Università
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DOCTORAL COURSE IN
ENVIRONMENTAL SUSTAINABILITY AND WELLBEING

CYCLE XXXV

Coordinator Prof. Spinozzi Paola

A multiple approach to reconstructing the impact of human activities on Alpine chamois (*Rupicapra rupicapra rupicapra*)

Scientific/Disciplinary Sector (SDS) BIO/18

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ABSTRACT

In the face of the current climate crisis and the impact of human activities on the natural ecosystems, it is increasingly essential to develop more accurate management practices based on detailed knowledge of the genetic status, evolutionary history, and ecological parameters of a wild species, to ensure their survival and adaptation to constantly changing environments. To achieve this goal, a multidisciplinary effort is necessary, that is, planning and decision-making processes should consider not only the biological aspects of species or the habitat to be protected, but also the economic and social feasibility of implementing management plans. As a case study, in this thesis, I integrated various techniques, including population genomics of modern and historical DNA, DNA metabarcoding for diet analysis, and an economic approach to decision-making, to the management and conservation of the Alpine chamois (*Rupicapra rupicapra rupicapra*). In particular, through the comparison of historical samples from the 1900s with modern samples, I studied how previous reintroduction and restocking events between different Alpine areas that occurred starting after World War II, influenced the current structure and genetic variability of this chamois. My results suggest that, while these events may have played a role in enhancing the existing genetic variability, it is crucial to carefully plan management strategies for animal translocation across diverse geographic areas to ensure their long-term success. The effectiveness of such conservation efforts can be enhanced by integrating genetic studies with other aspects of chamois ecology, such as the potential for interspecific competition with wild and domestic species. For this reason, the diet of the Alpine chamois was analyzed in relation to that of two other species that share its habitat: red deer and domestic sheep in three parks in Trentino. The results showed that trophic niche overlap was higher between chamois and red deer compared to that between chamois and domestic sheep, suggesting that coexistence of the two wild species may have a higher impact on chamois conservation than that between wild and domestic species with regard to food resources. Finally, in light of these results, four management alternatives were analyzed, aimed at reducing the aforementioned potential competition, through the use of a Multi-Criteria Decision- Making Analysis (MCDA) and the involvement of various types of stakeholders. Interestingly, the overall analysis identified ‘reducing deer densities through hunting plans’ as the preferred alternative. Furthermore, the socio-cultural dimension was found to be the most influential factor in choosing this option. In conclusion, this thesis shows how a multidisciplinary approach could contribute to a better management of the chamois, considering not only the biological aspects, but also economic and socio-cultural values.

RIASSUNTO

A fronte dell’attuale crisi climatica e dell’impatto delle attività umane sull’ambiente, diventa essenziale sviluppare pratiche di gestione sempre più accurate, basate su una profonda conoscenza dello stato genetico, della storia evolutiva e dei fattori ecologici di una specie, al fine di garantirne la sopravvivenza e l’adattamento ad ambienti in costante cambiamento. Tuttavia, per raggiungere questo obiettivo, è richiesto uno sforzo multidisciplinare: la pianificazione e i processi decisionali dovrebbero considerare non solo gli aspetti biologici della specie o dell'habitat da proteggere, ma anche la fattibilità economica e sociale dell'attuazione dei piani di gestione. Quindi, in questa tesi ho integrato diversi approcci, tra cui la genomica, il metabarcoding per lo studio della dieta, e l'analisi economica, per la gestione e conservazione del camoscio alpino (*Rupicapra rupicapra rupicapra*). In particolare, attraverso l’analisi di campioni storici risalenti all’inizio del ‘900 e campioni moderni di camoscio, ho studiato come i passati eventi di reintroduzione e restocking tra diverse zone alpine avvenuti a partire dal secondo dopoguerra, abbiano influito sull’attuale struttura e variabilità genetica del camoscio. I risultati suggeriscono che, sebbene questi eventi possano aver svolto un ruolo nell'aumentare la variabilità genetica esistente, è fondamentale pianificare attentamente strategie di gestione per la traslocazione degli animali in diverse aree geografiche al fine di garantirne il successo a lungo termine. L'efficacia di tali sforzi di conservazione può essere migliorata integrando gli studi genetici con altri aspetti dell'ecologia del camoscio, come ad esempio, il potenziale di competizione interspecifica con specie selvatiche e domestiche. Per questo motivo è stata analizzata la dieta del camoscio, nonché di altre due specie che condividono il suo habitat: il cervo e la pecora. I risultati hanno mostrato che la sovrapposizione delle nicchie trofiche era maggiore tra camoscio e cervo rispetto a quella tra camoscio e pecora, suggerendo dunque che la coesistenza tra specie selvatiche potrebbe avere un impatto più problematico rispetto alla coesistenza tra fauna selvatica e domestica per quanto riguarda le risorse alimentari. Alla luce di questi risultati, sono state analizzate quattro alternative di gestione volte alla riduzione della potenziale competizione attraverso l’utilizzo dell’Analisi Multicriterio e il coinvolgimento di vari stakeholder. L’analisi complessiva ha individuato come miglior alternativa quella di ridurre le popolazioni di cervo tramite piani di abbattimento. Inoltre, la dimensione socioculturale è risultata essere il fattore più influente nella scelta di tale opzione. I risultati ottenuti

in questa tesi, dunque, mostrano come un approccio multidisciplinare basato su aspetti biologici, economici e socioculturali possa contribuire a una migliore gestione del camoscio.

1. INTRODUCTION

1.1 A MULTIDISCIPLINARY APPROACH TO CHAMOIS CONSERVATION

Faced with the current biodiversity crisis, urgent actions are needed to mitigate the impact of natural and human-mediated activities on biodiversity and ecosystem. However, despite the numerous actions and efforts focused on environmental protection, global biodiversity is still rapidly decreasing (Pascual et al., 2021). As reported by the International Union for Conservation of Nature (IUCN) more than 42,100 species are threatened with extinction of which 27% are mammals (IUCN, 2022) (Figure 1.1).

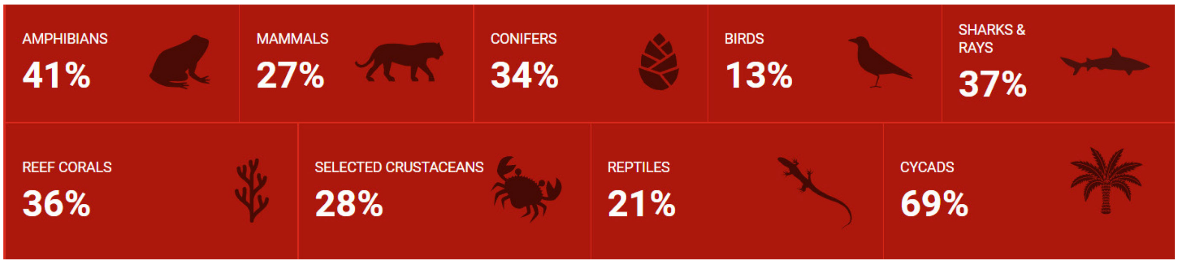

Figure 1.1 IUCN List of threatened species. From https://www.iucnredlist.org/

To respond to this challenge, the field of conservation biology was founded in the early 1980 (Gerber L., 2010) to describe the biological diversity of living organisms, identify the anthropogenic impact on species and ecosystems, and find ways to preserve this biodiversity, taking inspiration from social, economic, and philosophical sciences (Meine, 2010; Primack & Boitani, 2013).

The Convention on Biological Diversity (CBD) was adopted at the Earth Summit held in Rio de Janeiro. This international treaty seeks to promote the conservation of biodiversity, while also encouraging its sustainable use and the equitable sharing of derived benefits (CBD, 1992). Importantly, this agreement recognizes three main levels of biodiversity: ecosystem, species and genetic diversity. Ecosystem diversity refers to the diversity of habitats and ecosystems and can be defined as the variety of terrestrial and aquatic environments, ecological processes plus all the species characteristic of a region, while species diversity refers to the variety of different species, which are the fundamental units of biological organization (Gaston & Spicer, 2004; Kumar & Mina, 2018); instead, genetic diversity refers to the variation at levels of genes which provides the raw material for evolutionary processes enabling populations to adapt to changing environments and to respond to natural selection (Frankham et al., 2002; Gaston & Spicer, 2004; Kumar & Mina, 2018). Genetic diversity has also been included as a primary goal within the COP (Conference of Parties) framework, with the specific objective of maintaining and safeguarding the genetic diversity within populations of both wild and domesticated species (CBD, 2022).

Due to habitat fragmentation and population reduction and isolation many species are experiencing losses in genetic diversity as a consequence of the effects of inbreeding, resulting in increased homozygosity and a higher likelihood of fixation of deleterious alleles, which in turn increases the risk of extinction (Frankham et al., 2002; Reed & Frankham, 2003). By analyzing and monitoring the genetic diversity within a species or a population it is possible to understand how they have and may respond to changing environmental conditions and/or how they have been impacted by human activities, to develop effective and long-term conservation strategies. Additionally, genetic information can help to resolve taxonomic uncertainties and identify management units which can help develop more appropriate management actions (Frankham et al., 2002; Hohenlohe et al., 2021).

Even more recently, since biodiversity conservation actions can involve different stakeholders such as governments, private businesses, or local communities, each of them bringing their values, needs and priorities, to tackle complex conservation issues, it has been suggested that a holistic approach to wildlife conservation might be more successful. Such an approach would include not only ecological aspects, but also social, economic, and political dimensions (Daily & Matson, 2008; Norgaard, 2010). Indeed, to develop successful and effective conservation actions, a multidisciplinary approach identifying sustainable solutions may be required (Dick et al., 2016; Mallegowda, 2013). One of the main challenges to this approach is to promote collaboration and effectively integrate knowledge, data and goals of stakeholders with different mindsets and vocabulary (Le et al., 2017).

The need for a holistic approach is also consistent with the principles of the United Nation Agenda for Sustainable Development (AGENDA 2030) which highlight the interconnections between the social, economic, and environmental dimensions of sustainability (Transforming Our World: The 2030 Agenda for Sustainable Development, 2015). In this context, to achieve the 15 Sustainable Development Goal (SDG) "Protect, restore and promote sustainable use of terrestrial ecosystems, sustainably manage forests, combat desertification, and halt and reverse land degradation and halt biodiversity loss", a multidisciplinary approach would help to identify effective strategies for biodiversity conservation and ecosystem restoration and, interestingly, within this goal, the UN’s Agenda 2030 also recognizes the importance of mountain ecosystems.

Due to their varied physical, topographic, and climatic characteristics, mountainous environments form a diverse array of habitats across different elevations, resulting in a mosaic of landscapes (Beniston, 2006; Körner et al., 2011; Viterbi et al., 2013). They cover approximately one-quarter of the Earth's land surface and are a hotspot for biodiversity (Diaz et al., 2003), but, at the same time, they are particularly sensitive to the effects of climate change; in fact, the impacts of increasing temperature are amplified at high altitudes, so that mountain environments are expected to experience more rapid changes compared to those at lower elevation (Pepin et al., 2015). Increasing temperatures can lead to a shift of cold-adapted plant species to higher altitudes (Chen et al., 2011; Lenoir et al., 2008; Pauli et al., 2012; Telwala et al., 2013), which in turn prompts animal species feeding on them to change their distribution pattern as well (Lovari et al., 2020). Over the long term, such climatic changes can lead to a reduction of suitable habitat at higher altitudes with dramatic consequences on species adaptability and survival (Reiner et al., 2021). Moreover, mountain environment composition can greatly be affected by human activities, such as tourism, habitat fragmentation, intensification of exploitation as well as abandonment of traditional practices and pose an additional threat to species adaptation to changing environments (Chemini & Rizzoli, 2003). Moreover, mountain biodiversity offers a wide range of ecosystem services such as food, water, medicinal, energy, upon which humans heavily rely; consequently, any alteration or depletion of this biodiversity can have a significant impact on human well-being (Grêt-Regamey et al., 2012; Payne et al., 2017). Therefore, conserving mountain biodiversity is a complex issue that requires, the integration of the natural and human dimensions toward environmental sustainability and human well-being.

