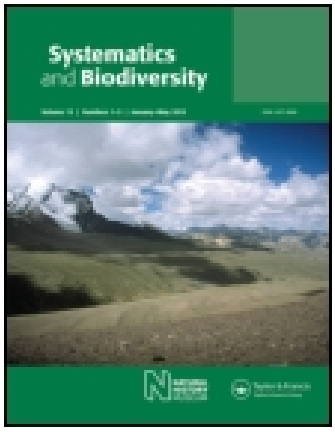

Systematics and Biodiversity
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/tsab20

Molecular DNA identity of the mouflon of Cyprus (Ovis orientalis ophion, Bovidae): Near Eastern origin and divergence from Western Mediterranean conspecific populations

Monica Guerrini[a], Giovanni Forcina[a], Panicos Panayides[b], Rita Lorenzini[c], Mathieu Garel[d], Petros Anayiotos[b], Nikolaos Kassinis[b] & Filippo Barbanera[a]

[a] Dipartimento di Biologia, Unità di Zoologia e Antropologia, Via A. Volta 4, 56126 Pisa, Italy

[b] Game Fund Service, Ministry of Interior, 1453 Nicosia, Cyprus

[c] Istituto Zooprofilattico Sperimentale delle Regioni Lazio e Toscana, Centro di Referenza Nazionale per la Medicina Forense Veterinaria, Via Tancia 21, 02100 Rieti, Italy

[d] Office National de la Chasse et de la Faune Sauvage, Centre National d'Études et de Recherche Appliquée Faune de Montagne, 5 allée de Bethléem, Z.I. Mayencin, 38610 Gières, France

Published online: 11 Jun 2015.

Systematics and Biodiversity (2015), **13(5)**: 472–483

Taylor & Francis
Taylor & Francis Group

Research Article

Molecular DNA identity of the mouflon of Cyprus (*Ovis orientalis ophion*, Bovidae): Near Eastern origin and divergence from Western Mediterranean conspecific populations

MONICA GUERRINI[1], GIOVANNI FORCINA[1], PANICOS PANAYIDES[2], RITA LORENZINI[3], MATHIEU GAREL[4], PETROS ANAYIOTOS[2], NIKOLAOS KASSINIS[2] & FILIPPO BARBANERA[1]

[1]Dipartimento di Biologia, Unità di Zoologia e Antropologia, Via A. Volta 4, 56126 Pisa, Italy
[2]Game Fund Service, Ministry of Interior, 1453 Nicosia, Cyprus
[3]Istituto Zooprofilattico Sperimentale delle Regioni Lazio e Toscana, Centro di Referenza Nazionale per la Medicina Forense Veterinaria, Via Tancia 21, 02100 Rieti, Italy
[4]Office National de la Chasse et de la Faune Sauvage, Centre National d'Études et de Recherche Appliquée Faune de Montagne, 5 allée de Bethléem, Z.I. Mayencin, 38610 Gières, France

(Received 2 February 2015; accepted 23 March 2015)

The mouflon population of Cyprus (*Ovis orientalis ophion*) comprises historically preserved feral descendants of sheep domesticated during the Neolithic. We determined genetic identity of this taxon in order to elucidate its systematic placement and enforce its protection. We used 12 loci of microsatellite DNA to infer genetic relationships between the Cypriot mouflon and either long-time isolated (Corsica, Sardinia) or recently introduced (central Italy) European mouflons (*O. o. musimon*). We also sequenced the mitochondrial DNA (mtDNA) Cytochrome-*b* gene to infer the origin of the Cypriot mouflon including many National Centre for Biotechnology Information (NCBI) entries of European and Near Eastern conspecifics. Microsatellites disclosed net divergence between Western Mediterranean and Cypriot mouflon. The latter was included in the highly heterogeneous Near Eastern *O. orientalis* mtDNA group, Iran representing the most credited region as the source for its ancient introduction to Cyprus. Both international and national legislation protect the mouflon of Cyprus as a wild taxon (*O. o. ophion*). However, the IUCN Red List of Threatened Species and NCBI include the Cypriot mouflon as subspecies of its respective domestic species, the sheep (*O. aries*). Unfortunately, people charged with crime against protected mouflon may benefit from such taxonomic inconsistency between legislation and databases, as the latter can frustrate molecular DNA forensic outcomes. Until a definitive light can be shed on Near Eastern *O. orientalis* systematics, we suggest that the Cypriot mouflon should be unvaryingly referred to as *O. o. ophion* in order not to impair conservation in the country where it resides.

Key words: Cyprus, domestic sheep, Mediterranean, microsatellite DNA, mitochondrial DNA, Mouflon, near eastern, *Ovis*, taxonomy, wild sheep

Introduction

The European mouflon (*Ovis musimon*, Bovidae – but see below) is thought to represent the relic of the first domesticated sheep readapted to feral life (e.g. Hiendleder, Kaupe, Wassmuth, & Janke, 2002). Historically preserved mouflon populations are presently restricted to the islands of Corsica, Sardinia and Cyprus. The conservation value of those introduced into continental Europe (in the 18th century) is varied and requires *ad hoc* investigation, as only some populations have a known history while others have multiple/mysterious origins (Andreotti et al., 2001; Boitani, Lovari, & Vigna Taglianti, 2003; Cugnasse, 1994; Piegert & Uloth, 2005; Türcke & Schmincke, 1965; Uloth, 1972).

Many revisions based on different criteria have made the systematics of the genus *Ovis* take on the appearance of a very complex puzzle (Hiendleder et al., 2002). Wilson and Reeder (2005) listed both European (*O. musimon*) and Near Eastern (*O. orientalis*) mouflon as domestic sheep (*O. aries*) subspecies (*O. a. musimon* and *O. a. orientalis*, respectively), the Cypriot mouflon being referred to as *O. a. ophion*. Other authors (e.g., Shackleton & IUCN/SSC Caprinae Specialist Group, 1997) argued

Correspondence to: Filippo Barbanera. E-mail: filippo.barbanera@unipi.it

ISSN 1477-2000 print / 1478-0933 online

http://dx.doi.org/10.1080/14772000.2015.1046409

that mouflon and domestic sheep should be considered as distinct species (*O. orientalis* and *O. aries*, respectively). Lately, DNA data suggested ranking the European mouflon as a subspecies (*O. o. musimon*) of the Near Eastern one (Rezaei et al., 2010).

Claimed for a long time as endemic to this island (Cugnasse, 1994; Hadjisterkotis, 1993, 1999; Shackleton et al., 1997), the mouflon of Cyprus (*O. orientalis ophion*) has never been included in any comparative molecular DNA study. Lawson Handley et al. (2007) investigated the genetic structure of European sheep breeds, yet only two Cypriot mouflons were genotyped at the microsatellite DNA (Short Tandem Repeats, STRs). Other authors either did not include (Rezaei et al., 2010) or used very marginally (Bruford & Townsend, 2006; Demirci et al., 2013) mitochondrial DNA (mtDNA) sequences of Cypriot mouflon. Chessa et al. (2009) investigated Eurasian sheep using endogenous retroviruses as markers and found relic genomic traits of ancestral sheep mostly in the Cypriot mouflon. While the Fertile Crescent hosted early domestication (11,000 BP), Cyprus acted as a stepping-stone since the first wave (10,500 BP) of sea-faring colonists dispersing Near Eastern livestock species westwards across the Mediterranean (Peters, von den Driesch, & Helmer, 2005; Vigne, Buitenhuis, & Davis, 1999, 2003; Zeder, 2008).

Here, in compliance with current legislation and taxonomy followed in Rezaei et al. (2010), we refer to Corsican/Sardinian and Cypriot mouflon as *O. o. musimon* and *O. o. ophion*, respectively. We attempted to determine the molecular DNA identity of the mouflon of Cyprus in order to elucidate its systematic placement and, accordingly, enforce its protection. We used a panel of STR loci to infer both genetic structure and relationships with either historically preserved (Corsica, Sardinia) or recently introduced (central Italy) populations. In addition, we employed the mtDNA to infer the origin of the Cypriot mouflon within a phylogeographic framework including many National Centre for Biotechnology Information (NCBI) entries from the Near East.

Materials and methods

The mouflon of Cyprus

In the last century, the mouflon of Cyprus faced serious challenges related to habitat loss/fragmentation, disease transmission through livestock and poaching (Ioannou et al., 2011). Population distribution range is limited to the mountainous Paphos forest (a state-owned area of about 620 km^2 managed by the Forestry Department) and adjacent forest areas in the western side of Cyprus. Census is stable (3,000 head), as recently assessed by the Game Fund Service of the Ministry of the Interior and cited in the Mouflon Management Plan (Sfouggaris, 2011). Protected by national legislation, the mouflon of Cyprus is included as *O. o. ophion* in both Annexes II/IV of 92/43 Habitats Directive and Appendix I of CITES (see online supplemental material, which is available from the article's Taylor & Francis Online page at http://dx.doi.org/10.1080/14772000.2015.1046409).

Biological sampling

The Cypriot Game Fund Service in collaboration with the Cypriot Veterinary Services collected 63 mouflon samples (dry blood spot on Whatman filter paper): 53 were from individuals captured in the Paphos forest (Fig. S1, see supplemental material online), eight from local captive animals and two of unknown origin. We also sampled 20 mouflons in Sardinia (6000 head; Apollonio, Luccarini, Giustini, Scandura, & Ghiandai, 2005) either in the wild (16: blood: Ogliastra Province) or in captivity (four: hairs: Breeding and Wildlife Recovery Centre, Bonassai, Sassari). These latter were originally from the Asinara National Park. We also collected many dry faecal samples of Corsican (1000 head, minimum; M. Garel, unpublished data) and central Italy (Tuscany) mouflons during winter (Maudet, Luikart, Dubray, Von Hardenberg, & Taberlet, 2004). Each sample was individually housed in a plastic tube, kept at 4 °C in the field and not extra dried before it was stored at −40 °C within 8h from its collection. We analysed one scat per sampling site to avoid duplicates from the same animal in both Corsican (19) and central Italy (23: Tuscan Archipelago National Park, 13; Tuscan-Emilian Apennines National Park, six; Apuan Alps Regional Park, four) populations. With the exception of faeces (no chemicals added: cf. Guerrini & Barbanera, 2009), all samples were preserved in 96% ethanol. Detailed sampling information is given in Fig. 1 and Table S1 (see supplemental material online).

DNA extraction

We extracted DNA from blood/hairs using Puregene Core Kit-A (Qiagen, Germany) and from faeces using QIAamp DNA Stool Mini Kit (Qiagen) following the manufacturer's instructions. In order to minimize the risk of contamination, we thoroughly swabbed laboratory equipment with 4.2% sodium hypochlorite and autoclaved all disposables in their containers. We monitored reliability of each DNA extraction through two negative controls (no tissue added). We determined DNA concentration and purity with an Eppendorf BioPhotometer (AG Eppendorf, Germany) (faeces excluded).

