuals from the Atlantic Sahara and Tunisia lineages (individual number 42 in Fig. 2); (iv) individuals from the introduced population in Sierra María-Los Velez cluster with individuals from the Atlantic and Central Sahara lineages (specifically with an individual from Niger). The populations kept at the EEZA are known to have originated from a wild couple captured in the Atlantic Sahara, and the population in Sierra María-Los Velez to have originated from individuals from the Frankfurt Zoo in Germany and the Ain Sebad Zoo in Casablanca (Morocco) (Stipoljev et al. 2021). The Frankfurt Zoo and the Ain Sebad Zoo were also the source for other European populations in Croatia (Mosor Mountain), the Czech Republic (surroundings of Plzeň), Murcia and La Palma (Spain) (Stipoljev et al. 2021). Since the individuals kept in the Ain Sebad Zoo likely belong to the Atlantic Sahara lineage (Stipoljev et al. 2021), we hypothesised that at least some individuals kept in the Zoo of Frankfurt belonged to a population in Niger (Central Sahara lineage). However, additional analyses involving genomic data are needed to test the actual origin and genetic adaptations of individuals from captive populations before future reinforcement of wild populations with captive bred individuals can be proposed.

Implications for taxonomy and systematics

In line with our phylogenetic reconstruction and genetic structure analyses from the sampled wild populations we recommend the following systematic subdivision: (i) The

subspecies *angusi, fassini, ornata*, and *sahariensis* should be considered as a single subspecies, distributed from north-western Algeria to Niger and Egypt; (ii) the Atlantic Sahara population, previously included to the subspecies *sahariensis*, should be considered as a unique taxonomic entity (potentially a new subspecies) distributed from the Atlantic Sahara region to the province of Bechar (Algeria); and (iii) the distribution of the nominal subspecies *lervia* should be restricted to Tunisia. The Egyptian individuals grouped together (although with low support; PP = 93; Fig. 2) in the phylogenetic analyses and STRUCTURE bar plot (in brown in Fig. 1) and it presents the highest F_{ST} values if compared with the other four populations (Table 3). This line of evidence may instead suggest the validity of the subspecies *ornata* endemic to Egypt, and previously described based on morphological traits (see Cassinello 1998). However, one individual from Algeria (number 4 in Fig. 2; retrieved from Derouiche et al. 2020) grouped within the Egyptian lineage in the phylogenetic tree. Although Derouiche et al. (2020) collected samples from this individual in the wild, it cannot be excluded that individual comes from a previous translocation of Egyptian individuals to Algeria aimed in restoring the Algerian populations that have been massively hunted in the past (i.e. as it is happening in Naâma; De Smet 1997; Derouiche et al. 2020). For this reason, further genetic and morphological data are needed to corroborate the existence of the subspecies *ornata*. The current study refrains from formally redefining the systematic of the aoudad and testing validity of the subspecies *ornata* and of the Atlantic Sahara lineage as new subspecies. These two topics should be addressed in an integrative taxonomy approach (Padial et al. 2010), including additional genetic (samples and markers), ecological, morphological, and distributional data.

Conclusions and conservation recommendations

Despite being protected by national law, the management of aoudad wild populations still needs improvements in most of the species' distribution. Future translocation projects might be needed to reinforce the native populations, but this may result ineffective if poaching and habitat loss endure (Silva et al. 2015; Gardner et al. 2021). Moreover, translocations are an important conservation tool, but their success depends also on retaining genetic fitness and evolutionary potential (Pacioni et al. 2019). Translocations ignoring the genetic diversity distribution could have deleterious effects on wild populations as diluting the adaptation to climate or pathogen load, leading to high mortality and poor success of the management intervention (Bertola et al. 2022). The data we provide in this study allowed determining the aoudad main genetic units in the native distribution of the species, laying the ground for a more comprehensive genetic analysis of its native populations. In light of the results found, we suggest the following conservation actions: (i) conducting regular census in all known wild populations to keep monitoring demographic trends; (ii) implementing a landscape genomic approach to uncover local genetic adaptations and ecological corridors used by the species; (iii) including samples from individuals of the subspecies *blainei* to formally redefining the subspecific taxonomic units through an integrative taxonomy approach; (iv) to promote the establishment of international protected areas where hunting is strictly prohibited or carefully regulated; (v) use genomic data to assess the genetic diversity of all European and American introduced populations, to highlight their potential role as source individuals for future reintroductions; (vi) collaborating with local communities to find alternative livelihood project to poaching (i.e. ecotourism); and (vii) reintroduction, translocation, and reinforcement programs should consider at first the design of management units, which delineation should be based on the partial reproductive isolation/outbreeding depression.

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Author contributions TA and JCB conceived the ideas; TLS, TA, and JCB collected the data; CP, TLS and GB analysed the data; CP led the writing with inputs from all authors.

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Data availability The datasets generated during and/or analysed during the current study are available as supporting material (Alignments 1 and 2) and in the GenBank.

Declarations

Competing interest The authors declare no competing interests.

Ethical approval Sample collection made under authorisation from the Haut-Commissariat aux Eaux et Forêts et à la Lutte Contre la Désertification of Morocco (permit 05/2016), and the Direction Générale des Fôrets of Algeria and of Tunisia (in the frame of collaboration con-

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