The aims of this thesis arise precisely from the necessity to integrate ecological and social approaches to animal conservation. Thus, I decided to integrate various methods (population genomics, metabarcoding for diet analysis, and a multi-criteria decision making analysis) with the aim of generating data that can be used to help develop future management policies of the Alpine chamois

(*Rupicapra rupicapra rupicapira*) (Anderwald et al., 2020; Corlatti et al., 2022a, Masini & Lovari, 1988).

Population genomics allowed me to test if past and current natural and human-mediated processes have affected the current levels of genetic diversity and adaptive potential of this species, since several previous papers show that the current genetic patterns found in some chamois populations are a result of various factors, including overhunting (Buzan et al., 2013; Lovari et al., 2010; Rezić et al., 2022; Zima et al., 2011), translocations (documented and undocumented) (Crestanello et al., 2009), hybridization with other introduced subspecies (Šprem & Buzan, 2016; Zemanová et al., 2015) habitat fragmentation, and population isolation (Crestanello et al., 2009; Soglia et al., 2010). One way of capturing past evolutionary changes and human-mediated activities and their consequences on genetic variability, is the analysis of the mitochondrial DNA (mtDNA) of historical and modern samples. Since mitochondrial DNA is haploid and maternally inherited, it is not involved in recombination processes, and the accumulation of mutations allows the tracing of matrilinear ancestry across multiple generations (Nesheva, 2014). In addition, the number of mtDNA copies per cell is much higher than the number of nuclear DNA copies (only two in diploid individuals). This means that it is easier to obtain mtDNA sequences from historical samples, such as bones or teeth, allowing for the investigation of patterns of genetic variation through time and for the reconstruction of animal evolutionary history (Merheb et al., 2019).

DNA analysis from both modern and historical samples can provide insight into the effects of population decline and relocation, thus indicating at risk situations that require specific management interventions. However, a thorough understanding of the ecology of the chamois could improve the success of these plans. For example, the impact of other wild ungulates and domestic livestock, as well as that of current human disturbances on chamois health is not well understood. One of the main causes of the decline of local chamois populations in Italy is competition with mountain agriculture such as sheep and goat livestock grazing (Carnevali et al., 2009). In addition to this, in the last few years, red deer numbers have been increasing, which has been hypothesized to impact chamois populations through interspecific competition (Corlatti et al., 2019). In this context, knowledge of livestock, deer and chamois diets could be fundamental to evaluating intraspecific competition (Nichols et al., 2016). Many diet studies (Espunyes et al., 2019; Karmiris et al., 2011; Mphinyane et al., 2015) have used traditional methods such as microscopic or histological analyses of faeces or stomach contents, but although these methods can be used to quantify the ingested food items, they may lead to biased results, especially for ruminants, as a result of the digestive process, as not all dietary components can be identified (Nichols et al., 2016). Instead, a powerful and efficient method to study animal diet from non-invasive samples such as faeces is DNA metabarcoding. With this technique, it is possible to simultaneously obtain multiple DNA sequences (barcodes) from many samples that can be used to identify the dietary components that are present (Nichols et al., 2016; Pompanon et al., 2012), and gain insight into species trophic niche overlap.

The issues related to the management and conservation of chamois led me to develop the final part of my project. Since conflicts between wildlife and human activities in mountain areas is becoming increasingly relevant, it is crucial to minimize these interactions in order to mitigate the impact of disturbances for chamois, but also to maintain an active and sustainable Alpine economy and cultural traditions. One potential approach to aid the decision-making process in nature conservation is Multi-Criteria Decision-Making Analysis (MCDMA), a method that effectively assists in the decision process by systematically analyzing the advantages and disadvantages of different options (Geneletti & Ferretti, 2015; McShane et al., 2011). A methodical and collaborative approach to assessing different intervention measures while considering their trade-offs can lead to outcomes that are both more efficient and more widely accepted (Davies et al., 2013; McShane et al., 2011; Treves et al., 2006), essential for making sustainable decisions that balance social, economic, and environmental concerns in the short and long term.

In conclusion, the main results of this thesis are twofold. First, I showed how different disciplines can be integrated to produce useful results for the future development or modification of management plans necessary to preserve biodiversity. Second, I applied this approach to the Alpine chamois, a taxon with a highly relevant ecological and cultural role in mountainous environments, introducing relevant aspects and suggestions for its conservation. Future studies should apply the multidisciplinary approach proposed here to concrete conservation actions, but we can safely conclude that this approach is the only way to proceed, considering that human needs and environment protection cannot be managed separately.

1.2 THE CHAMOIS

1.2.1 BIOLOGY AND ECOLOGY

The chamois (*Rupicapra* spp.), is a ruminant artiodactyl mammal of the family Bovidae, tribe Caprini, adapted to life on rocky mountain terrain. Two species are currently recognized (Figure 1.2): the Northern chamois, *R. rupicapra*, and the Southern chamois, *R. pyrenaica*, (Anderwald et al., 2020; Corlatti et al., 2011; Herrero, Lovari, Nores, et al., 2020; Masini & Lovari, 1988).

Figure 1.2 The Northern chamois, R. rupicapra on the left, and the Southern chamois, R. pyrenaica, on the right. Figures from https://www.iucnredlist.org (left) and https://eunis.eea.europa.eu/species/1552 (right).

The two chamois species, *R. rupicapra* and *R. pyrenaica*, can be distinguished by size, with the Pyrenean being smaller than the Alpine chamois. In addition, the winter coat of *R. rupicapra* is blackish–brown with a small anal spot and throat patch, which does not reach the chest (Catusse et al. 1996; Masseti &Salari, 2012; Salari et al. 2014), while that of *R. pyrenaica* differs slightly between subspecies: *R. p ornata*, has five large and one small spot, the coat is paler or cream-colored on the back and has a wide throat patch that reaches the chest; the Pyrenean chamois, *R. p. pyrenaica*, and the Cantabrian chamois, *R. p. parva*, have five large and one small spot, but a smaller throat patch: light gray in the Pyrenean population, and yellowish and dark gray in the Cantabrian populations (Alvarez-Busto et al., 2007; Masseti & Salari, 2012; Salari et al., 2014). In addition, the Northern chamois has sharply-pointed, backward curved horns, while the Southern chamois has less curved, slightly shorter and thinner horns (Walzer C., 2008).

Both species can cope with various climates and terrains, utilizing the full range of slopes, microreliefs, and forest cover to ensure optimal thermal conditions and maximize food accessibility (Catusse et al., 1996). The Northern chamois generally inhabit steep, rocky areas, and alpine meadows, while the Southern chamois can also be found in forested valleys and at lower slopes in mountainous areas (Anderwald et al., 2020; Catusse et al., 1996; Herrero, Lovari, Nores, et al., 2020).

Their capacity of chamois to adapt to a wide range of environments also includes a varied diet. Chamois are considered ‘intermediate’ feeders (Garcia-Gonzalez & Cuartas, 1996; Mustoni et al., 2017), meaning they either vary their diet between a grazing (bulk roughage feeders) or browsing (concentrate selectors) on a seasonal basis or have a mixed diet year-round (Hofmann, 1989). In general, herbaceous plants form the primary constituent of chamois diet, however, during winter when these plants become difficult to access due to snow cover, chamois incorporate tree and shrub components such as buds, twigs, leaves, bark of hardwoods and conifers, as well as mosses and lichens obtained from tree trunks into their diet (Catusse et al., 1996).

Mating takes place between November and December and after about 160-170 days, females give birth to single kid, generally between May and June (Anderwald et al., 2020; Catusse et al., 1996; Herrero, Lovari, Nores, et al., 2020). The kids are suckled for two months, but after few weeks they can already feed on grass. Over two months of age they occasionally suckle, until weaning (Catusse et al., 1996).

The population dynamics of Northern and Southern chamois can be strongly affected by various factors, such as climate change, competition with other ungulates, diseases and hunting. Severe snow conditions in late winter may negatively affect the total population size of Alpine chamois, limiting population growth (Willisch et al, 2013), while years characterized by late springs can negatively affect juvenile body mass (Garel et al, 2011). Moreover, increasing temperatures has been observed to negatively affect body mass of yearlings by leading animals to spend more time resting than feeding (Mason et al. 2014), as well as net recruitment, suggesting thus lower adaptability of younger animals to ongoing global changes (Chirichella et al., 2021).

Climatic changes also seem to affect the Southern chamois, especially the Apennine chamois, *R. p. ornata*. This subspecies inhabits low mountain ranges, which means there are no potential refugial areas at higher altitudes (Lovari et al., 2020). In addition, increasing temperatures affect the availability of nutritious food resources linked to long-lasting snow cover, with potential consequences on winter survival of kids (Corlatti, 2021; Ferrari et al., 1988). In the Pyrenees, the ongoing shrubification, i.e., the expansion of woody plant due to abandonment of traditional pastures, does not seem to negatively affect chamois feeding habits, although in the long-term shifts in forage availability and in habitat composition may potentially affect population dynamics (Espunyes et al., 2019). In addition, in the French Pyrenees, it has been observed that a higher snow cover from March to May, caused a decrease in the reproduction rate (Loison, 2004). Also in the Cantabrian mountains, the reproduction rate was observed to be negatively correlated with rainy springs and heavy snowfall (Palacios Alberti, 2009).

Outbreaks of diseases such as keratoconjunctivitis or sarcoptic mange combined with high densities and harsh winters can affect the population dynamics of the Northern chamois with overall mortalities over 30% and 80%, respectively (Corlatti et al., 2022b), although higher reproductive rates in subsequent years can substantially contribute to population recover (Loison et al., 1996; Rossi et al., 1996). Sarcoptic mange can also affect the population dynamics of the Southern chamois by altering the sex-ratio (Nores & González-Quirós, 2009), although it has only been diagnosed in the *parva* subspecies (Corlatti et al., 2022b; González-Quirós, 2009). Kerato-conjunctivitis outbreaks were detected in Southern chamois in the Pyrenees (Arnal et al., 2013; Pañella et al., 2010). Moreover, an exclusive outbreak of pestivirosis has been detected in this area with mortality rates ranging from 40% to 85% (Corlatti et al., 2022b; Marco et al., 2009).

Population dynamics can also be affected by interspecific competition with sympatric ungulates, with possible consequences on population growth, survival, and reproduction (Begon et al., 2006). Northern chamois share its habitat with wild and domestic herbivores, with mouflon, red deer, Alpine ibex and domestic sheep thought to be potential competitor. In the Italian Alps, physical displacement of chamois in areas with lower food availability has been observed in the presence of mouflon (Chirichella et al., 2013). In addition, Chirichella et al. (2015) reported an increase in the size of female chamois groups when in closer proximity with mouflon group, either to avoid or as a consequence of spatial displacement by mouflon. A potential for competition was also observed by Bertolino et al. (2009) who reported a considerable dietary overlap between the two species.

Spatial displacement has also been observed in response to red deer in the European Alps. For instance, in the Swiss National Park, Anderwald et al. (2016)

reported a negative effect of red deer population size on the use of meadows (in favor of scree slopes) by chamois and on horn growth in young chamois. In Italy, in the Stelvio National Park, Corlatti et al. (2019) reported that high red deer densities was negatively associated with chamois growth rate. Additional studies (Andreoli et al., 2016; Bertolino et al., 2009; Homolka & Heroldová, 2001; Redjadj et al., 2014) reported a high similarity of diet between chamois and red deer, based on micro-histological data, suggesting the potential for interspecific competition.

Chamois also share their habitat with Alpine ibex but, Anderwald et al. (2015) and Herfindal et al. (2019) reported that it is probably the chamois that influences ibex habitat use. In addition, although high dietary overlap between the two species has been reported (Anderwald et al., 2015), there is no evidence that this overlap effects on population density or body condition of either species.

With regard to domestic animals, it has been suggested that domestic sheep could be potential competitor of chamois. La Morgia & Bassano (2009) reported a shift in chamois diet including a reduced consumption of forbs when domestic sheep were in the same range. Additionally, Mason et al. (2014) and (Chirichella et al., 2013) reported spatial displacement of chamois to suboptimal areas in response to domestic sheep presence.