Microsatellite DNA

We genotyped all Corsica, Sardinia, central Italy and Cyprus (19 + 20 + 23 + 63 = 125) samples at 12 STR

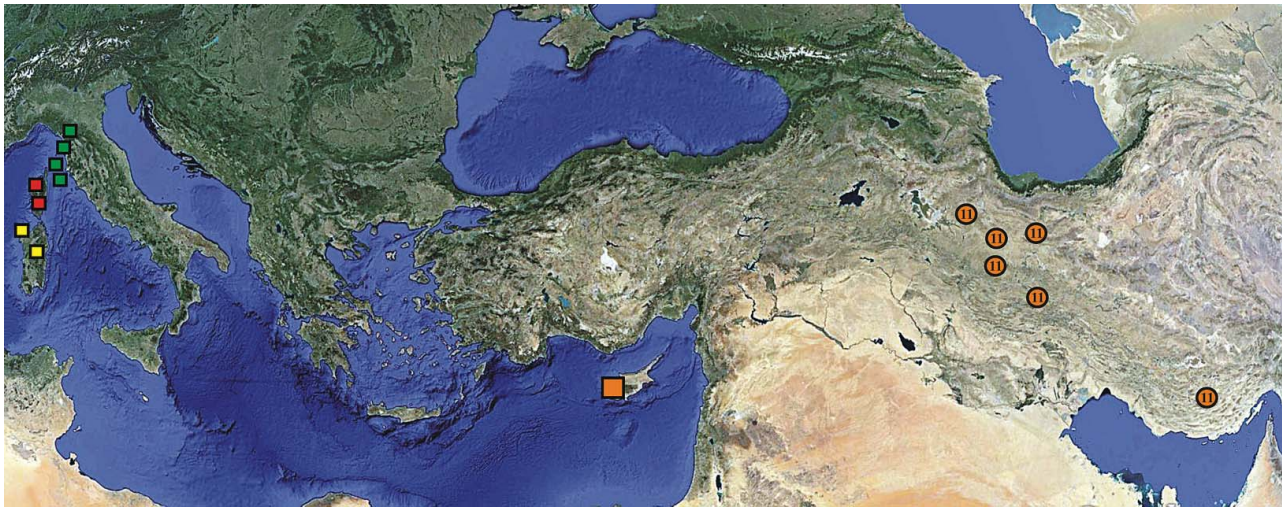

Fig. 1. Map of the study area. Red squares: Corsica (upper: Mt. Cinto population; lower: Bavella population); yellow squares: Sardinia (upper: Asinara National Park; lower: Ogliastra Province); green squares, central Italy (from the upper to the lower square: Tuscan-Emilian National Park, Apuan Alps Regional Park, Capraia Island and Elba Island); large orange square: Paphos Forest, Cyprus. Near Eastern (Iran) localities hosting H11 (the single haplotype disclosed in Cyprus, see Results) are indicated with an orange circle (cf. Fig. 4). See Table S1, online supplementary material for detailed information for each population.

loci isolated from domestic sheep (*O. aries*), goat (*Capra hircus*) and cattle (*Bos taurus*) genome (Table 1). We performed PCRs (12.5 μL) as in Barbanera et al. (2012). However, we added 0.3 μL of 1:4 diluted not-acetylated bovine serum albumin (20 mg/mL; Sigma Aldrich) to reactions including DNA from faeces/hair. We carried out gene sizing on an ABI Prism 3730 DNA automated sequencer with GENESCAN (Applied Biosystems). Only for faeces/hairs, we genotyped each locus from two to five times according to the comparative multiple-tubes approach of Frantz et al. (2003). Then, we used GIMLET (v. 1.3.3; Valière, 2002) to reconstruct consensus genotypes.

We evaluated the discriminatory power of the whole panel of loci by estimating the probability that two individuals drawn at random from the populations showed identical multilocus genotypes by chance (P_{ID} and P_{ID} sib: for the latter, we assumed sibling relationships: Paetkau et al., 1998; Waits, Luikart, & Taberlet, 2001). We used ARLEQUIN (v. 3.5.1; Excoffier & Lischer, 2010), FSTAT (v. 2.9.3; Goudet, 2001) and GENEPOP (v. 3.4; Raymond & Rousset, 1995) to (i) compute the

Table 1. Characteristics of STR loci. T_M (°C), annealing temperature; TD, touch-down PCR; H_O, mean observed heterozygosity; H_E, mean expected heterozygosity; P_{ID}, probability that two individuals drawn at random share identical genotypes by chance; P_{ID}sib, probability of identity among siblings. STR loci are sorted according to the increasing order of their P_{ID} and P_{ID}sib single-locus values (the locus at the top is the most informative one), and a sequentially multi-loci P_{ID} (P_{ID}sib) is reported for each locus.

Locus	T_M (°C)	Size-range (bp)	Repeat motif	H_O	H_E	P_{ID}	P_{ID}sib	Literature record
OarFCB48	TD 58-55	134–168	$(GT)_{13}$	0.63	0.88	2.84×10^{-2}	3.20×10^{-1}	Buchanan et al. (1994)
ILSTS028	TD 55-50	125–175	$(AC)_{13}$	0.62	0.85	1.04×10^{-3}	1.08×10^{-1}	Kemp et al. (1995)
OarFCB304	TD 58-55	141–189	$(TC)_{7}$	0.57	0.80	6.09×10^{-5}	3.92×10^{-2}	Buchanan & Crawford (1993)
SR-CRSP8	50	211–247	$(GT)_{11}$	0.34	0.80	3.93×10^{-6}	1.44×10^{-2}	Bhebhe et al. (1994)
OarJMP58	TD 60-55	138–174	$(TG)_{18}$	0.49	0.79	2.32×10^{-7}	5.29×10^{-3}	Crawford et al. (1995)
MCM527	TD 58-55	155–179	$(GT)_{11}$	0.43	0.79	1.72×10^{-8}	1.99×10^{-3}	Hulme et al. (1994)
BM415	50	131–177	$(TG)_{13}$	0.45	0.78	1.17×10^{-9}	7.49×10^{-4}	Bishop et al. (1994)
OarAE129	TD 55-50	137–165	$(AC)_{12}$	0.30	0.76	1.08×10^{-10}	2.95×10^{-4}	Penty et al. (1993)
MAF70	TD 60-55	121–137	$(AC)_{16}$	0.33	0.69	1.31×10^{-11}	1.28×10^{-4}	Buchanan & Crawford (1992)
SR-CRSP7	50	152–192	$(GT)_n\,(AT)_n$	0.18	0.68	1.56×10^{-12}	5.64×10^{-5}	Bhebhe et al. (1994)
ILSTS011	TD 58-55	262–292	$(TC)_{9}$	0.37	0.64	2.54×10^{-13}	2.67×10^{-5}	Brezinsky et al. (1993)
SR-CRSP9	TD 58-55	99–141	$(GT)_{5}$	0.33	0.48	7.69×10^{-14}	1.57×10^{-5}	Bhebhe et al. (1994)

number of alleles per locus, the number of unique alleles and the allelic richness; (ii) calculate expected (H_E) and observed (H_O) heterozygosity; (iii) infer deviations from both Hardy–Weinberg Equilibrium (HWE) and Linkage Disequilibrium (LE) (10,000 dememorization, 100 batches, 5,000 iterations per batch); (iv) investigate the partition of the STR diversity within and among populations by AMOVA; (v) infer the degree of genetic differentiation among populations by estimating average pairwise F_{ST} distance values. These latter were also plotted on the first two axes of a Principal Components Analysis (PCA) using STATISTICA 5.0/W (Statsoft Inc., USA). We adopted Bonferroni correction (Hochberg, 1988) to adjust the significance level of each statistical test.

Bayesian clustering analysis was performed with STRUCTURE (v. 2.3.4; Pritchard, Stephens, & Donnelly, 2000) to investigate the spatial structure of the genetic diversity. We attempted to determine the K (unknown) clusters of origin of the sampled individuals and to assign them to each cluster. Simulations were performed with 10^6 Markov Chain of Monte-Carlo iterations (burn-in: 10^5 iterations) and replicated five times per each K-value (1 to 12). We choose the correct K-value using the maximum of the function $\Delta K = m(|L(K+1) - 2\,L(K) + L(K-1)|)/s[L(K)]$, where $L(K)$ stands for 'log estimated likelihood' calculated for each K value, m for "mean" and s for "standard deviation" (Evanno, Regnaut, & Goudet, 2005). An identification threshold (Q_i) to each cluster was set to 0.90 (Väha & Primmer, 2006).

Only for the Cypriot mouflon population, we computed maximum likelihood estimates of relatedness (i.e., the likelihood that a pair of individuals would be classified as either full-siblings, half-siblings or unrelated) with ML-RELATE (Kalinowski, Wagner, & Taper, 2006), and we calculated the inbreeding coefficient (f; Weir & Cockerham, 1984) using GENETIC DATA ANALYSIS (v. 1.1) (1,000 bootstrapping replicates across loci). We used BOTTLENECK (v. 1.2.02: Piry, Luikart, & Cornuet, 1999) with a Two Phase Mutation (TPM) model (1,000 replicates; Di Rienzo et al., 1994) to find evidence of genetic bottlenecks, and carried out a qualitative mode signed-rank test.

Mitochondrial DNA

We amplified the entire mtDNA Cytochrome-*b* gene (Cyt-*b*, 1140 bp) using primers Cytb_F and Cytb_R of Pedrosa et al. (2005). PCR (50 μL) reactions contained 1 μL of Ampli*Taq* Gold DNA Polymerase (1 U/μL), 4 μL of 25 mM $MgCl_2$, 5 μL of 10 × PCR Gold buffer (Applied Biosystems, USA), 5 μL of 2.5 mM dNTP (Sigma Aldrich, Italy), 3 μL of each primer (1 μM) and *c*. 20 ng of DNA template (for faeces: 3 μL, final elution). We performed PCRs in a MyCycler thermal cycler (v. 1.065, Biorad) with the following profile: 3 min 94 °C, 35 cycles of 1 min 94 °C, 2 min at 55 °C and 1 min 72 °C, followed by 7 min 72 °C. For faecal samples only, however, when we could not visualize any PCR product after the gel electrophoresis, we re-amplified first amplicon in a semi-nested PCR as described by Guerrini and Barbanera (2009). PCR products were purified (Genelute PCR Clean-up Kit, Sigma Aldrich) and directly sequenced on both DNA strands using the BigDye Terminator v. 3.1 Cycle Sequencing Kit on an ABI 3730 DNA automated sequencer (Applied Biosystems).

We sequenced the Cyt-*b* gene for 41 Cypriot and all remaining (Corsica + Sardinia + central Italy: 62) samples (41 + 62 = 103). In order to include in the alignment 57 GenBank entries (Corsica, two; Turkey, nine; Armenia, one; Iran, 45: Table S1, see supplemental material online) we cut our sequences at both 5'- (positions: 1–21) and 3'- (positions: 1064–1140) ends. Hence, we aligned 160 sequences (final length: 1042 bp) with CLUSTALX (v. 1.81: Thompson, Gibson, Plewniak, Jeanmougin, & Higgins, 1997) and inferred haplotype composition with DNASP (v. 5.00: Librado & Rozas, 2009).

We selected the HKY85 (Hasegawa, Kishino, & Yano, 1985) + I + G substitution model using MODELTEST (v. 3.06: Posada & Crandall, 1998) and the Akaike Information Criterion (AIC = 3945.1; Akaike, 1974). Then, we performed a Maximum Likelihood (ML) tree reconstruction using PHYML (v. 3.0: Guindon et al., 2010) platform (www.atgc-montpellier.fr) and setting main parameters as follows: I = 0.77, α = 0.017 and T_i/T_v = 7.50. We employed *O. ammon argali* (Argali or mountain sheep) AJ867266 sequence of Bunch, Wu, and Zhang (2006) as outgroup, and evaluated the statistical support for each node by bootstrapping (BP, with 10,000 replicates: Felsenstein, 1985). We also constructed a haplotype network with DNA ALIGNMENT (v. 1.3.3.2; 2003–2013 Fluxus Technology) and the Median Joining method (Bandelt, Forster, & Röhl, 1999) as in NETWORK (v. 4.6.1.2; 2004–2014 Fluxus Technology). We excluded Armenia from our dataset (one sequence: Table S1; see supplemental material online) before using ARLEQUIN to calculate haplotype diversity (h), mean number of pairwise differences (k), and nucleotide diversity (π) for each population. The AMOVA was performed among and within the populations using the ϕ_{ST} analogous to Wright's (1965) F-statistics (1,000 permutations).

