



POPULATION GENETICS AND FORENSIC DNA FOR CONSERVATION MANAGEMENT OF THE CYPRIOT MOUFLON (*OVIS ORIENTALIS OPHION*)

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The mouflon (*Ovis orientalis ophion*) is the largest wild terrestrial mammal of Cyprus. Considered as the flagship species of the island, its population (c. 3000 head) has a distribution range limited to the mountainous Paphos Forest and adjacent areas including parts of Troodos National Forest (> 700 Km²). The species is protected by a rigorous national legislation supported since 1996 by management plans carried out by the Game and Fauna Service, and, together with its habitat, by the National Law 152 (I)/2003 for the Protection & Management of Wild Birds and Game Species. The species is listed in the Annexes II and IV of 92/43 Habitats Directive, in the Appendix I of CITES, and classified as “vulnerable” by the IUCN. Poaching, habitat loss, road network building and livestock intrusion (i.e., increased risk of pathogen infection) represent the main threatening factors. We aimed at elucidating the systematic placement of the Cypriot mouflon to enforce its protection within an adaptive conservation framework. Therefore, we attempted to determine its genetic structure and relationships with either historically preserved (Corsica, Sardinia) or recently introduced (central Italy) populations including also GenBank entries from the historical range of the species (Near East). The Game and Fauna Service in collaboration with the Cyprus Veterinary Service collected 63 blood samples: 53 were from mouflons captured in the Paphos forest, eight from captive individuals and two of unknown origin. We also sampled 20 mouflons in Sardinia either in the wild (16) or in captivity (4), and collected scats of both Corsican (19) and central Italy (23: Tuscan Archipelago National Park, 13; Tuscan-Emilian Apennines National Park, 6; Apuan Alps Regional Park, 4) mouflon populations in order to increase geographical scope. We genotyped each sample at the entire mitochondrial DNA Cytochrome-*b* codifying gene (Cyt-*b*, 1140 bp) and up to 12 microsatellite DNA markers (Short Tandem Repeats, STR) isolated from goat, sheep and cattle genomes. We found that the Cypriot mouflon strongly diverged from western Mediterranean conspecifics, while North West Iran appeared as the most credited geographic region as the source for its ancient introduction to Cyprus. Although we disclosed much lower mitochondrial and nuclear DNA diversity in the Cypriot than in other island populations, neither evidence of genetic bottleneck nor significant low level of both average pairwise relatedness and inbreeding coefficient was detected. Overall, present mitochondrial and STR dataset worked reliably as crime-fighting tool to tackle illegal mouflon killing in Cyprus. Between 2008 and 2013, the Police and the Game and Fauna Service, in collaboration with the Cyprus Veterinary Services, confiscated 29 samples (meat, hairs, bloodstains) dealing with nine episodes of supposed poaching against the Cypriot mouflon. In all cases, we identified the species in point by sequencing the mtDNA Cyt-*b* gene. In one case, we were specifically requested to establish if there was a link between three dead mouflons recovered at a roadside and 12



bloodstains collected in the car of suspected poachers at the crime scene. With reference to this case, we were able to match nine bloodstains to two out of the three carcasses (seven with very strong support: Likelihood Ratio >3000 and Random Match Probability $<10^{-3}$), overall assigning 22 out of 29 samples to the Cypriot mouflon and the remaining ones to wild boar, cow, domestic goat, horse and hare. These results included the first genetic reference for the Cypriot mouflon and the first published material of forensic wildlife investigations in Cyprus.



Cyprian mouflon male and female during the breeding season. Photo: © E. Hadjisterkotis.