For Southern chamois, there are only a few reports of potential competition only with red deer, including spatial and dietary overlap and possible consequences for winter survival of kids (Ferretti et al., 2015; Lovari et al., 2014). In some areas of the Cantabrian Mountains, a spatial displacement of chamois to higher altitudes has been associated with the presence of livestock (J. Pérez-Barbería & Pérez-Fernández, 2009).

Hunting also plays a key role in chamois population dynamics, thus, monitoring population size and distribution is fundamental for its management (Corlatti et al., 2022b). Most of the Northern chamois subspecies are hunted under varying hunting regimes, from simplified age class or more complex grouping systems (Corlatti et al., 2022b). With few local exceptions, hunting of the Southern chamois, *R. p. pyrenaica* and *R. p. parva*, is generally sustainable and well regulated (Herrero et al., 2020).

However, to ensure long-term viability and persistence of a given population, it has been suggested that conservative harvest quotas should be decided according to current demographic structure, with the aim of avoiding strong skews in sex- and age-ratios (Corlatti et al., 2022a). Currently, however, other factors such as presence of potential wild or domestic competitors, and/or quality of diet are not taken into account.

1.2.2 DISTRIBUTION, TAXONOMY AND CONSERVATION

Chamois are distributed over most of the medium to high-altitude mountain ranges of central and southern Europe, as well as Asia Minor (Anderwald et al., 2020; Herrero, Lovari, Nores, et al., 2020). In addition, the Alpine chamois is also found in New Zealand, where it was introduced in 1907 for hunting purposes (Corlatti et al., 2011; Martínková et al., 2012).

The Northern chamois, *R. rupicapra*, includes seven subspecies (*R. r. asiatica*, *balcanica*, *carpatica*, *cartusiana*, *caucasica*, *rupicapra* and *tatrica*). The Southern chamois, *R. pyrenaica*, includes three subspecies (*R. p. pyrenaica*, *parva* and *ornata*) (Corlatti et al., 2011; Masini & Lovari, 1988). The majority of the populations of the Northern and Southern chamois are relatively secure, thus the two species are assessed as Least Concern in the IUCN Red List (Anderwald et al., 2020; Corlatti et al., 2011; Herrero, Lovari, Nores, et al., 2020). On the other hand, the status of the different subspecies is variable, and several are considered globally threatened, requiring urgent conservation actions (Anderwald et al., 2020; Aulagnier et al., 2008; Corlatti et al., 2011; Herrero, Lovari, Nores, et al., 2020).

Considering that the phylogenetic analyses conducted in the second chapter of this thesis were performed on chamois samples collected across its entire range, the following paragraphs provide an overview of the status of the various subspecies.

The Northern chamois, R. rupicapra

The Northern chamois is distributed throughout mountainous regions in central and southern Europe, as well as in Asia Minor (Anderwald et al., 2020) (Figure 1.3). It is listed in Annexes V of the EU Habitats and Species Directive and Appendix III of the Bern Convention. Table 1.1 shows the status and distribution of the *R. rupicapra* subspecies.

Figure 1.3 Distribution map of the Northern chamois (Rupicapra rupicapra). From IUCN (iucnredlist.org)

Table 1.1 Status and distribution of R. rupicapra subspecies. Modified from (Corlatti et al., 2022a).

Subspecies	**Distribution**	**Population size**	**Habitat directive annexes**	**IUCN 2020-2021**
R. r. rupicapra	Alps of France, Italy, Switzerland, Germany, Liechtenstein, Austria, Slovenia, Croatia	~ 500,000		
R. r. tatrica	Tatra mountains of Poland and Slovakia	1100	II, IV	EN, B1+2ab
R. r. cartusiana	Chartreuse limestone massif	1500		VU, D1+2
R. r. asiatica	Northeastern mountains of the Black Sea, eastern Anatolia, Turkey, southwestern Georgia	800		EN, C1+2a(i)
R. r. balcanica	Croatia, Bosnia and Herzegovina, Serbia, Kosovo, Montenegro, north Macedonia, Albania, Bulgaria, Greece	< 10 000	II, IV	
R. r. carpatica	Transylvania Alps, Carpathian Mountains	8000		
R. r. caucasica	Caucasus Mountains of southern Russia, Georgia, Azerbaijan	> 9000		VU, C1

The Alpine subspecies, *R. r. rupicapra*, is the most abundant, and is widespread across the Alps of France, Italy, Switzerland, Austria, Liechtenstein, Germany, Slovenia, Croatia, and New Zealand (Corlatti et al., 2011), with a population size estimated at 500,000 individuals (Corlatti et al., 2022a). In most of its range, this subspecies is not threatened, but several factors negatively affect specific Alpine chamois populations, such as climate change (Willisch et al., 2013) and competition with domestic (la Morgia & Bassano, 2009) and wild species (Bertolino et al., 2009; Chirichella et al., 2013; Corlatti et al., 2019).

R. r. tatrica, listed as Endangered by the IUCN Red List (Anderwald et al., 2020), is found in the Tatra mountains of Poland and Slovakia (Anderwald et al., 2020; Bačkor & Velič, 2008). Since the second half of the 19th century, populations here have experienced significant fluctuations mainly due to poaching and hunting, with a long-term decline starting in 1964 (Janiga & Zámečnícová, 2002; Zemanová et al., 2015). In addition, introgressive hybridization with the introduced Alpine chamois has compromised the integrity of the Tatra chamois genome and may interfere with locally adapted genetic characteristics (Zemanová et al., 2015). However, there is still no information on the consequences of hybridization. This subspecies is listed in Annexes II and IV of the EU Habitats and Species Directive and it is strictly protected by law (Anderwald et al., 2020). Its population is estimated to be around 1100 individuals, but this number also includes about 100 chamois hybridized with Alpine chamois (Corlatti et al., 2022a).

R. r. cartusiana is endemic to France and restricted to an area of the Chartreuse limestone massif, and listed as Vulnerable (Anderwald et al., 2020). Potential hybridization with introduced *R. r. rupicapra*, competition with domestic and wild species, poaching, overharvesting, and disturbance from summer tourism and winter cross-country skiing seriously threaten this subspecies. Translocations have been undertaken to restore population sizes (Anderwald et al., 2020; Berducou, 1990), and in 2017-2018, there were approximately 1500 individuals (Prévost, 2018).

R. r. asiatica, the Anatolian chamois, is limited to three largely isolated populations found in the northeastern mountains of the Black Sea, eastern regions of Anatolia in Turkey, and southwestern areas of Georgia (Anderwald et al., 2020). Due to trophy hunting, poaching, habitat degradation and intensive human disturbance, numbers have declined sharply. There is very little data on its conservation status, but over the last 20 years the number of individuals has decreased drastically, and is still declining (Ambarlı, 2014; Anderwald et al., 2020). For this reason, Anderwald et al. (2020) proposed to list the subspecies as Endangered (EN).

The subspecies *R. r. balcanica* occurs in the Dinaric area through Scardo-Pindic Mountain in Croatia, Bosnia and Herzegovina, Serbia, Kosovo, Montenegro, North Macedonia, Albania, Bulgaria, and Greece and is listed in Annexes II and IV of the European Union Habitats Directive. The overall population size is estimated as less than 10,000 individuals (Anderwald et al., 2020). Poaching, habitat fragmentation, human disturbance and introduction of *R. r. rupicapra*, are major threats for this subspecies (Anderwald et al., 2020; Iacolina et al., 2021; Šprem & Buzan, 2016).

R. r. carpatica inhabits the Transylvania Alps and the Carpathian Mountains. In 2014 its population size was estimated to be between 8000 and 9000 individuals (Verghelet & Ionescu, 2014). Growth is constrained by several factors including the carrying capacity of winter refuges and predation in protected areas. Additionally, poaching, competition for food with sheep and goats, as well as disease, represents significant limiting factors (Anderwald et al., 2020; Verghelet & Ionescu, 2014).

R. r. caucasica is distributed in the Caucasus Mountains of southern Russia, Georgia and Azerbaijan. Overall, it has a population estimate of slightly more than 9,000 individuals, but in general, it is still decreasing, therefore, it is classified as Vulnerable by the IUCN. Poaching related is a major threat and, in some areas, probably also competition with livestock, tur, red deer and roe deer (Anderwald et al., 2020).

The Southern chamois, R. pyrenaica

The Southern chamois is endemic to south-west Europe (Figure 1.4). It is listed on Appendix III of the Bern Convention and Annex V of the EU Habitats and Species Directive. It is also IUCN-assessed as Least Concern, although the subspecies *R. r. ornata* is considered Vulnerable, due to its restricted geographic range and a very small population size (Herrero, Lovari, Nores, et al., 2020). The status and distribution of the three subspecies, *R. p. pyrenaica, R. p. parva* and *R. p. ornata* are listed in Table 1.2.

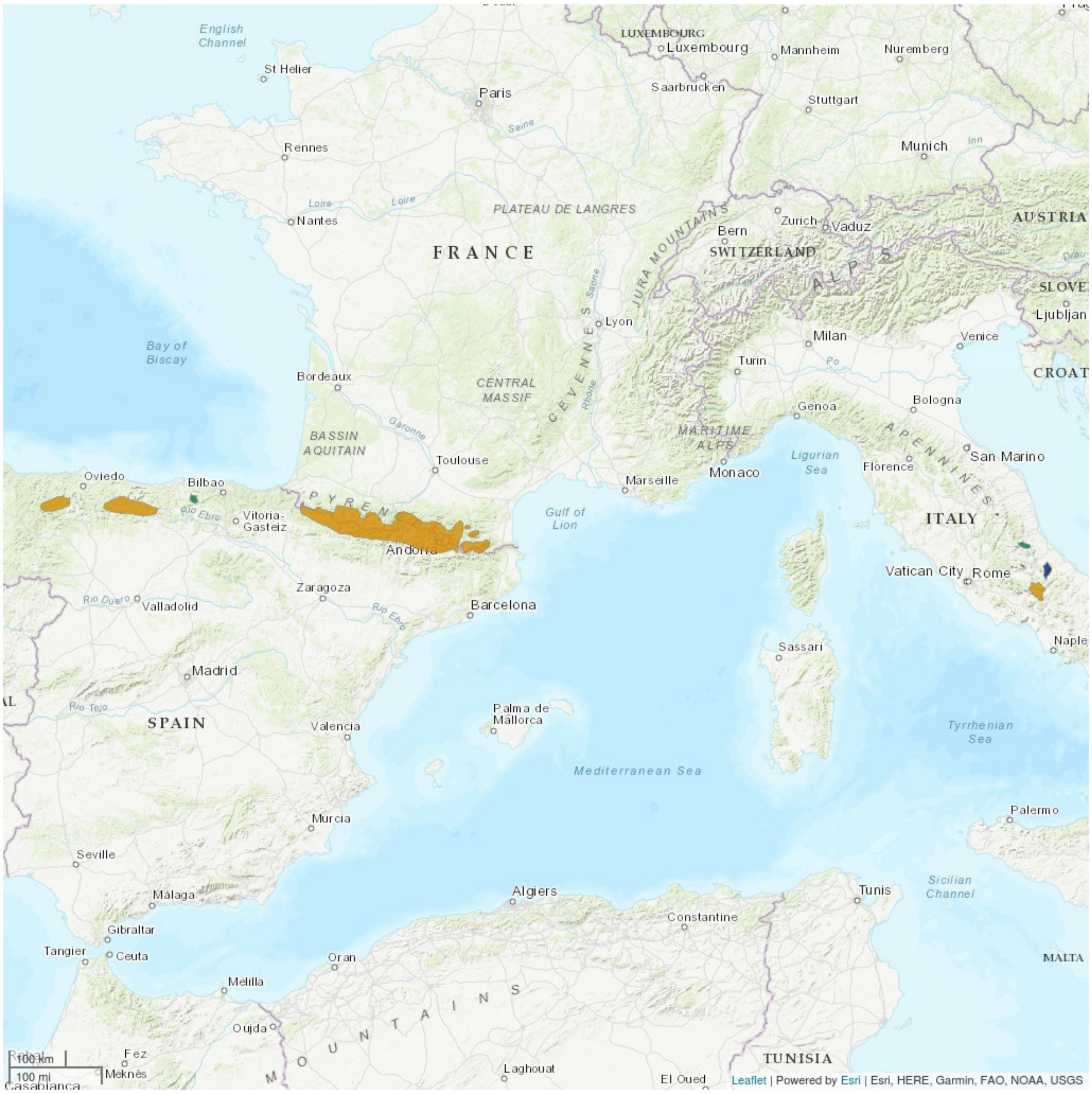

Figure 1.4 Distribution map of the Southern chamois (Rupicapra pyrenaica). From IUCN (iucnredlist.org)

Table 1.2 Status and distribution of R. pyrenaica subspecies. Modified from (Corlatti et al., 2022a).