Results

Microsatellite DNA

The STR panel was powerful in discriminating individuals (n = 125: $P_{ID} = 7.69 \times 10^{-14}$ and $P_{ID}sib = 1.57 \times 10^{-5}$; Table 1), as values lower than 0.001 can be considered satisfactory (Waits et al., 2001). All loci were highly polymorphic with the exception of SR-CRSP7 and SR-CRSP9

Table 2. STR genetic variability for each Mediterranean population: n, sample size; n_a, average number of alleles/locus; A_r, allelic richness; A_u, number of unique alleles; H_O, observed heterozygosity; H_E, expected heterozygosity; p_{HWE}, probability value for the Hardy–Weinberg Equilibrium test; χ^2 test with relative degrees of freedom (*df*) (Fisher exact test, all loci). Departure from HWE was significant in all populations after Bonferroni correction ($\alpha = 0.05$, $\alpha' = 0.05/48 = 0.001$).

Population	n	n_a	A_r	A_u	H_O	H_E	p_{HWE}	χ^2 (*df*)	Average gene diversity
Corsica	19	7.9	7.7	21	0.54	0.77	< 0.001	∞ (24)	0.69
Sardinia	20	5.4	5.2	8	0.55	0.66	< 0.001	81.8 (24)	0.61
Central Italy	23	7.2	6.8	14	0.48	0.74	< 0.001	∞ (24)	0.64
Cyprus	63	5.5	3.6	30	0.39	0.49	< 0.001	∞ (20)	0.39

(monomorphic within the Cypriot population). The total number of alleles at each locus ranged between nine and 15 (13.3, on average): the mouflon of Cyprus hold either the lowest average number of alleles per locus (5.5) or the highest total number of private alleles (30) (Table 2).

All populations showed significant departure from HWE due to heterozygote deficiency after Bonferroni correction (Fisher test: $P < 0.001$: Table 2). Such deviation was highly significant at four, three and five loci in Cyprus, Corsica and central Italy, respectively ($p < 0.001$, Table S4, see supplemental material online). Average level of both H_O and H_E (0.39 and 0.49, respectively: Table 2) was lower in the Cypriot mouflon populations than in all of the other ones. LE test carried out for all pairs of loci across all populations was significant only for one (MAF70 versus OarJMP58) in 45 comparisons, only in the population from Corsica ($P < 0.001$, $P < \alpha' = \alpha/180 = 0.05/180 = 0.0003$, after Bonferroni correction) (data not shown).

We found that 66.2% of the STR variability was partitioned within populations and 33.8% among them ($F_{st} = 0.34$, $p < 0.001$). In the PCA plot (Fig. 2, upper part), the first two components explained the 98.2% of the total variability. The Cypriot population diverged from all the western Mediterranean ones ($0.38 < F_{st} < 0.47$, $p < 0.001$: Table 3), while mouflons of Corsica and central Italy ($P = 0.11$: Table 3) were closer to each other than to Sardinia (Fig. 2 and Table 3).

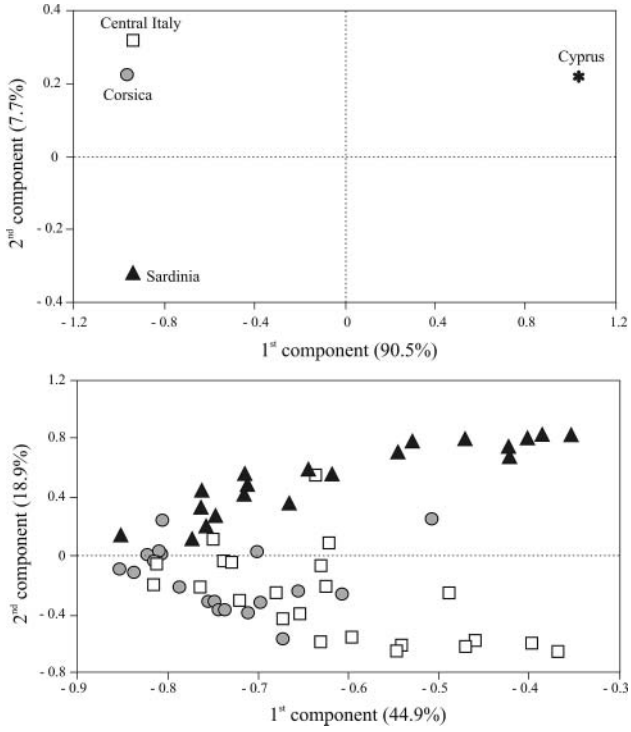

Fig. 2. The Principal Component Analysis performed using average pairwise F_{ST} distances among STR genotyped populations (upper part) and single mouflons (lower part: Cyprus excluded). The percentage of total variance explained by each of the first two components is given. Symbols are the same in both parts.

Table 3. Above diagonal: average pairwise distance values (F_{ST}) computed for the STR genotyped populations. Below diagonal: average pairwise distance values (ϕ_{ST}) computed among mtDNA genotyped populations (Armenia was excluded as it includes only one GenBank entry). All *p* values were highly significant ($p < 0.001$) except for Iran versus Turkey ($P = 0.01$) and Corsica versus Central Italy ($P = 0.11$) comparisons.

	Corsica	Sardinia	Central Italy	Cyprus	Turkey	Iran
Corsica	–	0.127	0.064	0.392	–	–
Sardinia	0.329	–	0.147	0.465	–	–
Central Italy	0.044	0.331	–	0.384	–	–
Cyprus	0.956	0.973	0.947	–	–	–
Turkey	0.612	0.629	0.607	0.810	–	–
Iran	0.555	0.520	0.559	0.387	0.112	–

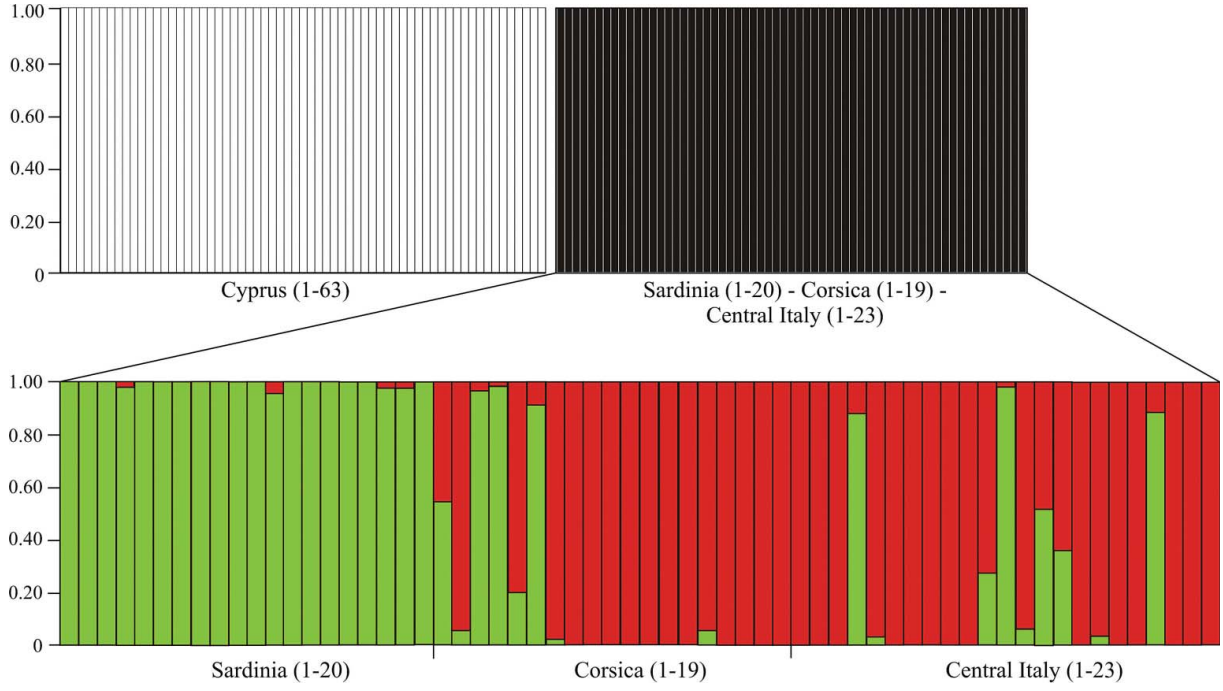

Fig. 3. Bayesian admixture analysis of STR genotypes computed by STRUCTURE with $K = 2$. Upper part: all populations. Lower part: only Sardinia, Corsica and central Italy. Each individual is represented as a vertical bar partitioned in K segments, whose length is proportional to the estimated membership in the K clusters.

Some deviation from HWE notwithstanding, we felt confident in using the entire STR panel for all individuals in the Bayesian clustering analysis, as it has been proved this may have a negligible effect on simulated assignment tests (Cornuet, Piry, Luikart, Estoup, & Solignac, 1999). In the STRUCTURE analysis mouflons were partitioned into two groups: the first included all individuals from Cyprus, while the second all those from Corsica, Sardinia and central Italy (Fig. 3, upper part: $Q_I = 1.00$, all populations). We repeated the analysis excluding the Cypriot population. We found that all mouflons from Sardinia were assigned to the cluster I ($Q_I = 0.99$: Fig. 3, lower part). Corsica and central Italy hold low assignment value to cluster II ($Q_{II} = 0.80$ and 0.82, respectively: data not shown), as their individuals clustered into group II (Corsica: 14; central Italy: 17), I (Corsica: three; central Italy: one) or were admixed (Corsica: two; central Italy: five).

As far as the population of the island of Cyprus is concerned, the PCA carried out using STR data from each sampling locality marked out a slight longitudinal gradient of genetic differentiation across the Paphos forest (Fig. S1, see supplementary material online). Nonetheless, the Bayesian clustering analysis did not confirm this result (see below). Coming to the single Cypriot mouflons, the average pairwise relatedness ranged from zero (1014 pairs) to one (one pair). We found that 78.5% of individuals were unrelated (1891 comparisons), 11.7% half siblings, 5.2% parent/offspring and 4.6% full siblings; the value of the coefficient of inbreeding (f) was 0.190. The frequency distribution of the STR alleles (Fig. S2, see supplementary material online) as well as all tests that were performed (Table S2, see supplementary material online) did not point to the occurrence of genetic bottlenecks.

Mitochondrial DNA

We found 36 haplotypes (H1-H36; accession codes: LN651259- LN651268, Table S1, see supplementary material online). The Iranian population showed the highest value for all diversity indexes, whereas one haplotype (H11) only was disclosed in Cyprus (Fig. 4, Table S3, see supplementary material online). The 66.4% of the variability was partitioned among populations while the 33.6% within them ($\phi_{ST} = 0.66$, $p < 0.001$).

ML tree and network concordantly disclosed two main groups of haplotypes. In the phylogenetic reconstruction (Fig. 4), first clade (BP = 80) included western European mouflons from Corsica, Sardinia and central Italy, one *O. orientalis gmelini* from Iran (H7) being the only exception. However, several Turkish and Iranian individuals shared haplotype H1 (see also Table S1, see supplementary material online), which was sister lineage to the previously mentioned group. Second clade (BP = 85) included most of Near Eastern *Ovis orientalis* ssp. In particular, the single Cypriot mouflon haplotype (H11) fell into a sub-clade (BP = 77) including mostly Iranian

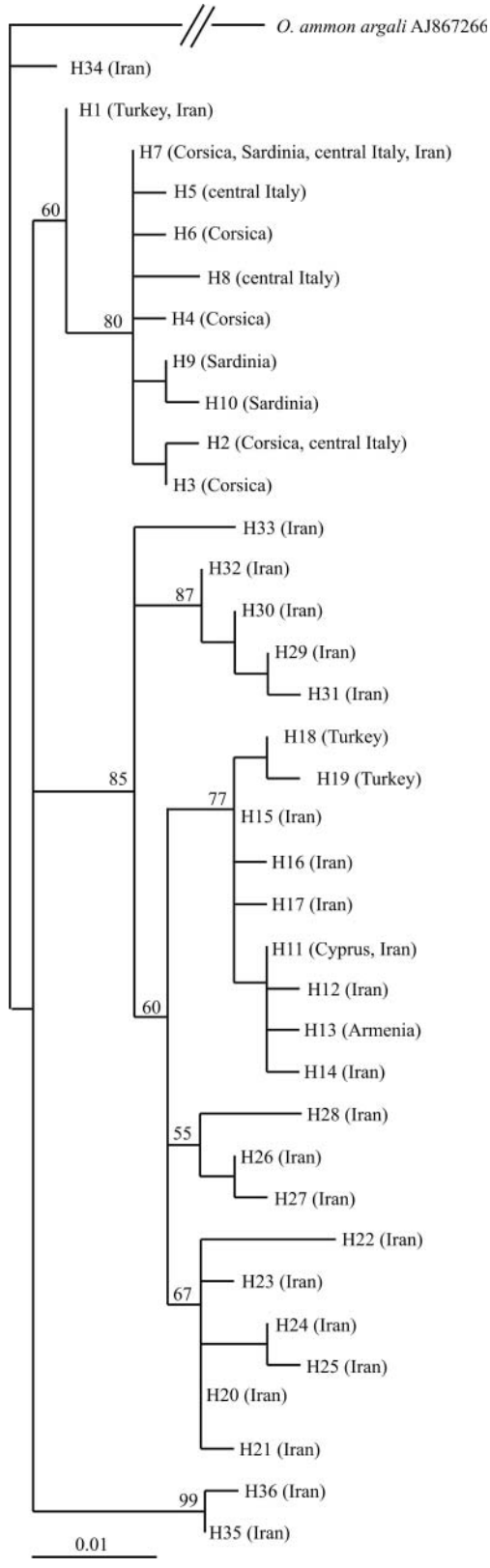

Fig. 4. ML tree computed by PHYML for the aligned haplotypes (H) and using *O. ammon argali* as outgroup. Statistical support (bootstrapping percentage) was reported above each node. Scale bar is proportional to the number of substitutions per site.

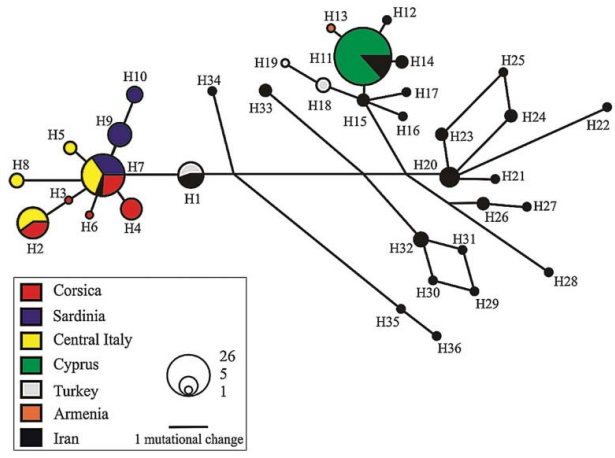

Fig. 5. Haplotype network. A scale to infer the number of haplotypes for each pie is provided together with a length bar to compute the number of mutational changes.

mouflons (with some Turkish/Armenian individuals). In the network (Fig. 5), first cluster included haplotypes (H11−H36) held by mouflons from Cyprus, Turkey, Armenia and Iran. Six Iranian individuals (*O. o. gmelini* and *O. o. laristanica* from North West and South Iran, respectively: Fig. 1 and Table S1, see supplementary material online) shared single Cypriot haplotype (H11), while others from Iran and Armenia (private H13) were only one mutational step away from the latter. In the second cluster (H2−H10), which included all *O. o. musimon* individuals, haplotype H7 was shared by all West Mediterranean populations and one Iranian mouflon (Table S1, see supplementary material online). Haplotype H1 lay between the two clusters and included both Turkish and Iranian mouflons with various taxonomic assignations (Demirci et al., 2013; Rezaei et al., 2010; Table S1, see supplementary material online). We reported in Table 3 the ϕ_{ST} distance values obtained from all population pair comparisons.

Discussion

With the exception of a preliminary investigation carried out in Corsica (Maudet & Dubray, 2002), this study represents the first survey on Mediterranean mouflon populations relying on a panel of microsatellite DNA loci. Principal Component Analysis of STR variability, Bayesian clustering of individual multilocus genotypes, and average F_{ST} pairwise distance values computed among all population pairs concordantly disclosed net genetic separation between the mouflon of Cyprus and those from the western Mediterranean (Figs 2, 3 and Table 3). Among these, Corsican and central Italy populations were much more closely related to each other than to Sardinian ones,

which diverged from both of them. Although confirmed by mtDNA ϕ_{ST} distance values computed among all population pairs (Table 3), such a result was unexpected. The very large majority of mouflons introduced into the Italian Peninsula after the 1970s (e.g., all sampled populations of this study: Table S1, see supplementary material online) were originally from the Wildlife-Hunting Company of Miemo (Tuscany) (Masseti, 2003). Here, a balanced stock of Sardinian and Corsican mouflons was kept in captivity since the 1960s. While the export of mouflons from central Italy to Corsica can be excluded, it sounds possible that present-study small sample sizes available for each area in central Italy (Tuscan-Emilian Apennines, Apuan Alps, Elba Island, and Capraia Island: Table S1, see supplementary material online) have probably allowed for a non-random sorting of Corsican versus Sardinian genotypes. Nevertheless, in the mountain habitat where all these sampled populations were introduced about 40 years ago, selection might have also differently shaped genetic diversity of descendants of Corsican/Sardinian source stocks. Kaeueffer, Coltman, Chapuis, Pontier, and Denis Réale (2007), for instance, attributed to selection an unexpectedly high level of heterozygosis found in a sub-Antarctic island mouflon population established in 1957 by a single pair of captive French individuals.

There is a huge body of evidence that diversity can be rapidly lost in small populations because of genetic drift and related inbreeding (e.g., Reed & Frankham, 2003). In the Mediterranean mouflons, geographic partition of mtDNA diversity was much larger than that disclosed at microsatellite DNA loci. The ratio of mtDNA ϕ_{ST} to microsatellite F_{ST} was, indeed, $0.66/0.34 = 1.95$. Contrasting results between the two genetic systems can be attributed to the fact that the effective population size of mtDNA genome is 1/4 of that of the nuclear DNA (Birky, Fuerst, & Maruyama, 1989). Decline in mtDNA diversity can be much faster in fragmented populations or, similarly, in those derived from a few founders. Hence, comparatively low nuclear and null mitochondrial DNA diversity of the Cypriot mouflon did not come as a surprise (Table 2 and Table S3, respectively, see supplementary material online). This population has been isolated for thousands of years, as there is no evidence for further introductions since the Neolithic. However, neither average pairwise relatedness nor inbreeding coefficient values disclosed in this study arouse concern over the long-term survival of such population. On the contrary, although detected only by some molecular tools (cf. Fig. 3 versus Fig. S1, see supplementary material online), evidence of population genetic structure was found across the Paphos forest. Furthermore, it is known that in the 1930s hunting pressure had reduced the mouflon population of Cyprus to only about 20 individuals (<1% of the present-day population; Forestry Department, 2012; Maisels, 1988). It is worth recalling here that only severe reduction in the population size (at least by 100-fold) can be detected by software such as BOTTLENECK using a number of STR loci comprised between 10 and 20 (Cristescu, Sherwin, Handasyde, Cahill, & Cooper, 2010). In the present study, however, three tests for mutation drift equilibrium relying on 12 STR loci did not disclose any sign of a genetic bottleneck in the Cypriot mouflon (Table S2, see supplementary material online). We also analysed the frequency distribution of STR alleles, which allows detection of a bottleneck efficiently when it has occurred 2-4 $\times$ N_e generations ago (N_e, effective size, i.e., the number of reproductive mouflons). Setting $N_e = 10$ for the mouflon population in the 1930s, time elapsed would have definitely allowed for disclosure of genetic bottlenecks. Once more, high frequency of rarest STR alleles strongly pointed against such an occurrence (Fig. S2, see supplementary material online).

About 11,000 years BP, at the onset of the very first wave of human-mediated dispersal of livestock across the Mediterranean Basin, the island of Cyprus acted as a staging ground for introductions towards western regions, which indeed were reached by such expansion only a few thousands of years later (Guilaine, 2003; Masseti, 1997; Zeder, 2008). According to this, Chessa et al. (2009) found relic genomic traits of ancestral sheep mostly in the Cypriot mouflon. We realize that present-day genetic structure of Mediterranean mouflon populations represents the outcome of many historical events. Nonetheless, we found the lowest value of both nuclear (Table 2) and mitochondrial (Table S3, see supplementary material online) DNA diversity in the mouflon of Cyprus, while the highest ones were disclosed in the populations of Corsica and Sardinia. Such a pattern closely resembles that discovered by Pereira, van Asch, Bradley, and Amorim (2005, 2006) in both Mediterranean sheep and goats. These authors found unexpected high genetic diversity at the westernmost periphery of the Mediterranean Basin, in Portugal, and attributed the latter to multiple introductions of caprinae into the Iberian Peninsula (Zeder, 2008). Furthermore, our mitochondrial DNA results (Figs 4, 5) pointed to the subdivision of *Ovis orientalis* into two groups (cf. Valdez, 1982), the first including *O. o. musimon* from Corsica, Sardinia and central Italy, and the second a few *O. orientalis* morphological subspecies (also *O. o. ophion* from Cyprus, see below) from the Near East. More importantly, both mtDNA tree and network acknowledged the ancestral position of H1 (Turkey, Iran) and H7 (Iran) haplotypes with respect to the western *O. orientalis* group, as well as H34−H36 (Iran) intermediate placement between Near Eastern and western *O. orientalis* group. As to the latter, genetic drift (e.g., see long branches for H35 and H36: Figs 4, 5) likely affecting small populations could also be assumed. Overall, mtDNA suggests that *O. o. musimon* derived from the Near Eastern *O. orientalis* group, present-day mouflons

still retaining a few oriental haplotypes along a westwards decreasing gradient across the Mediterranean. MtDNA also pointed to North West Iran as the most credited geographic region as the source for its ancient introduction to Cyprus. This result was in agreement with genetic data of Bruford and Townsend (2006) and the known archaeozoological pattern for livestock domestication and diffusion across the Mediterranean (Zeder, 2008). In spite of the geographic range reported for *O. gmelinii* (= *O. o. anatolica*, *O. o. gmelini*, *O. o. isphahanica* and *O. o. laristanica*) by Demirci et al. (2013), however, we could not untangle Cypriot mouflon (*O. o. ophion*) identity as well as any of the members of its group (Figs 4, 5).