Subspecies	Distribution	Population size	Habitat directive annexes	CITES appendix	Bern convention appendix	IUCN 2020-2021
R. p. pyrenaica	Pyrenees (France and Spain)	53,000				
R. p. parva	Cantabrian Mountains	16,000				
R. p. ornata	Abruzzo, Lazio and Molise National Park, reintriduced in the Majella, Gran Sasso, Monti della Laga, Monti Sibillini National Parks and the Sirente-Velino Regional Park	2500	II, IV	II	II	VU

The isard, *R. p. pyrenaica*, occurs in the Pyrenees, on the border between France and Spain. In 2002, population was estimated as 53,000 individuals (Herrero et al., 2004). Since 2004, mortality has been high as a result of viral diseases; in fact, keratoconjunctivitis (Mycoplasma conjunctivae; Arnal et al., 2013) and pestivirus (Marco et al., 2009; Serrano et al., 2015) are currently the major threats for this subspecies.

The subspecies *R p. parva*, or Cantabrian chamois, is found in Spain across the Cantabrian mountains, with around 16,000 individuals (J. Pérez-Barbería & Pérez-Fernández, 2009). There are no significant threats for this subspecies, although sarcoptic mange outbreaks have caused local declines (Nores & González-Quirós, 2009; Palomo & Gisbert, 2002).

The only native population of the Apennine chamois, *R. p. ornata*, occurs in Italy, in the Abruzzo, Lazio and Molise National Park, but it has also been introduced to the Majella, Gran Sasso, Monti della Laga, Monti Sibillini National Parks and the Sirente-Velino Regional Park (Bocci et al., 2016; Di Domenico G. et al., 2015; Herrero, Lovari, Nores, et al., 2020). After several population contractions, following the application of conservation actions, numbers have increased to about 2500 individuals. It is included in Appendix II of the Bern Convention, as well as Annexes II and IV of the EU Habitats and Species Directive. Additionally, it is listed in Appendix I of CITES and designated as a "specially protected species" under Italian hunting law (Herrero, Lovari, Nores, et al., 2020). Due to the small number of individuals and the very low genetic variability (Corlatti et al., 2011; Lorenzini, 2005), this species is particularly vulnerable to many factors, such as climate change (Lovari et al., 2020) and competition with wild and domestic species (F. Ferretti et al., 2015; Herrero, Lovari, Nores, et al., 2020; Lovari et al., 2014).

As the above descriptions demonstrate, chamois species, subspecies and local populations are all subject to several risk factors, both natural and human mediated, that can affect their state of conservation. The development of an integrated management, which considers a variety of biotic and abiotic factors involved and their interconnection, may be more useful for providing successful conservation strategies.

1.3 AIM AND STRUCTURE OF THE THESIS

This doctoral thesis aims to investigate how natural and human-mediated processes in the past and present have influenced the current genetic pattern and adaptive potential of the chamois, as well as identifying the management practice that take into account ecological, social and economic aspects. In particular, the thesis is structured as follow:

Chapter 2: Exploring chamois phylogenetics and genetic variability in historical and modern mitochondrial DNA. In this chapter I analyzed the mitochondrial DNA of chamois museum specimens collected from across its range, preserved approximately one hundred years ago and, along with the re-analysis of modern samples, I directly compared modern and ancient genetic variation patterns to investigate the possible impact of recent human activities on genetic variability.

Chapter 3: Diet and trophic niche overlap between chamois, domestic sheep and red deer. Here, I applied DNA metabarcoding to explore the dietary habits of chamois, domestic sheep, and red deer, with the aim of determining the degree of overlap in their trophic niches.

Chapter 4: A Multi-Criteria Decision-Making Analysis for chamois management in the Alps. In this chapter, I carried out a Multi-Criteria Decision-Making Analysis (MCDA), an approach commonly used in Economics, to rank and evaluate the trade-offs of four intervention options, taking into account not only biological aspects, but also the economic and socio-cultural dimensions of Alpine chamois conservation.

Chapter 5: General Conclusions. In the concluding chapter, I outline the primary outcomes of my research, discuss the merits of an interdisciplinary approach to identify the best management practices, and provide suggestions for further investigations.

2. EXPLORING CHAMOIS PHYLOGENETICS AND GENETIC VARIABILITY IN HISTORICAL AND MODERN MITOCHONDRIAL DNA

2.1 INTRODUCTION

Chamois (*Rupicapra* spp.) are mountain ungulates found across Europe and Asia, predominantly inhabiting mountain pastures above the tree line and lower, shrubby areas where rocky slopes are available. This habitat is shrinking and increasingly fragmented due to the effects of a warmer climate. Although neither of the two species of chamois under current taxonomic classification, known as the Northern (*R. rupicapra*) and Southern chamois (*R. pyrenaica*) (Corlatti et al., 2011) is at risk, the status of the numerous subspecies is variable, and some are considered threatened, requiring urgent conservation actions (Anderwald et al., 2020, see also Chapter 1 of this thesis).

Over the last two centuries, chamois populations have been affected by overhunting, leading to local extinctions or severe bottlenecks. This is the case of the Apennine chamois (*R. p. ornata*), as well as the Tatra (*R. r. tatrica*) and Balkan chamois (*R. r. balcanica*), which all experienced severe population bottlenecks during the two World Wars due to poaching (Lovari et al., 2010; Rezić et al., 2022; Zima et al., 2011). In the Dinaric Mountains of Slovenia, in the 19th century, overhunting led to the near extinction of the Alpine subspecies population, which only started to recover in the 1950s thanks to the improvement of hunting management, reintroductions and restocking (Buzan et al., 2013). In the Italian Alps, from the second half of the XVIII century, when the Northern chamois was widely distributed, until the 1950s there was a strong decline in numbers as well as a reduction in the range of this species, caused by the increased human presence and direct persecution (Carnevali et al., 2009). In addition to this, several outbreaks of keratoconjunctivitis (IKC) (Arnal et al., 2013; Degiorgi et al., 2000; Grattarola et al., 1999; Tschopp et al., 2005) and sarcoptic mange (Fernández-Morán et al., 1997; Rossi et al., 1996) caused strong population declines in some Alpine areas. In order to mitigate the impact of diseases and human activities, several reintroduction programs were undertaken; however, genetic considerations have often been overlooked in management efforts (Buzan et al., 2013; Shackleton, 1977), which may have influenced the current distribution, population size and genetic structure of the chamois (Bertorelle et al., 2009; Soglia et al., 2010).

In the last two decades, several studies have investigated the impact of natural and human-mediated activities on population genetic variability and to define the phylogeographic structure of the chamois. Thus far, mitochondrial DNA (mtDNA) and microsatellites have been the markers to study the phylogeny, taxonomy and historical and current patterns of population genetic structure in this genus, but they often reported contrasting results (Crestanello et al., 2009, Rodríguez et al., 2009, 2010). For example, mitochondrial analysis showed three different geographically separated clades (Clade West: mtW, Central: mtC, Clade East: mtE, corresponding to *R. r. rupicapra*, *R. p. pyrenaica,* and *R. p. parva*; *R. r. ornata* and *R. r. cartusiana*; *R. r. rupicapra*, *R. r. balcanica*, *R. r. tatrica*, *R. r. asiatica*, *R. r. carpatica*, *R. r. caucasica,* respectively), while microsatellite analyses aligned more closely with morphological classification, with two main clades: 1) the Iberian (Clade µsatW) and Eastern chamois (Clade µsatE), and 2) a well separated clade corresponding to the Apennine chamois (Clade µsatC) (Rodríguez et al., 2009).

In order to develop successful management plans that aim at preserving adaptive diversity and protecting evolutionarily significant units, it is considered critical to resolve issues related to the taxonomy of this genus (Crestanello et al., 2009; Corlatti et al., 2022a). Here, I further investigate how the genus *Rupicapra* diversified in order to resolve taxonomic questions, but also to evaluate how past bottleneck and subsequent translocations or restocking events may have impacted the current genetic structure. To identify the genetic consequences of past evolutionary changes and human-mediated activities I decided to analyse historical samples using ancient DNA methods. Mitochondrial DNA was the marker of choice since this molecule is often well-preserved in every ancient museum specimens. Moreover, since it is haploid and maternally inherited in most animals (Sun et al., 2020), has different gene evolution rates (Cann et al., 1984; Neupert, 2016), and a faster accumulation of mutations in non-coding regions compared to coding regions (Iacolina et al., 2021), the analysis of whole mtDNA can aid in exploring various phylogenetic aspects (Iacolina et al., 2021; Sun et al., 2020).

In this study we analyzed the complete mtDNA and the concatenated Cyt b – D-Loop sequence of historical and modern samples from across the range of the Northern and Southern chamois to better understand the processes and events that led to the current pattern of genetic diversity. More specifically, the goals of this study were (i) to explore the phylogenetic relationships within the genus *Rupicapra* through time and (ii) to identify temporal changes in the genetic diversity in *R.*

rupicapra and *R. pyrenaica* chamois populations to better understand historical and modern impacts of natural and human-mediated processes. I discuss how use of these results could improve the genetic health of this species and aid in the development of effective management guidelines.

2.2 MATERIALS AND METHODS

SAMPLE COLLECTION

Bone-cartilage fragments of the nasal cavity (vomer, nasal concha) or teeth of 63 samples of taxidermied chamois (‘historical specimens’) dated between 1900-1915 and originating from across the chamois range (from the Pyrenees to Western and Eastern Alps, as well as the Caucasus) were collected using sterile methods from the Museum of Natural Sciences of Turin in 2006 by B. Crestanello. Three additional historical samples carbon dated between 1800-1850 and collected from glaciers in Valle d’Aosta, in Gran Paradiso National Park, were included in the analysis. Thirty-six modern samples from carcasses in the Abruzzo, Lazio and Molise National Park, Gran Paradiso National Park, Dolomiti Bellunesi National Park and Province of Bolzano were available at the Fondazione E. Mach from previous projects (samples from Dolomiti Bellunesi) or were received from parks and government offices during this project (Abruzzo, Lazio and Molise National Park, Gran Paradiso National Park and Province of Bolzano). Moreover, a dataset of mtDNA sequences (n = 66) analyzed by Crestanello et al. (2009) for the Cyt b and D-Loop regions was used for additional analysis of this genomic region along with the ancient and modern samples. The geographical origins of the collected samples are shown in Figure 2.1. The taxonomic (subspecies) identification was noted for all samples as recorded by the museums and other collectors.