Cypriot mouflon conservation: systematics, legislation and DNA database

Systematics of the genus *Ovis* is a very complex matter. Wild sheep found on Mediterranean islands are recognized as introduced by humans. Some authors (e.g., Gentry, Clutton-Brock, & Groves, 2004; Gippoliti & Amori, 2004; Wilson & Reeder, 2005) proposed to include them in the domestic species (*O. aries*) and not as subspecies in wild taxa. However, Rezaei et al. (2010) drawn a mtDNA phylogenetic picture where the majority of the morphological species of Nadler et al. (1973) were confirmed and Mediterranean mouflons figured as *O. orientalis* (cf. Shackleton et al., 1997). On the other hand, taxonomic information not merely pertains to systematics and evolution as such but also to conservation management. If, on the one hand, DNA-based studies lead sometimes to taxonomic over-splitting and species inflation (e.g., Groves & Grubb, 2011), on the other hand they have warranted long-awaited breakthroughs in the knowledge and protection of biodiversity (e.g., Zachos et al., 2013; Zachos, Mattioli, Ferretti, & Lorenzini, 2014). In addition, rapid growth of forensic DNA analysis in crimes against protected wildlife made uniform recording of taxonomic information in legislation and DNA databases inevitable (Alacs, Georges, FitzSimmons, & Robertson, 2010; Iyengar, 2014). DNA sequences, indeed can be of high relevance in court cases and the genus *Ovis* is not an exception in this regard (Barbanera et al., 2012; Lorenzini, Cabras, Fanelli, & Carboni, 2011).

The mouflon of Cyprus is included as subspecies of either wild (*O. o. ophion*) or domestic (*O. aries*) within international (Habitats Directive, CITES) as well as national legislation and the IUCN Red List of Threatened Species plus NCBI database (based on Wilson & Reeder, 2005), respectively. Regardless of the IUCN use of 'Cyprus mouflon' as the common name for vulnerable *O. orientalis*, Cyprus is not included in the geographic range of the species nor is *O. o. ophion* reported in Valdez (2008). Unfortunately, quotation of Cypriot mouflon DNA entries under *O. aries* represents an Achilles heel in court cases, as taxonomic inconsistency between NCBI and national legislation may favour people charged with crime against protected wildlife by frustrating molecular DNA forensic outcomes (Barbanera et al., 2012) and undermining conservation efforts to protect the species.

In this study, microsatellite DNA disclosed significant divergence between West Mediterranean *O. o. musimon* and the Cypriot mouflon. The latter was included in a mtDNA group with *O. o. anatolica*, *O. o. gmelini*, *O. o. isphahanica* and *O. o. laristanica* individuals. MtDNA also pointed to the introduction of the mouflon from Iran to Cyprus. However, lack of Iranian samples prevented us from testing at the microsatellite DNA level whether long-time isolation eventually allowed the Cypriot mouflon to diverge from its source population. On one hand, we recommend this investigation be highly prioritized as it can certainly convey further conservation value to the mouflon of Cyprus. On the other hand, until definitive light can be shed on taxonomically heterogeneous Near Eastern *O. orientalis* group, we suggest that the mouflon of Cyprus should be unvaryingly acknowledged as *O. orientalis ophion* not to impair conservation in the country where it resides. In the light of the genetic divergence disclosed between Cypriot and European mouflon, we also recommend to ban importation of any mouflon into Cyprus to preserve the integrity of the island population.

Acknowledgements

The authors are grateful to: the staff of the Cypriot Game Fund Service as well as to I. Ioannou and the staff of the Cypriot Veterinary Service for the sampling in Cyprus; P. Benedetti and F. Sanchis (Office National de la Chasse et de la Faune Sauvage, Cellule Technique, Moltifao) for the sampling in Corsica; G. L. Carboni (Forestry Corps, Lanusei) and P. Cabras (Istituto Zooprofilattico Sperimentale della Sardegna) for providing the mouflon samples from Ogliastra Province; M. Muzzeddu (Breeding and Wildlife Recovery Centre, Bonassai, Sassari) for part of the Sardinian mouflon samples; C. Beccani and F.P. Frontini (Department of Biology, University of Pisa) for their support in the laboratory work.

Disclosure Statement

No potential conflict of interest was reported by the authors.

Funding

This work was given a grant by the Game Fund Service, Ministry of Interior, Nicosia (Cyprus).

Supplemental data

Supplemental data for this article can be accessed here.

References

Akaike, H. (1974). Information theory as an extension of the maximum likelihood principle. In B. N. Petrov & F. Csaki (Eds.), *Second International Symposium on Information Theory* (pp. 276−281). Budapest: Akademiai Kiado.

Alacs, E. A., Georges, A., FitzSimmons, N. N., & Robertson, J. (2010). DNA detective: A review of molecular approaches to wildlife forensic. *Forensic Science, Medicine and Pathology*, *6*, 180−194.

Andreotti, A., Baccetti, N., Perfetti, A., Besa, M., Genovesi, P., & Guberti, V. (2001). Mammiferi ed Uccelli esotici in Italia: Analisi del fenomeno, impatto sulla biodiversità e linee guida gestionali. *Quaderni di Conservazione della Natura*, Ministero dell'Ambiente e Istituto Nazionale di Fauna Selvatica.

Apollonio, M., Luccarini, S., Giustini, D., Scandura, M., & Ghiandai, F. (2005). *Carta delle vocazioni faunistiche della Sardegna*. *Studio relativo agli Ungulati*. Regione Autonoma della Sardegna.

Bandelt, H.-J., Forster, P., & Röhl, A. (1999). Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution*, *16*, 37−48.

Barbanera, F., Guerrini, M., Beccani, C., Forcina, G., Anayiotos, P., & Panayides, P. (2012). Conservation of endemic and threatened wildlife: Molecular forensic DNA against poaching of the Cypriot mouflon (*Ovis orientalis ophion*, Bovidae). *Forensic Science International: Genetics*, *6*, 671−675.

Bhebhe, E., Kogi, J., Holder, D. A., Arevalo, E., Derr, J. N., Linn, R. A., & Taylor, J. F. (1994). Caprine microsatellite dinucleotide repeat polymorphisms at the SR-CRSP-6, SR-CRSP-7, SR-CRSP-8, SR-CRSP-9 and SR-CRSP-10 loci. *Animal Genetics*, *25*, 203.

Birky, C. W., Fuerst, P., & Maruyama, T. (1989). Organelle diversity under migration, mutation and drift: Equilibrium expectations, approach to equilibrium, effects of heteroplasmic cells and comparisons to nuclear genes. *Genetics*, *121*, 613−627.

Bishop, M. D., Kappes, S. M., Keele, J. W., Stone, R. T., Sunden, S. L. F.,... & Beattie, C. W. (1994). A genetic linkage map for cattle. *Genetics*, *136*, 619−639.

Boitani, L., Lovari, S., & Vigna Taglianti, A. (2003).(Eds.). *Fauna d'Italia Mammalia III Carnivora-Artiodactyla*. Bologna: Edizioni Calderini.

Brezinsky, L., Kemp, S. J., & Teale, A. J. (1993). Five polymorphic bovine microsatellites. *Animal Genetics*, *24*, 75−76.

Bruford, M. W., & Townsend, S. J. (2006). Mitochondrial DNA diversity in modern sheep: Implication for domestication. In M. A. Zeder et al. (Eds.), *Documenting domestication: New genetic and archaeological paradigms* (pp. 307−317). Oakland: University of California Press.

Buchanan, F. C., & Crawford, A. M. (1992). Ovine dinucleotide repeat polymorphism at the MAF70 locus. *Animal Genetics*, *23*, 185.

Buchanan, F. C., & Crawford, A. M. (1993). Ovine microsatellites at the OarFCB11, OarFCB128, OarFCB193, OarFCB266 and OarFCB304 loci. *Animal Genetics*, *24*, 145.

Buchanan, F. C., Galloway, S. M., & Crawford, A. M. (1994). Ovine microsatellites at the OarFCB5, OarFCB19, OarFCB20, OarFCB48, OarFCB129 and OarFCB226 loci. *Animal Genetics*, *25*, 60.

Bunch, T. D., Wu, C., & Zhang, Y. P. (2006). Phylogenetics analysis of snow sheep (*Ovis nivicola*) and closely related taxa. *Journal of Heredity*, *97*, 21−30.

Chessa, B., Pereira, F., Arnaud, F., Amorim, A., Goyache, F., Mainland, I., & Palmarini, M. (2009). Revealing the history of sheep domestication using retrovirus integrations. *Science*, *324*, 532−536.

Cornuet, J. M., Piry, S., Luikart, G., Estoup, A., & Solignac, M. (1999). New methods employing multilocus genotypes to select or exclude populations as origins of individuals. *Genetics*, *153*, 1989−2000.

Crawford, A., Dodds, K., Ede, A., Pierson, C. A., Montgomery, G. W., & Garmonsway, H. G. (1995). An autosomal genetic linkage map of the sheep genome. *Genetics*, *140*, 703−724.

Cristescu, R., Sherwin, W. B., Handasyde, K., Cahill, V., & Cooper, D. W. (2010). Detecting bottlenecks using BOTTLENECK 1.2.02 in wild populations: The importance of the microsatellite structure. *Conservation Genetics*, *11*, 1043−1049.

Cugnasse, J. M. (1994). Révision taxonomique des mouflons des îles méditerranéennes. *Mammalia*, *3*, 507−512.

Demirci, S., Koban, B., Evren, D., Nihan, D., Pişkin, E., Engin, A., & Togan, I. (2013). Mitochondrial DNA Diversity of Modern, Ancient and Wild Sheep (*Ovis gmelinii anatolica*) from Turkey: New Insights on the Evolutionary History of Sheep. *Public Library of Science ONE, 8*, e81952.

Di Rienzo, A., Peterson, A. C., Garza, J. C., Valdes, A. M., Slatkin, M., & Freimer, N. B. (1994). Mutational processes of simple sequence repeat loci in human populations. *Proceedings of the National Academy of Sciences of the United States of America*, *91*, 3166−3170.

Evanno, G., Regnaut, S., & Goudet, J. (2005). Detecting the number of clusters of individuals using the software STRUCTURE: A simulation study. *Molecular Ecology*, *14*, 2611−2620.

Excoffier, L., & Lischer, H. E. L. (2010). Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources*, *10*, 564−567.

Felsenstein, J. (1985). Confidence limits on phylogenies: An approach using the bootstrap. *Evolution*, *39*, 783−791.

Forestry Department. (2012). *The Cyprus mouflon*. Republic of Cyprus Press and Information Office, Ref no. 128/2012. Nicosia, Cyprus: Othon Press Ltd.

Frantz, A. C., Pope, L. C., Carpenter, P. J., Roper, T. J., Wilson, G. J., Delahay, R. J., & Burke, T. (2003). Reliable microsatellite genotyping of the Eurasian badger (*Meles meles*) using faecal DNA. *Molecular Ecology*, *12*, 1649−1661.