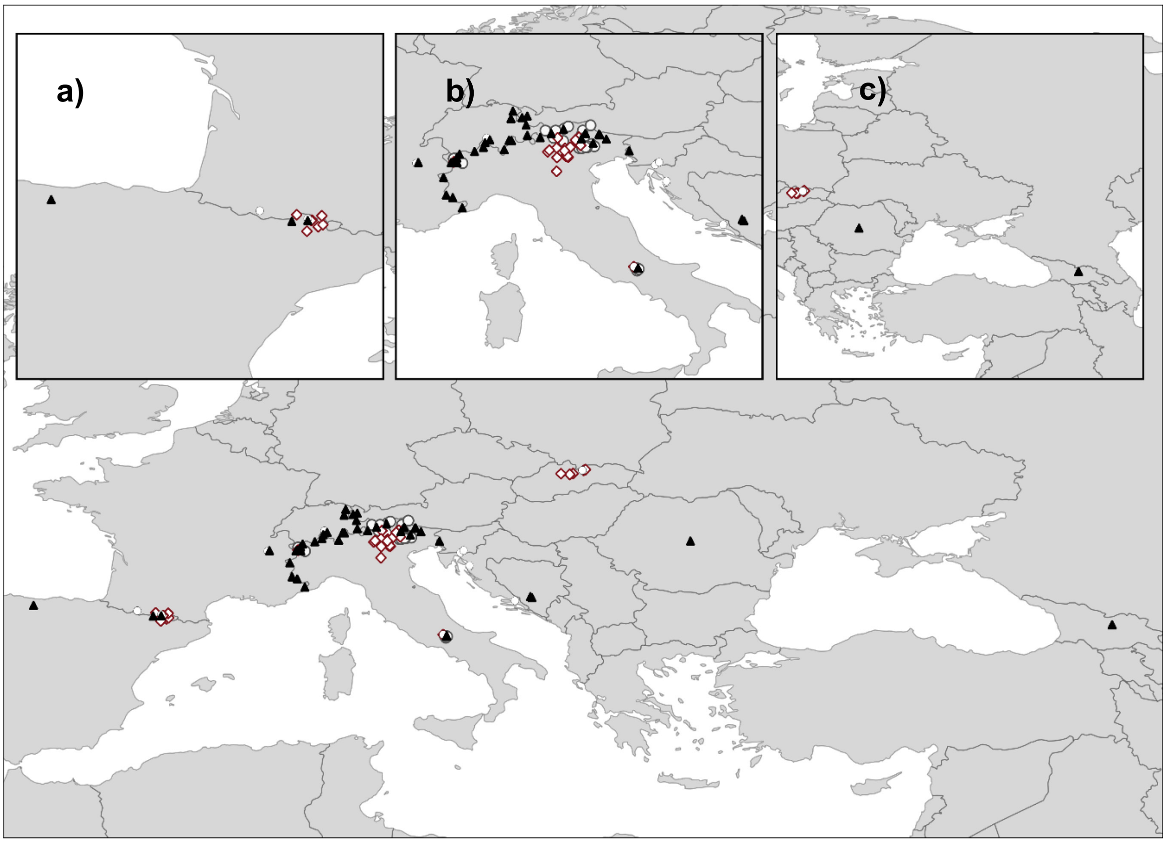

Figure 2.1 Sample distribution of the collected samples. Historical samples are represented with black triangles, modern samples with white circles and Cyt b – D-Loop samples with red-bordered white diamonds. Details of the Pyrenees and Cantabrian Mountains (a), the Alps, Apennines and the Balkans (b) and Slovakia, Romania and the Caucasus (c) are shown.

LABORATORY ANALYSIS

DNA Extraction of historical samples

Preparation of historical samples, including DNA extraction and library preparation, was carried out in a dedicated ancient DNA (aDNA) laboratory of the Animal, Environmental and Ancient DNA Platform (Conservation Genomics Unit), Fondazione E. Mach. To avoid sample contamination, this laboratory is physically separated from any laboratory where modern DNA samples are handled. Bone-cartilage fragments of the nasal cavity were fragmented manually with a scalpel, whereas teeth and bones were pulverized using a freezer mill and genomic DNA was extracted following a phenol-chloroform protocol (Sambrook & Russell, 2006). Details on DNA extraction and subsequent steps are described in Supplementary Materials 1.

Library Preparation

Library preparation for each sample, along with negative controls, followed the modified multiplex protocol developed by Meyer & Kircher (2010) with blunt-end repair, adapter ligation, adapter fill-in and indexing PCR steps (details in Supplementary Materials 1).

Probe design and mtDNA capture

For the generation of the probes, the quality of modern DNA samples was visualized by running them on 1% agarose gel. Selected samples were used as template for two long range PCR in order to obtain the entire mitochondrial genome amplified in two partially overlapping portions. We used two pair of primers with the following sequences:

First primer pair:
Forward: 5'-ATAGGAATTGAACCTACTCC-3'
Reverse: 5'-CATGTTGTTTATTTCTAGGG-3'

Second primer pair:
Forward: 5'-CAAATCTTCATACTAAAACCA-3'
Reverse: 5'-TATGTTATTTATCCGAGGAA-3'

The procedure consists of a first step of fragment sonication, followed by blunt-end repair and adapter ligation, with a purification phase after each step. Each produced aliquot is then checked on an Agilent BioAnalyzer (Agilent - DNA 1000 Kit) and the final product quantified on Nanodrop 2.

The capture of the mtDNA was performed following the protocol developed by Maricic et al. (2010), which involves the use of magnetic beads as support for the probes in order to capture the mtDNA by using a magnet (details in Supplementary Materials 1).

DNA extraction of modern samples

Modern DNA was extracted using the QIAgen DNeasy® Blood and Tissue Kit (Qiagen, Inc., Valencia, CA, USA) following manufacturer instructions. DNA was eluted in 200 µl of elution buffer and fragments were checked on 1% agarose gel.

Long-range DNA amplification

The amplification of modern mtDNA was done using long-range PCR, with the same primers designed for probe construction. For each sample the mix was prepared with 30 µl of water, 10 µl of 5x PrimeSTAR GXL Buffer, 4 µl of dNTP mixture 2.5 mM, 1 µl of Forward Primer 100 µM, 1 µl of Reverse Primer 100 µM, 1 µl of PrimeSTAR GXL DNA Polymerase and 3 µl of template, for a final volume of 50 µl. Thermocycling conditions consisted of 34 cycles of 98°C for 10 s, 50°C for 15 seconds and 68 °C for 10 minutes. PCR products were checked on 1% agarose gel and then purified with QIAquick PCR purification kit (Qiagen, Inc., Valencia, CA, USA) following the manufacturer instructions, with a modification of the elution step that was done in 130 µl of TE LOW buffer.

The amplified fragments were then sonicated with COVARIS S220 to obtain fragments of a length between 300 and 400 bp with the following settings run: 10% duty factor, 68 peak incident power (W), 200 cycles per burst, time of 60 seconds of standby, 88 run, 30 seconds standby and 85 run and a temperature of 7 °C. Sonicated fragments were then checked on an Agilent BioAnalyzer with the DNA 1000 kit and sent to the sequencing platform for library preparation and sequencing.

BIOINFORMATIC ANALYSIS

Bioinformatic analysis of historical samples

Sequence data were processed using PALEOMIX 1.2.12 (Schubert et al., 2014): first, Illumina adapters were removed and overlapping pairing reads were collapsed with AdapterRemoval 2.2.2 with a minimum overlap length of 30 and a maximum of 1/3 allowed mismatches. Collapsed reads were then aligned to reference mitochondrial genomes of *R. rupicapra* (GenBank: FJ207539.1), *R. pyrenaica* (GenBank: NC_020789.1) and *R. p. ornata* (GenBank: KJ184173.1) with bwa 0.7.15 with a minimum mapping quality of hits to retain set to 10. PCR duplicates were removed and Indel realignment was performed with GATK 3.5.0.

To call the consensus, we used ANGSD with the following parameters: -dofasta2 to create a FASTA file from a sequencing BAM file using the most common base, -remove_bads 1 to remove bad reads, -uniqueOnly 1 to remove reads that have multiple hits, -minMapQ 20 to retain only sites with a minimum mapping quality of 20, -only_proper_pairs 0 to use all reads, -minQ 20 to include only sites with a minimum qscore of 20, -C 50 to reduce the effect of reads with excessive mismatches, -setMinDepthInd 3 to retain sites with a minimum depth of coverage of 3 and -doCounts 1 to calculate the various count statistics.

Modern sequences were processed using a bash pipeline which first collapsed pairing reads with AdapterRemoval. Collapsed reads were then aligned to reference *R. rupicapra* mitochondrial genome (GenBank: FJ207539.1), and to *R. pyrenaica ornata* mitochondrial genome (GenBank: KJ184173.1) with bwa aligner. 5 bases of the R1 reads were trimmed at the left end and 0 at the right end, a fraction of 0.01 edit distance of missing alignments was chosen given 2% uniform base error rate, a seed length of 32, a maximum of 1 gap opens, a maximum insert size of 500 for a read pair to be considered being mapped properly, and an optical duplicate pixel distance of 100.

The final consensus was called with ANGSD using the same parameters used for historical samples consensus, but without the -C 50 value.

Sequence Alignment

Multiple sequence alignment of the complete mtDNA sequences of historical and modern samples was performed using MAFFT v7.490 (Katoh & Standley, 2013) after removing six samples due to low mitogenome coverage and including 14 chamois sequences already available in GenBank.

Sequence Alignment of Cyt b – D-Loop database

The haplotypes already analyzed by Crestanello et al. (2009) for the Cyt b and D-Loop regions were aligned with the historical and modern sequences, and the sequences available in GenBank using MAFFT v7.490 (Katoh & Standley, 2013). Subsequently, in order to obtain the same fragment from the complete mtDNA sequences, only the Cyt b and the D-Loop regions were selected in each sequence and the two regions were concatenated using Geneious v.8.1.9 (https://www.geneious.com).

STATISTICAL ANALYSIS

Bayesian Mitochondrial Phylogeny. The phylogenetic relationship of these sequences was investigated using the mountain goat (*Oreamnos americanus*) as an outgroup. Aligned sequences were collapsed using FaBox (Villesen, 2007), and only unique haplotypes were considered for the analysis with BEAST. The final dataset for the complete sequence was composed by 59 historical samples, 32 modern samples and 10 chamois sequences already available in GenBank, making a total of 101 unique sequences; the Cytb and D-Loop database was composed by 58 historical samples, 48 modern samples and 10 sequences from GenBank, making a total of 116 sequences.

The best substitution model, used to estimate the substitution rate for each node of the tree, was selected with MEGA11 (Tamura et al., 2021) using the AIC criteria resulting in the Hasegawa–Kishino–Yano model with gamma distributed rates (HKY + I + G), for both the complete mtDNA sequences and the Cyt b – D-Loop database. Phylogenetic tree for both datasets was constructed using BEAST 1.7.1 (Drummond et al., 2012) with 50,000,000 MCMC generations sampled every 1,000 iterations and a Strict Clock model and Coalescent Constant Population as prior. The molecular clock was calibrated using a normal prior and mean age of 8.9 Ma (standard deviation = 2 Ma) for the divergence of Caprini (Bibi, 2013; Pérez et al., 2022), and 1.93 Ma (standard deviation = 0.43 Ma) for the *Rupicapra* node (Pérez et al., 2014a, 2022). Chain convergence was assessed with Tracer v.1.7.2 (Rambaut et al., 2017). The Maximum Clade Credibility Tree was obtained using TreeAnnotator v.1.10.4 (Drummond & Rambaut, 2007) by discarding the first 10% of iterations as burn-in and with mean node heights. The resulting phylogenetic tree was visualized with FigTree v.1.4.4 (Rambaut, 2018).

Phylogenetic network. From the alignment of the complete mtDNA sequences six samples were removed because they contained more than 10% of missing data; in the remaining sequences all ambiguities/missing data were removed. In addition to this, four samples were removed from the dataset because they were of uncertain origin. The final dataset was composed of 101 samples, 52 historical samples and 49 modern samples.

For the Cyt b – D-Loop database, the same nine samples were removed for the reasons discussed above. The final dataset was composed of 168 samples, 52 historical and 116 modern. In addition, in 26 samples identified as *R. r. ornata* and three samples of *R. r. pyrenaica* containing an indel of 41 bp starting (position 739 to 780), the indel was removed from all sequences.

To investigate the relationships between haplotypes, a median-joining network was constructed for both the complete mtDNA and the Cyt b – D-Loop databases using PopArt v.1.7 (Leigh & Bryant, 2015).

Genetic Analysis. For both databases, I identified four geographic regions for each of the two groups, historical and modern samples, for a total of eight populations distributed among the Western Alps, Eastern Alps, Apennines, and Pyrenees. The distinction between Western and Eastern Alps was defined by the SOIUSA classification (Marazzi, 2005).