Gentry, A., Clutton-Brock, J., & Groves, C. P. (2004). The naming of wild animal species and their domestic derivatives. *Journal of Archaeological Science*, *31*, 645−651.

Gippoliti, S., & Amori, G. (2004). Mediterranean island mammals: Are they a priority for biodiversity conservation? *Biogeographia*, *25*, 135−144.

Goudet, J. (2001). FSTAT, a program to estimate and test gene diversities and fixation indices (Version 2.9.3). Retrieved from http://www.unil.ch/izea/softwares/fstat.html (accessed 24 April 2015).

Guerrini, M., & Barbanera, F. (2009). Non-invasive genotyping of the red-legged partridge (*Alectoris rufa*, Phasianidae): Semi-nested PCR of mitochondrial DNA from feces. *Biochemical Genetics*, *47*, 873−883.

Guilaine, J. (2003). Parekklisha-Shillourokambos. Périodisation et aménagements domestiques. In J. Guilaine & A. Le Brun (Eds), *Le Néolithique de Chypre: Actes du colloque*

international organisé par le Département des Antiquités de Chypre et l'Ecole Française d'Athènes, Nicosie 17-19 Mai 2001 (pp. 3−14). Bulletin de Correspondance Hellénique, supplément 43. Athens: Ecole Française d'Athènes.

Guindon, S., Dufayard, J. F., Lefort, V., Anisimova, M., Hordijk, W., & Gascuel, O. (2010). New algorithms and methods to estimate maximum-likelihood phylogenies: Assessing the performance of PhyML 3.0. *Systematic Biology*, *59*, 307−321.

Groves, C., & Grubb, P. (2011). *Ungulate taxonomy*. Baltimore, Maryland: The Johns Hopkins University Press.

Hadjisterkotis, E. (1993). *The Cyprus mouflon, Ovis gmelini ophion*, *management, conservation and evolution* (PhD Thesis). McGill University, Montreal, Canada.

Hadjisterkotis, E. (1999). Dangers facing the Cyprus mouflon from being one population in one reserve. *European Journal of Wildlife Research*, *45*, 27−34.

Hasegawa, M., Kishino, H., & Yano, T. (1985). Dating the human-ape splitting by a molecular clock of mitochondrial DNA. *Journal of Molecular Evolution*, *22*, 160−174.

Hiendleder, S., Kaupe, B., Wassmuth, R., & Janke, A. (2002). Molecular analysis of wild and domestic sheep questions current nomenclature and provides evidence for domestication from two different subspecies. *Proceedings of the Royal Society B: Biological Sciences*, *269*, 893−904.

Hochberg, Y. (1988). A sharper Bonferroni procedure for multiple tests of significance. *Biometrika 75*, 800−802.

Hulme, D. J., Silk, J. P., Redwin, J., Barense, W. R., & Beh, K. J. (1994). Ten polymorphic ovine microsatellites. *Animal Genetics*, *25*, 434−435.

Ioannou, I., Sandalakis, V., Kassinis, N., Chochlakis, D., Papadopoulos, B., Loukaides, F., & Psaroulaki, A. (2011). Tick-borne bacteria in mouflons and their ectoparasites in Cyprus. *Journal of Wildlife Diseases*, *47*, 300−306.

Iyengar, A. (2014). Forensic DNA analysis for animal protection and biodiversity conservation: A review. *Journal for Nature Conservation*, *22*, 195−205.

Kaeueffer, R., Coltman, D. W., Chapuis, J. L., Pontier, D., & Denis Réale, D. (2007). Unexpected heterozygosity in an island mouflon population founded by a single pair of individuals. *Proceedings of the Royal Society B: Biological Sciences*, *274*, 527−533.

Kalinowski, S. T., Wagner, A. P., & Taper, M. L. (2006). ML-Relate: A computer program for maximum likelihood estimation of relatedness and relationship. *Molecular Ecology Notes*, *6*, 576−579.

Kemp, S. J., Hishida, O., Wambugu, J., Rink, A., Longeri, M. L., Ma, R. Z., & Teale, A. J. (1995). A panel of polymorphic bovine ovine and caprine microsatellite markers. *Animal Genetics*, *26*, 299−306.

Lawson Handley, L. J., Byrne, K., Santucci, F., Townsend, S., Taylor, M., Bruford, M. W., & Hewitt, G. M. (2007). Genetic structure of European sheep breeds. *Heredity*, *99*, 620−631.

Librado, P., & Rozas, J. (2009). DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. *Bioinformatics*, *25*, 1451−1452.

Lorenzini, R., Cabras, P., Fanelli, R., & Carboni, G. L. (2011). Wildlife molecular forensics: Identification of the Sardinian mouflon using STR profiling and the Bayesian assignment test. *Forensic Science International, 5*, 345−349.

Maisels, F. G. (1988). *The feeding ecology of the Cyprus mouflon Ovis orientalis Gmelin 1774, in the Paphos Forest, Cyprus* (PhD Thesis). University of Edinburgh, UK.

Masseti, M. (1997). The prehistorical diffusion of the Asiatic mouflon, *Ovis gmelini* Blyth, 1841, and of the Bezoar goat, *Capra aegagrus* Erxleben, 1777, in the Mediterranean region beyond their natural distributions. In E. Hadjisterkotis (Ed.), *Proceedings of the 2nd International Symposium on Mediterranean Mouflon*, (pp 12−27), *The Mediterranean Mouflon: Management, Genetics and Conservation*., Cyprus: Ministry of Interior, Nicosia.

Masseti, M. (2003). *Fauna Toscana. Galliformi non migratori, Lagomorfi e Artiodattili*. Firenze: Edizioni Arsia.

Maudet, C., & Dubray, D. (2002). *Comparison of the genetic structures of two corsican mouflon (Ovis gmelini musimon) populations (Cinto/Asco and Bavella)*. Poster session presented at the IV International Mouflon Symposium, Italy: Nuoro.

Maudet, C., Luikart, G., Dubray, D., Von Hardenberg, A., & Taberlet, P. (2004). Low genotyping error rates in wild ungulate faeces sampled in winter. *Molecular Ecology Notes*, *4*, 772−775.

Nadler, C. F., Korobitsyna, K. V., Hoffmann, R. S., & Vorontsov, N. N. (1973). Cytogenetic differentiation, geographic distribution and domestication in Paleartic sheep (*Ovis*). *Zeitschrift für Säugetierkunde*, *38*, 109−125.

Paetkau, D., Waits, L., Clarkson, P. L., Craighead, L., Vyse, E., Ward, R., & Strobeck, C. (1998). Variation in genetic diversity across the range of North American brown bears. *Conservation Biology*, *12*, 418−429.

Pedrosa, S., Uzun, M., Arranz, J. J., Gutierrezz-Gil, B., San Primitivo, F., & Bayon, Y. (2005). Evidence of three maternal lineages in near eastern sheep supporting multiple domestication events. *Proceedings of the Royal Society B: Biological Sciences, 272*, 2211−2217.

Penty, J. M., Henry, H. M., Ede, A. J., & Crawford, A. M. (1993). Ovine microsatellites at the OarAEl6, OarAE54, OarAE57, OarAE119 and OarAE129 loci. *Animal Genetics*, *24*, 219−219.

Pereira, F., Pereira, L., van Asch, B., Bradley, D. G., & Amorim, A. (2005). The mtDNA catalogue of all Portuguese autochthonous goat (*Capra hircus*) breeds: High diversity of female lineages at the western fringe of European distribution. *Molecular Ecology*, *14*, 2313−2318.

Pereira, F., Davis, S. J. M., Pereira, L., McEvoy, B., Bradley, D. G., & Amorim, A. (2006). Genetic signatures of a Mediterranean influence in Iberian Peninsula sheep husbandry. *Molecular Biology and Evolution*, *23*, 1420−1426.

Peters, J., von den Driesch, A., & Helmer, D. (2005). The upper Euphrates-Tigris Basin, cradle of agro-pastoralism? In J. D. Vigne, J. Peters, & D. Helmer (Eds.), *The first steps of animal domestication* (pp. 96−124). Oxford: Oxbow Books.

Piegert, H., & Uloth, W. (2005). *Der Europäische Mufflon*. DSV-Verlag GmbH, Edition Natur Life, Hamburg, Germany.

Piry, S., Luikart, G., & Cornuet, J. M. (1999). Bottleneck: A computer program for detecting recent reductions in the effective population size using allele frequency data. *Journal of Heredity*, *90*, 502−503.

Posada, D., & Crandall, K. A. (1998). Modeltest: Testing the model of DNA substitution. *Bioinformatics*, *14*, 817−818.

Pritchard, J. K., Stephens, M., & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics*, *155*, 945−959.

Raymond, M., & Rousset, F. (1995). GENEPOP (version 1.2): Population genetics software for exact tests and ecumenicism. *Journal of Heredity*, *86*, 248−249.

Reed, D. H., & Frankham, R. (2003). Correlation between fitness and Genetic Diversity. *Conservation Biology*, *17*, 230−237

Rezaei, H. R., Naderi, S., Chintaunan-Marquier, I. C., Taberlet, P., Virk, A. T., Naghash, H. R., & Pompanon, F. (2010). Evolution and taxonomy of the wild species of the genus *Ovis* (Mammalia, Artiodactyla, Bovidae). *Molecular Phylogenetics and Evolution*, *54*, 315−326.

Sfouggaris, A. (2011). Management Plan for the Cypriot Mouflon (*Ovis orientalis ophion*). *Preparation of an integrated Management Plan for the Pafos Forest* (pp. 1−77). Nicosia, Cyprus: Department of Forests.

Shackleton, D. M. (Ed.), & IUCN/SSC Caprinae Specialist Group (1997). *Wild sheep and goats and their relatives. Status survey and conservation action plan for Caprinae*. Gland, Switzerland: IUCN.

Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F., & Higgins, D. G. (1997). The ClustalX windows interface: Flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research*, *24*, 4876−4882.

Türcke, F., & Schmincke, S. (1965). Das Muffelwild. Naturgeschichte, Ökologie, Hege und Jagd. Germany: Paul Parey Verlag.

Uloth, W. (1972). To the history of the distribution, introduction and cross-breeding of the Tyrrhenis mouflon in Europe and oversea. *Acta Theriologica*, *17*, 412−413.

Väha, J. P., & Primmer, C. R. (2006). Efficiency of model-based Bayesian methods for detecting hybrid individuals under different hybridization scenarios and with different numbers of loci. *Molecular Ecology*, *15*, 63−72.

Valdez, R. (1982). W*ild sheep and wild sheep hunters of the New World*. Mesilla, New Mexico: Wild Sheep and Goat International.

Valdez, R. (2008). *Ovis orientalis*. The IUCN Red List of Threatened Species. Version 2014.2. Retrieved from www.iucnredlist.org (accessed on 14 November 2014).

Valière, N. (2002). Gimlet: A computer program for analyzing genetic individual identification data. *Molecular Ecology Notes*, *2*, 377−379.

Vigne, J. D., Buitenhuis, H., & Davis, S. (1999). Premiers (Les) pas de la domestication animale à l'ouest de l'Euphrate: Chypre et l'Anatolie Centrale. *Paléorient*, *25*, 49−62.

Vigne, J. D., Carrère, I., & Guilaine, J. (2003). Unstable status of early domestic ungulates in the Near East: The example of Shillourokambos (Cyprus, IX-VIIIth millennia Cal. B.C.). *Bulletin de correspondance hellénique*, *43*, 239−51.

Waits, L. P., Luikart, G., & Taberlet, P. (2001). Estimating the probability of identity among genotypes in natural populations: Cautions and guidelines. *Molecular Ecology*, *10*, 249−256.

Weir, B. S., & Cockerham, C. C. (1984). Estimating, *F*-statistics for the analysis of population structure. *Evolution*, *38*, 1358−1370.

Wilson, D. E., & Reeder, D. M. (2005).(Eds). *Mammal Species of the World. A Taxonomic and Geographic Reference* (3rd ed.). Baltimore, Maryland: Johns Hopkins University Press.

Wright, S. (1965). The interpretation of population structure by *F*-statistics with special regard to systems of mating. *Evolution*, *19*, 395−420.

Zachos, F. E., Apollonio, M., Bärmann, E. V., Festa-Bianchet, M., Gölich, U., Habel, J. C., & Suchentrunk, F. (2013). Species inflation and taxonomic artefacts: A critical comment on recent trends in mammalian classification, *Mammalian Biology*, *78*, 1−6.

Zachos, F. E., Mattioli, S., Ferretti, F., & Lorenzini, R. (2014). The unique Mesola red deer of Italy: Taxonomic recognition (*Cervus elaphus italicus* nova ssp., Cervidae) would endorse conservation, *Italian Journal of Zoology*, *81*, 136−143.

Zeder, M. A. (2008). Domestication and early agriculture in the Mediterranean Basin: Origins, diffusion, and impact. *Proceedings of the National Academy of Sciences of the United States of America*, *105*, 11597−11604.

Associate Editor: Christoph Bleidorn

Taxon	Country	Region	Locality (number, only for Cyprus)	Coordinates	Tissue	Size (n)	Haplotype	Literature record	GenBank accession code
O. o. ophion	Cyprus	Paphos forest	Orkontas (1)	35.05N, 32.83E	blood	5	H11	This study	LN651259
	Cyprus	Paphos forest	Stavros tis Psokas (2)	35.03N, 32.63E	blood	4	H11	This study	LN651259
	Cyprus	Paphos forest	Panayia (3)	34.93N, 32.63E	blood	2	H11	This study	LN651259
	Cyprus	Paphos forest	Potamos tou Kampou (4)	35.12N, 32.76E	blood	2	H11	This study	LN651259
	Cyprus	Paphos forest	Lefka (5)	35.08N, 32.85E	blood	2	H11	This study	LN651259
	Cyprus	Paphos forest	Vretsia (6)	34.88N, 32.67E	blood	3	H11	This study	LN651259
	Cyprus	Paphos forest	Gerakies (7)	35.00N, 32.80E	blood	2	H11	This study	LN651259
	Cyprus	Paphos forest	Kampos (8)	35.05N, 32.73E	blood	9	H11	This study	LN651259
	Cyprus	Paphos forest	Pedoulas (9)	34.97N, 32.83E	blood	2	H11	This study	LN651259
	Cyprus	Paphos forest	Ambelykou (10)	35.11N, 32.79E	blood	3	H11	This study	LN651259
	Cyprus	Paphos forest	Ayios Nikolas (11)	34.88N, 32.75E	blood	1	H11	This study	LN651259
	Cyprus	Paphos forest	Ayios Merkourios (12)	35.03N, 32.55E	blood	1	H11	This study	LN651259
	Cyprus	Paphos forest	Gyalia (13)	35.10N, 32.52E	blood	1	H11	This study	LN651259
	Cyprus	Paphos forest	Lemythou (14)	34.95N, 32.81E	blood	1	H11	This study	LN651259
	Cyprus	Paphos forest	Anadiou (15)	34.95N, 32.57E	blood	1	H11	This study	LN651259
	Cyprus	Paphos forest	Livadei (16)	35.11N, 32.60E	blood	1	H11	This study	LN651259
	Cyprus	Paphos forest	Limnitis (17)	35.12N, 32.72E	blood	2	H11	This study	LN651259
	Cyprus	Paphos forest	Trypillos (18)	35.02N, 32.61E	blood	1	H11	This study	LN651259
	Cyprus	Paphos forest	Ayios Ioannis (19)	34.88N, 32.70E	blood	1	H11	This study	LN651259
	Cyprus	Paphos forest	Nea Dymmata (20)	35.14N, 32.54E	blood	1	H11	This study	LN651259
	Cyprus	Paphos forest	Pera Vasa (21)	34.90N, 32.73E	blood	1	H11	This study	LN651259
	Cyprus	Paphos forest	Kykkos (22)	34.98N, 32.73E	blood	1	H11	This study	LN651259
	Cyprus	Paphos forest	Cedar Valley (23)	35.00N, 32.70E	blood	1	H11	This study	LN651259
	Cyprus	Paphos forest	Tzielefos Bridge (24)	34.89N, 32.75E	blood	2	H11	This study	LN651259
	Cyprus	Paphos forest	Arminou (25)	34.87N, 32.72E	blood	1	H11	This study	LN651259
	Cyprus	Paphos forest	Foini (26)	34.90N, 32.84E	blood	1	H11	This study	LN651259
	Cyprus	Paphos forest	Kalopanayiotis (27)	35.00N, 32.82E	blood	1	H11	This study	LN651259
	Cyprus	Paphos forest	Platania *	34.95N, 32.93E	blood	3	H11	This study	LN651259
	Cyprus	Paphos forest	Public Municipal Park, Nicosia *	35.18N, 33.36E	blood	1	H11	This study	LN651259
	Cyprus	Paphos forest	Empa village *	34.81N, 32.43E	blood	1	H11	This study	LN651259
	Cyprus	Paphos forest	Bird Park *	34.90N, 32.35E	blood	3	H11	This study	LN651259
	Cyprus	Paphos forest	Unknown 1	-	blood	1	H11	This study	LN651259
	Cyprus	Paphos forest	Unknown 2	-	blood	1	H11	This study	LN651259
O. o. musimon	Italy	Sardinia	Breeding and Wildlife Recovery Centre, Bonassai, Sassari *	-	hairs	4	H7	This study	LN651265
	Italy	Sardinia	Ogliastra Province	40.03N, 09.54E	blood	16	H7, H9, H10	This study	LN651265, -67, -68
	Italy	Central Italy	Tuscan Archipelago National Park (Elba island)	42.78N, 10.19E	faeces	10	H2, H7	This study	LN651260, -65
	Italy	Central Italy	Tuscan Archipelago National Park (Capraia island)	43.04N, 09.82E	faeces	3	H8	This study	LN651266
	Italy	Central Italy	Tuscan-Emilian Apennines National Park	44.21N, 10.36E	faeces	6	H7	This study	LN651265
	Italy	Central Italy	Apuan Alps Regional Park	44.07N, 10.27E	faeces	4	H2, H5, H7	This study	LN651260, -63, -65
	France	Corsica	Asco (Cinto population)	42.43N, 08.97E	faeces	3	H3, H7	This study	LN651261, -65
	France	Corsica	Tartagine (Cinto population)	42.51N, 08.97E	faeces	3	H6, H7	This study	LN651264, -65
	France	Corsica	Omita (Cinto population)	42.34N, 08.83E	faeces	3	H2	This study	LN651260
	France	Corsica	Niolu (Cinto population)	42.35N, 08.93E	faeces	2	H2	This study	LN651260
	France	Corsica	Tova (Bavella population)	41.86N, 09.25E	faeces	3	H4, H7	This study	LN651262, -65
	France	Corsica	Bavella (Bavella population)	41.81N, 09.26E	faeces	3	H4, H7	This study	LN651262, -65
	France	Corsica	Castellucciu (Bavella population)	41.73N, 09.22E	faeces	2	H4, H7	This study	LN651262, -65
	France	Corsica	Unknown population	41.81N, 09.22E	-	2	H2, H4	Rezaei et al. (2010)	EU365977, EU365990
O. g. anatolica	Turkey	Central Anatolia	Konya	37.87N, 32.48E	-	2	H1	Demirci et al. (2013)	KF677304, KF677306
	Turkey	Central Anatolia	Konya	37.87N, 32.48E	-	2	H18	Demirci et al. (2013)	KF677305, KF677307

O. o. gmelini	Turkey	-		38.69N, 44.14E	-	2	H1	Rezaei et al. (2010)	FJ936198, FJ936199
O. o. anatolica	Turkey	-		38.04N, 32.27E	-	3	H1, H18, H19	Rezaei et al. (2010)	EU365973, FJ936185, EU365987
O. o. gmelini	Iran	-		36.66N, 47.67E	-	2	H1, H11	Rezaei et al. (2010)	FJ936194, FJ936193
	Iran	-		38.85N, 45.24E	-	2	H7, H21	Rezaei et al. (2010)	EU365975, EU365989
	Iran	-		38.85N, 45.24E	-	2	H20	Rezaei et al. (2010)	FJ936191, FJ936192,
	Iran	-		38.85N, 45.24E	-	2	H23	Rezaei et al. (2010)	EU365998, EU366000
	Iran	-		36.07N, 47.51E	-	3	H1, H11, H24	Rezaei et al. (2010)	FJ936188, FJ936186, EU365997
	Iran	-		36.07N, 47.51E	-	2	H14	Rezaei et al. (2010)	EU365996, FJ936203
	Iran	-		34.20N, 48.95E	-	1	H11	Rezaei et al. (2010)	FJ936189
	Iran	-		36.12N, 49.56E	-	3	H11, H29, H31	Rezaei et al. (2010)	FJ936197, EU366053, EU366003
	Iran	-		37.75N, 46.43E	-	3	H11, H12, H20	Rezaei et al. (2010)	EU365979, EU365980, FJ936202
	Iran	-		38.83N, 46.50E	-	1	H20	Rezaei et al. (2010)	FJ936190
	Iran	-		36.66N, 47.67E	-	2	H24, H25	Rezaei et al. (2010)	FJ936195, EU366002
	Iran	-		33.35N, 46.08E	-	2	H35, H36	Rezaei et al. (2010)	FJ936200, FJ936201
O. o. isphahanica	Iran	-		33.45N, 49.36E	-	2	H1	Rezaei et al. (2010)	EU365976, FJ936204
	Iran	-		33.45N, 49.36E	-	1	H34	Rezaei et al. (2010)	EU366016
	Iran	-		32.85N, 51.21E	-	2	H33	Rezaei et al. (2010)	FJ936205, FJ936206
O. o. laristanica	Iran	-		27.68N, 54.33E	-	2	H11, H20	Rezaei et al. (2010)	FJ936209, FJ936210
O. orientalis	Iran	-	Sh population	32.10N, 50.08E	-	1	H28	Rezaei et al. (2010)	FJ936211
	Iran	-	Ko population	34.80N, 46.47E	-	2	H17, H22	Rezaei et al. (2010)	FJ936207, FJ936208
O. orientalis x *O. vignei*	Iran	-		29.71N, 52.70E	-	2	H15	Rezaei et al. (2010)	FJ936234, FJ936235
	Iran	-		29.71N, 52.70E	-	1	H16	Rezaei et al. (2010)	EU365982
	Iran	-		28.86N, 56.45E	-	1	H26	Rezaei et al. (2010)	FJ936226
	Iran	-		28.86N, 56.45E	-	1	H27	Rezaei et al. (2010)	EU366035
	Iran	-		34.71N, 52.19E	-	1	H26	Rezaei et al. (2010)	EU366027
	Iran	-		35.63N, 51.72E	-	2	H30, H32	Rezaei et al. (2010)	EU365991, EU366009
	Iran	-		36.70N, 55.43E	-	1	H32	Rezaei et al. (2010)	FJ936228
	Iran	-		35.97N, 53.51E	-	1	H32	Rezaei et al. (2010)	FJ936223
O. o. gmelini	Armenia	-		39.01N, 46.29E	-	1	H13	Rezaei et al. (2010)	EU366040

Table S1. The sample size of this study (n = 103) is given with the mtDNA sequences downloaded from the GenBank (n = 57); * = captive mouflon.