In order to investigate changes in genetic diversity between historical and modern populations, we used Arlequin (Excoffier & Lischer, 2010) to calculate gene diversity (He), nucleotide diversity (π), number of haplotypes (K), number of polymorphic sites (S), Theta S (θs) and Theta π (θπ) for the modern and historical samples overall and within the four mountain areas. For both datasets the analysis was repeated twice: once including the samples collected in the Western and Eastern Alps that clustered with *R. p. pyrenaica* and *R. p. ornata*, and once excluding these samples. In addition, allelic richness was calculated using the software HP-RARE 1.0 which uses the rarefaction method in order to compensate for different sample sizes (Kalinowski, 2004, 2005). More specifically, we selected samples that originated from approximately the same geographical region as shown in Figure 2.2 for the complete mtDNA sequence and Figure 2.3 for the Cyt b – D-Loop database.

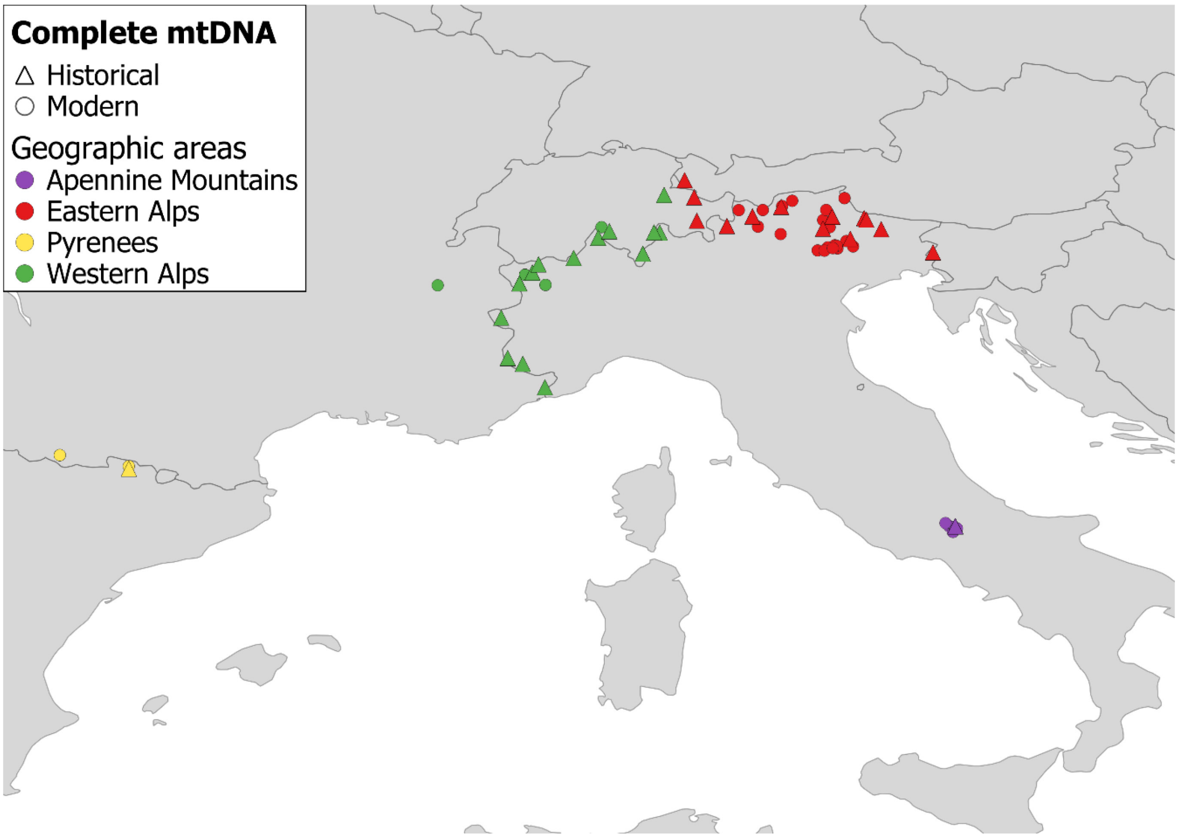

Figure 2.2 Sample distribution of historical and modern samples selected for the genetic analysis of the complete mtDNA.

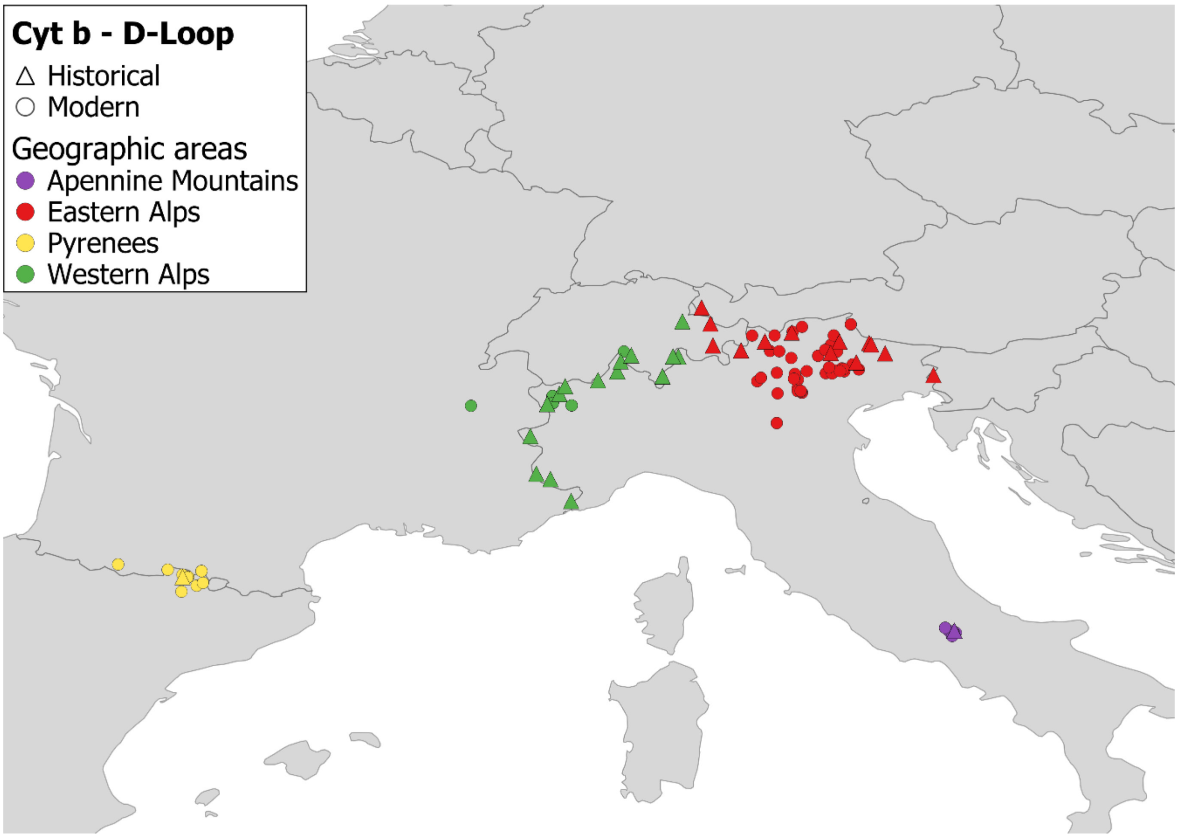

Figure 2.3 Sample distribution of historical and modern samples selected for the genetic analysis of Cyt b – D-Loop.

2.3 RESULTS

We successfully sequenced 61 out of 66 historical samples and 36 modern samples. Five historical samples were removed from downstream analysis because of low coverage. The final alignment length of the complete mtDNA sequence was 15,113 bp (N = 111), while the cytb-dloop database resulted in an alignment length of 1,604 bp (N = 177).

BAYESIAN MITOCHONDRIAL PHYLOGENY

The Effective Sample Size (ESS), an indicator of effective independent draws from the posterior distribution resulted in values higher than 200 for all the parameters considered, which is the recommended value to ensure chain convergence of the Bayesian analysis (Drummond & Rambaut, 2007).

The Bayesian analysis of both the complete mtDNA (Figure 2.5) and Cyt b-D-Loop (Figure 2.6) sequences showed a clear partition into three main genetic groups, consistently with what was found in previous studies (Rodríguez et al., 2009, 2010): Clade West (mtW), Clade Central (mtC) and Clade East (mtE), with mtW and mtC more closely related to each other than they are to mtE. The distribution of the mitochondrial clades as proposed by Rodriguez et al. (2010), is shown in Figure 2.4.

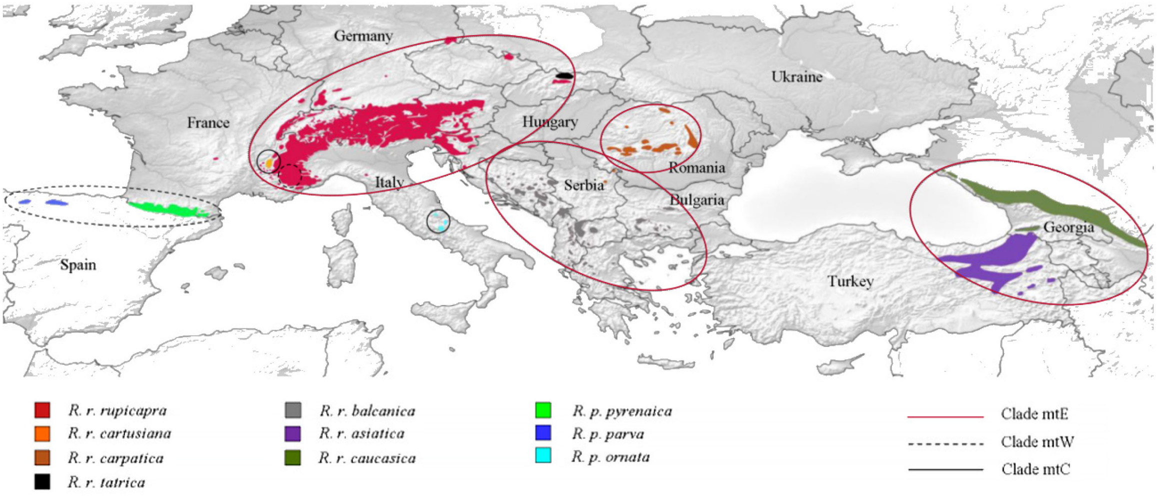

Figure 2.4 Maps showing the mitochondrial clades of the extant species of R. rupicapra and R. pyrenaica as proposed by (Rodríguez et al., 2010) modified from (Fioravanti et al., 2019)

More specifically, Clade mtW consisted of chamois from the Pyrenees and the Western Alps (corresponding to *R. p. pyrenaica,* and some individuals from the Western Alps in the *R. r. rupicapra* range); Clade mtC was observed only in individuals from the Apennines and the Chartreuse Massif (i.e. *R. p. ornata* and *R.*

r. cartusiana), and Clade mtE was represented by haplotypes found in individuals from across the Alps and central Europe, the Balkans, Carpathian Mountains, and the Caucasus (in the ranges of *R. r. rupicapra*, *R. r. balcanica*, *R. r. carpatica* and *R. r. caucaisca*).

Clade mtW (Figure 2.5 A) included historical (yellow triangles) and modern (yellow circles) haplotypes from the Pyrenees and historical (green triangle) and modern (green circle) haplotypes from the Western Alps in the complete mtDNA dataset and also one modern individual from the Eastern Alps (red circle) in the case of the larger dataset of concatenated Cyt b – D-Loop sequences (Figure 2.6 A).

Clade mtC (Figure 2.5 B and 2.6 B) was shared by historical (purple triangles) and modern haplotypes (purple circles) from Apennine Mountains and the modern haplotype from the Massif of Chartreuse (dark green circle), in accordance with what already found in other studies (Iacolina et al., 2021; Pérez et al., 2014; Rodríguez et al., 2009, 2010).