Bottleneck test	TPM
Sign test (number of loci with heterozygosity excess)	Expected = 5.7 Observed= 6.0 (P = 0.23)
Standardized differences test	T_2 = -1.5 (P = 0.07)
Wilcoxon sign rank test (probability of heterozygosity excess)	P = 0.93
Distribution of alleles frequency	Normal L-shaped (see Fig. S2)

Table S2. Tests for mutation drift equilibrium in the Cypriot population using BOTTLENECK. TPM: Two Phase Mutation model.

Population	Sample size (n)	Haplotypes (n)	Haplotype diversity (h ± s.d.)	Number of pairwise differences (k ± s.d.)	Nucleotide diversity (π ± s.d., %)
Corsica*	21	5	0.76 ± 0.04	1.43 ± 0.91	0.14 ± 0.09
Sardinia	20	3	0.65 ± 0.06	0.79 ± 0.60	0.08 ± 0.06
Central Italy	23	4	0.70 ± 0.06	1.65 ± 1.01	0.16 ± 0.11
Cyprus	41	1	-	-	-
Turkey**	9	3	0.64 ± 0.12	4.79 ± 2.58	0.46 ± 0.28
Iran**	45	25	0.96 ± 0.01	6.13 ± 2.97	0.59 ± 0.31

Table S3. Estimates of mtDNA genetic diversity (average ± standard deviation). *, includes two GenBank entries (see Table S1); **, includes only GenBank entries (see Table S1). Armenia was not listed as it includes only one GenBank entry.

	Corsica						Sardinia						Central Italy						Cyprus					
Locus	n	n_a	A_r	A_u	H_O/H_E	P_{HWE}	n	n_a	A_r	A_u	H_O/H_E	P_{HWE}	n	n_a	A_r	A_u	H_O/H_E	P_{HWE}	n	n_a	A_r	A_u	H_O/H_E	P_{HWE}
BM415	18	7	6.9	0	0.50/0.84	<0.001	17	6	5.9	0	0.35/0.56	0.001	21	6	5.7	2	0.52/0.72	*ns*	63	8	6.5	2	0.44/0.69	<0.001
ILSTS011	16	5	5.0	2	0.25/0.62	*ns*	20	6	5.5	2	0.45/0.64	*ns*	19	4	3.8	1	0.21/0.50	*ns*	60	4	3.3	3	0.43/0.54	*ns*
ILSTS028	19	11	10.5	7	0.74/0.89	*ns*	18	5	4.9	1	0.39/0.72	*ns*	20	9	8.7	1	0.55/0.87	<0.001	62	7	5.0	3	0.68/0.61	*ns*
MAF70	19	8	7.8	2	0.53/0.80	*ns*	20	6	5.8	2	0.60/0.74	0.001	21	5	4.9	0	0.67/0.70	*ns*	63	3	2.3	0	0.08/0.11	*ns*
MCM527	19	8	7.4	0	0.63/0.78	*ns*	20	5	4.9	0	0.60/0.63	*ns*	23	8	7.3	0	0.35/0.72	<0.001	63	6	3.9	4	0.35/0.42	0.001
OarAE129	17	4	4.0	0	0.53/0.58	*ns*	20	3	2.8	0	0.30/0.34	*ns*	22	4	3.9	0	0.36/0.68	0.001	63	5	3.5	5	0.22/0.44	<0.001
OarFCB304	16	8	8.0	1	0.63/0.74	*ns*	20	8	7.2	2	0.85/0.79	*ns*	22	7	6.4	1	0.45/0.76	<0.001	61	5	3.7	4	0.51/0.45	*ns*
OarFCB48	17	6	5.9	0	0.59/0.76	*ns*	20	4	3.8	0	0.70/0.59	*ns*	22	8	7.6	2	0.68/0.82	*ns*	63	10	7.4	6	0.60/0.78	<0.001
OarJMP58	19	11	10.2	3	0.58/0.81	*ns*	20	7	6.9	0	0.75/0.81	*ns*	22	9	8.4	2	0.59/0.87	0.001	63	3	2.4	0	0.35/0.39	*ns*
SRCRSP7	16	12	12.0	2	0.50/0.90	<0.001	17	6	5.9	0	0.29/0.73	*ns*	18	11	10.4	2	0.39/0.87	<0.001	63	1	1.0	1	Monomorphic locus	
SRCRSP8	19	8	7.6	2	0.37/0.77	<0.001	19	5	4.8	1	0.58/0.73	*ns*	23	9	8.2	2	0.39/0.82	<0.001	61	4	2.7	2	0.25/0.44	<0.001
SRCRSP9	19	7	6.7	2	0.63/0.80	*ns*	19	4	3.9	0	0.74/0.67	*ns*	22	7	6.0	1	0.64/0.62	*ns*	63	1	1.0	0	Monomorphic locus	

Table S4

STR genetic variability (each locus) as computed for each Mediterranean mouflon population. Legend: n, sample size; n_a, number of alleles; A_r, allelic richness; A_u, number of unique alleles; H_O, observed heterozygosity; H_E, expected heterozygosity; P_{HWE}, probability value for the Hardy-Weinberg Equilibrium test; *ns*, not significant departure from HWE after Bonferroni correction ($\alpha = 0.05$, $\alpha' = 0.05/48 = 0.001$).

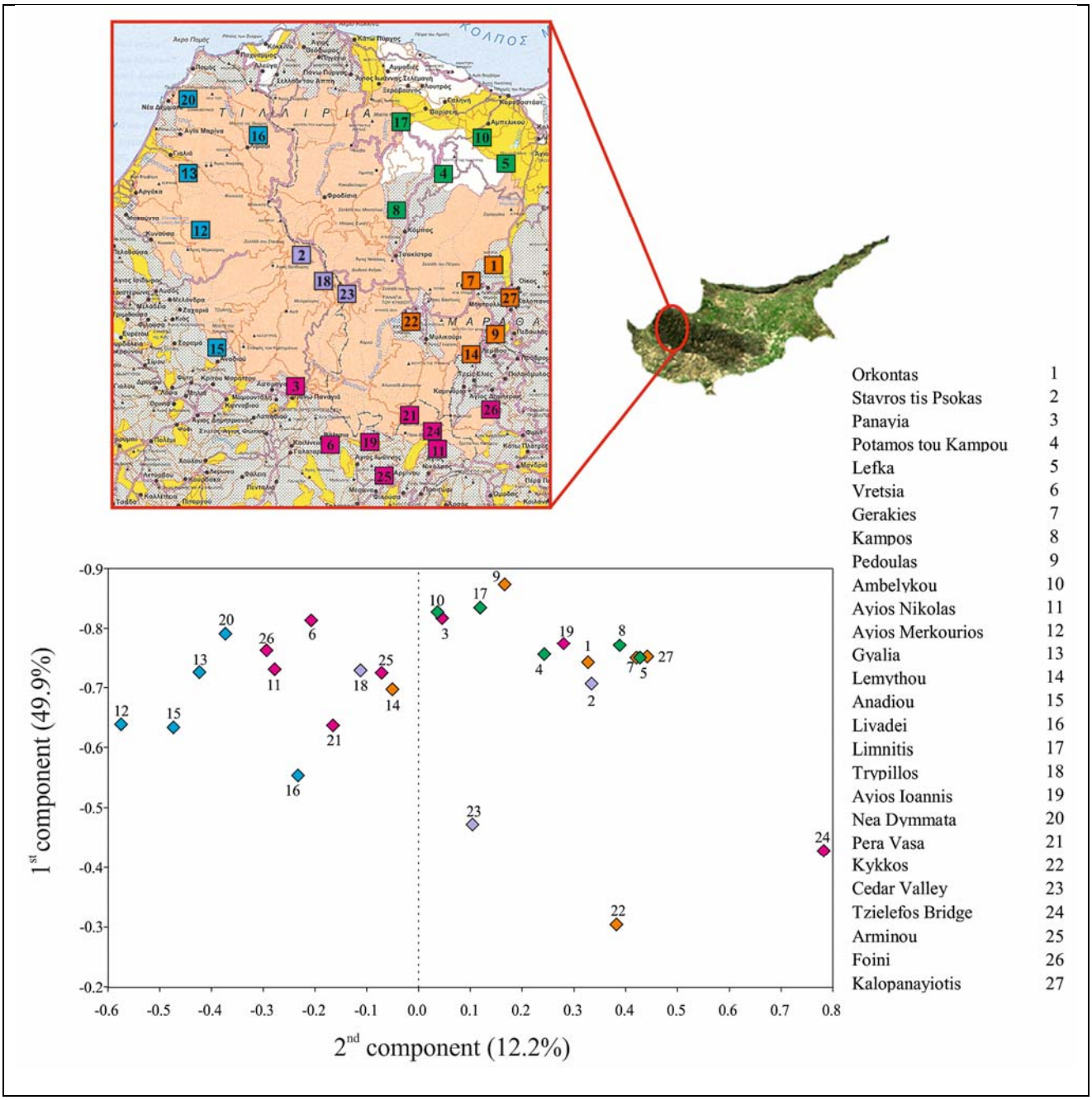

Figure S1. Upper part: map showing the 27 mouflon sampling localities (see list to the right side) in the Paphos Forest of Cyprus. Lower part: Principal Component Analysis performed using average pairwise F_{ST} distances among STR genotyped mouflons from each sampling locality. The percentage of total variance explained by each of the first two components is given. See also Table S1 for further details.

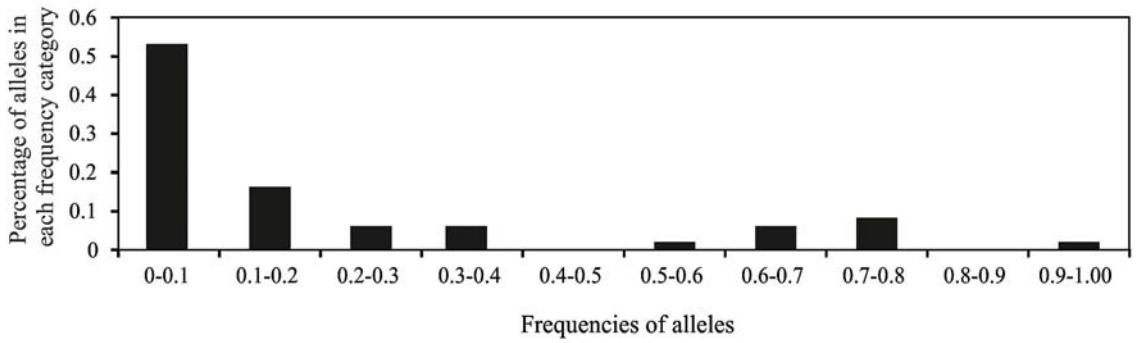

Figure S2. L-shaped frequency distribution of STR alleles. Rarest alleles are highly represented, this pointing against the occurrence of recent genetic bottlenecks.