Clade mtE (Figures 2.5 C, D, E) showed a clear geographic division in Western (D) and Eastern Alps (E). Within this clade, the historical Western Alps group included one haplotype from the Caucasus (blue triangle) and one from the Carpathian Mountains (brown triangle), while modern haplotype only included haplotypes from the Western Alps (green circles). The historical Eastern Alps group included seven haplotypes from the Western Alps (green triangles), three from the Caucasus (blue triangles) and two from the Balkans (pink triangle), while modern samples that grouped with Eastern Alps included haplotypes from this geographic area and one haplotype from the Balkan (pink circle).

In addition, within this clade (C), two modern haplotypes from the Balkans (pink circles) with two modern haplotypes from the Eastern Alps (red circles), formed a separate group with three historical haplotypes from the Caucasus (blue triangles), one historical haplotype from the Eastern Alps (red triangle) and one modern haplotype from the Tatra Mountains (teal circle).

The Bayesian tree constructed with the concatenated Cyt b – D-Loop haplotypes (Figure 2.6), showed the same division into three main clades (mtW, mtC and mtE) and a similar geographic distribution in the Eastern (E) and Western Alps (D) within the Clade mtE. In addition, as for the complete mtDNA sequence a separate group can be observed (C), but in this case three historical haplotypes from the Caucasus (blue triangle) with one haplotype from the Tatra Mountains (teal circle) grouped with four modern individuals from the Eastern Alps (red circles) and four modern individuals from the Tatra Mountains (teal circles). One haplotype was found to be shared between historical and modern samples within the Clade mtC (black arrow).

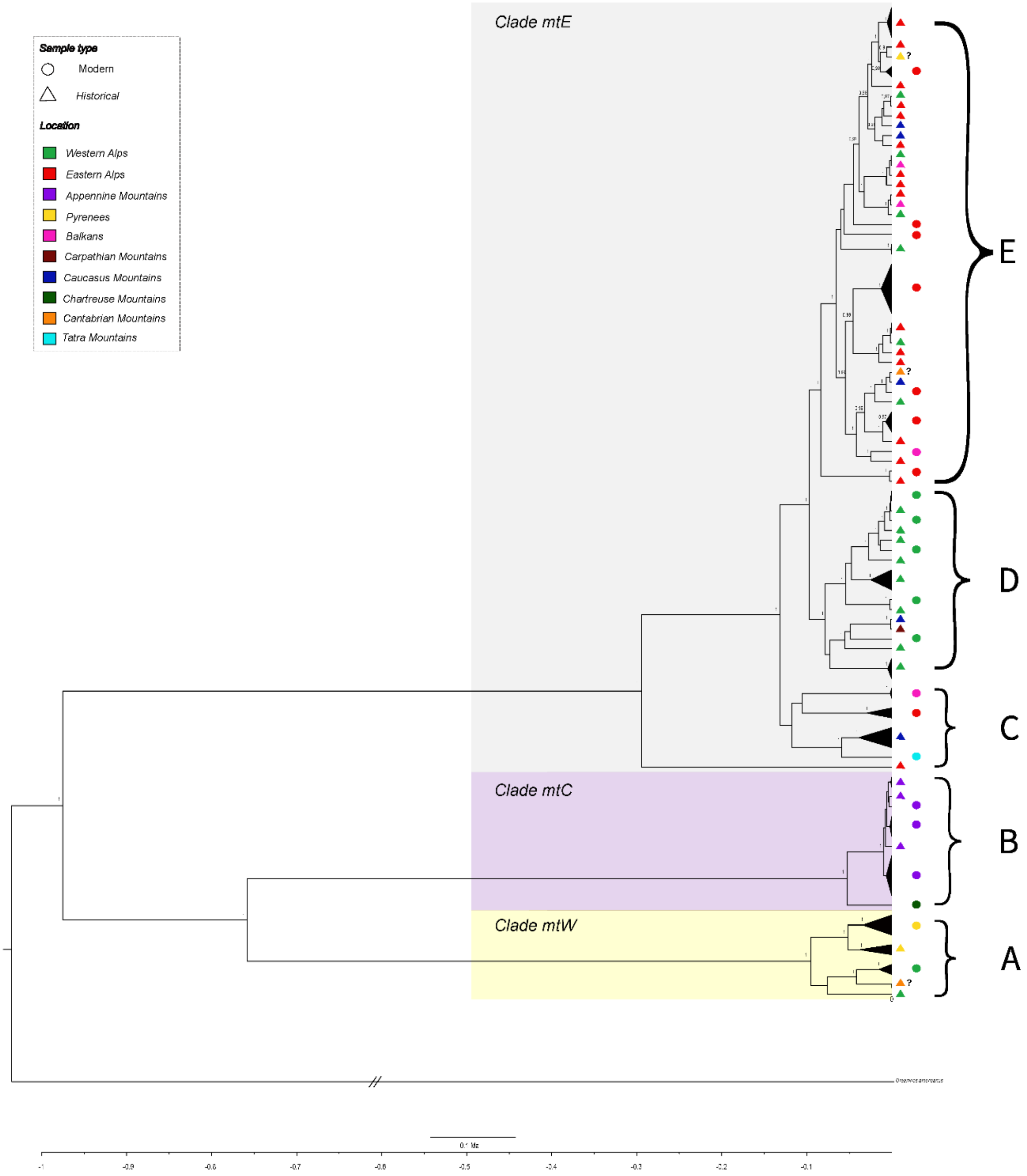

Figure 2.5 The Bayesian tree obtained with BEAST includes complete mtDNA sequences from historical and modern samples of chamois. The tree was rooted with Oreamnos americanus. The Clades mtE, mtC and mtW are indicated. Samples indicated with (?) are of unknown origin. A correspond to the Clade mtW, B to the clade mtC and C, D, E to the clade mtE.

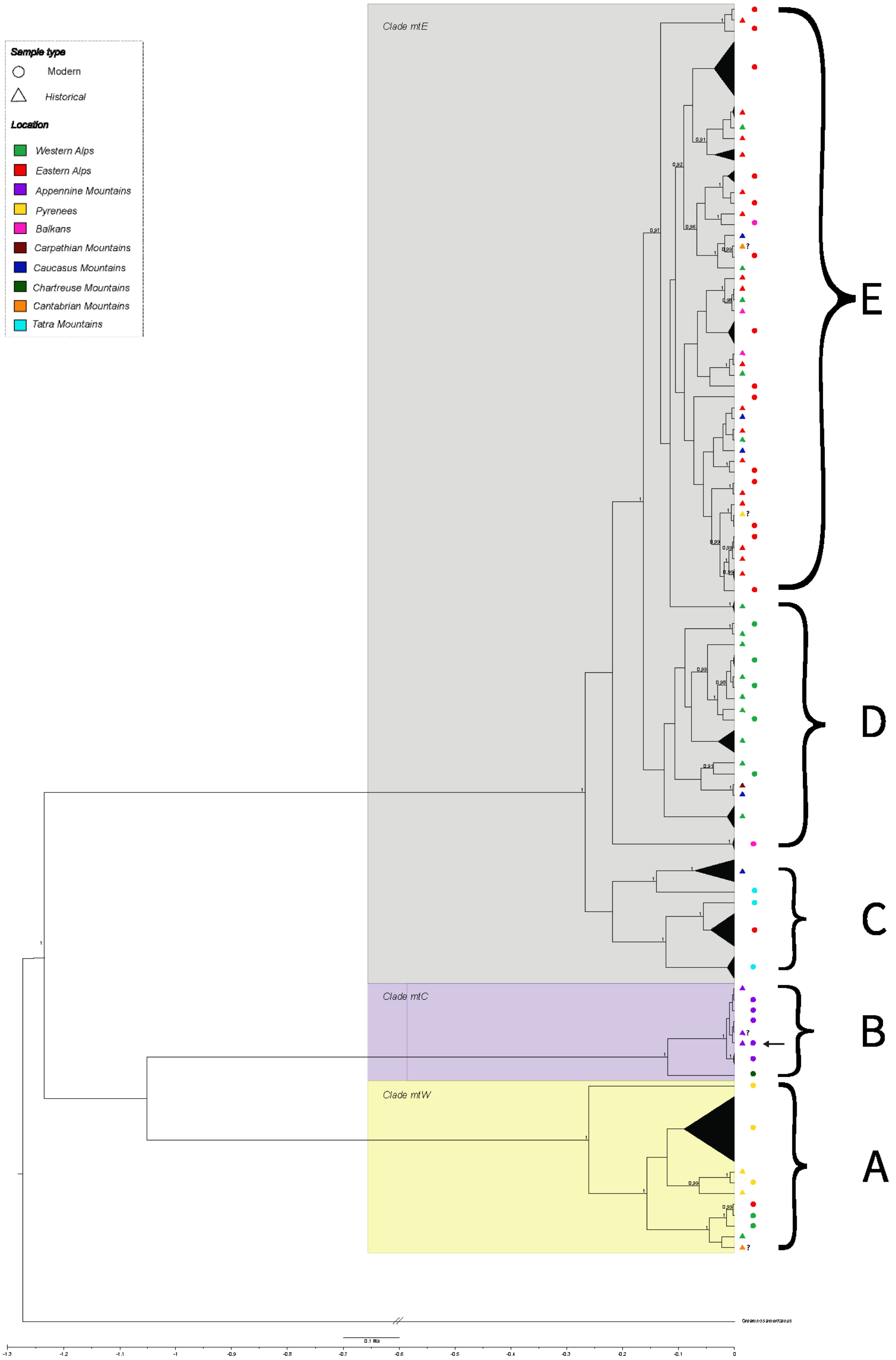

Figure 2.6 The Bayesian tree obtained with BEAST includes the concatenated Cyt b – D-Loop sequences from historical and modern samples of chamois. The tree was rooted with Oreamnos americanus. The Clades mtE, mtC and mtW are indicated. Samples indicated with (?) are of unknown origin. A correspond to the Clade mtW, B to the clade mtC and C, D, E to the clade mtE.

PHYLOGENETIC NETWORK

The haplotype networks for the complete mtDNA (Figure 2.7) and the Cyt b – D-Loop sequences database (Figure 2.10), confirmed the presence of three main clades and the geographical distribution of the haplotypes.

For the complete mtDNA sequence, Clade mtW was represented by five haplotypes from the Pyrenees and three haplotypes from the Western Alps (Figure 2.7). Haplotypes shared between historical and modern samples were found in the Western Alps (green arrow), Eastern Alps (red arrow) and Apennine Mountains (purple arrow). The Clade mtC was represented by seven haplotypes from the Apennine Mountains and one haplotype from the Massif of Chartreuse. The Clade mtE was the more diverse and was represented by 55 haplotypes.

Almost all haplotypes were limited to a single geographic region, except for one haplotype shared between Western Alps and Balkans (1), one haplotype shared between Western Alps, Eastern Alps, and Balkans (2), and one haplotype shared between Eastern Alps and Western Alps (3) within the Eastern Alps group. In the Western Alps one haplotype was shared between the Carpathian Mountains and the Caucasus Mountains (4).

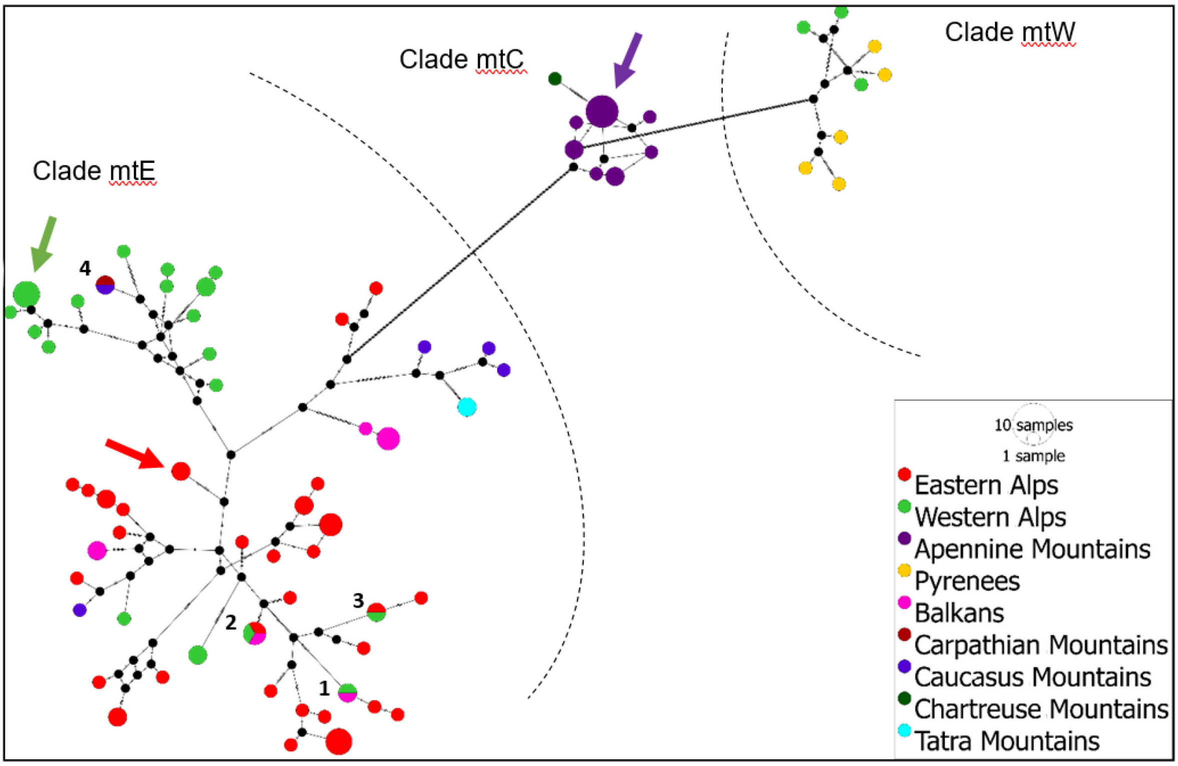

Figure 2.7 Median-joining network of the complete mtDNA sequences. Each circle depicts a unique haplotype, and the size of the circle is proportional to the number of individuals showing that haplotype. Branch lengths are not scaled. The red, green and purple arrows indicate shared haplotypes between historical and modern samples. Numbers refer to haplotypes shared between geographic areas.

In order to investigate how the haplotype distribution changed over time, a separated haplotype network for historical (Figure 2.8) and modern (Figure 2.9) samples that cover approximately the same geographic distribution is shown. Both historical and modern haplotypes confirmed a genetic separation between the Western and Eastern Alps.

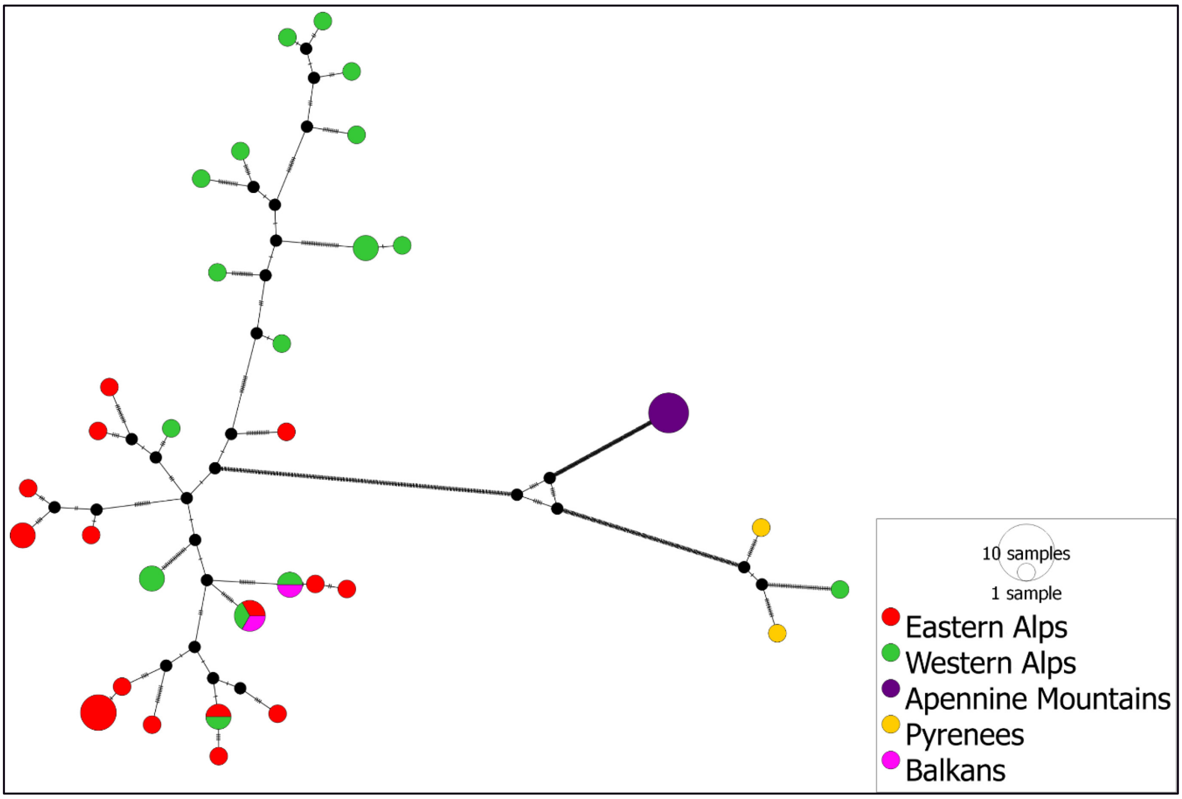

Figure 2.8 Median-joining network of the historical complete mtDNA sequences. Each circle depicts a unique haplotype, and the size of the circle is proportional to the number of individuals showing that haplotype. Branch lengths are not scaled.

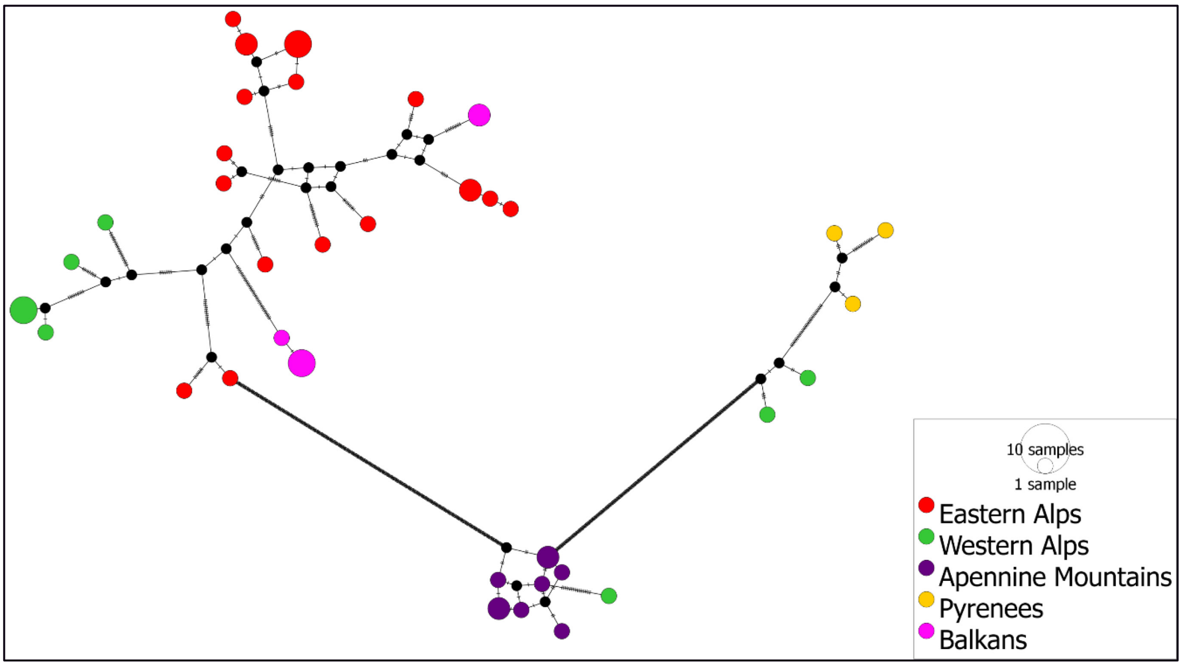

Figure 2.9 Median-joining network of the modern complete mtDNA sequences. Each circle depicts a unique haplotype, and the size of the circle is proportional to the number of individuals showing that haplotype. Branch lengths are not scaled.

In the Cyt b -D-Loop database (Figure 2.10), Clade mtW was represented by ten haplotypes from the Pyrenees and one haplotype from the Eastern and Western Alps; Clade mtC by two haplotypes from the Apennine Mountains and one haplotype from the Massif of Chartreuse; and Clade mtE was again the most diverse with 45 haplotypes. Haplotypes shared between historical and modern samples were found in the Western Alps (green arrow), Eastern Alps (red arrow) and Apennine Mountains (purple arrow).

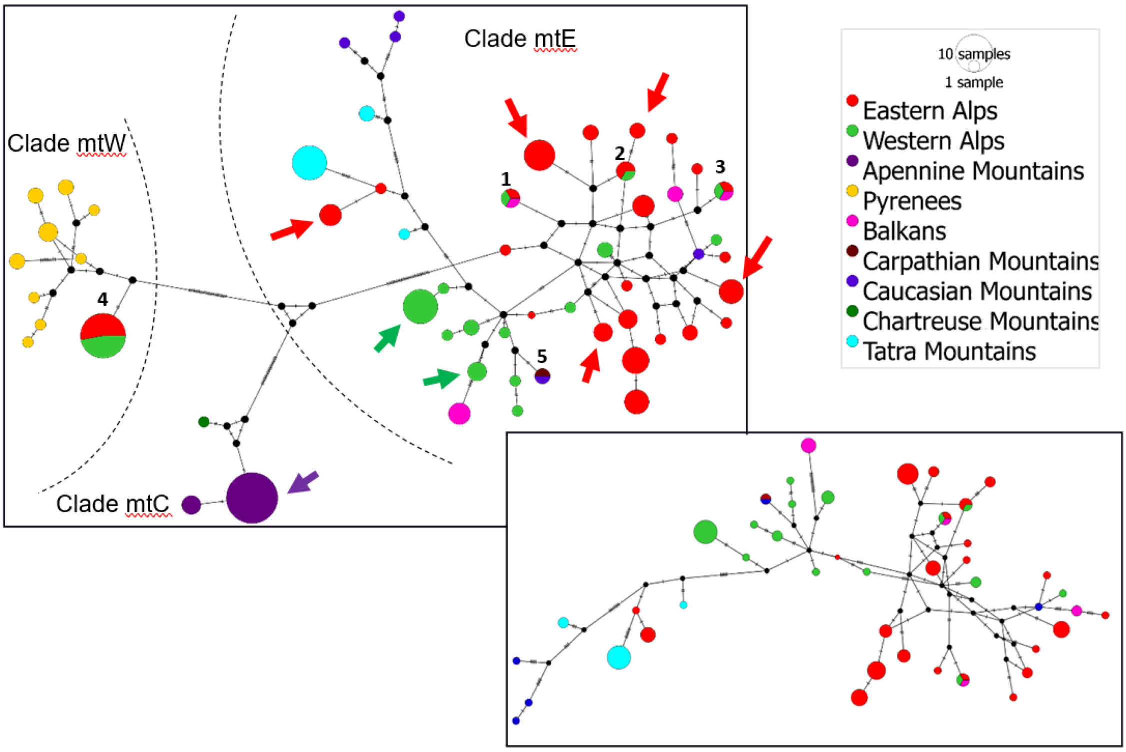

Figure 2.10 Median-joining network of the cytb-dloop sequences. Each circle depicts a unique haplotype, and the size of the circle is proportional to the number of individuals showing that haplotype. Branch lengths are not scaled. On the right a detail of the haplotype network of only R. rupicapra haplotypes only. The red, green and purple arrows indicate shared haplotypes between historical and modern samples. Numbers refere to haplotypes shared between geographic areas.

GENETIC VARIATION ANALYSIS

Since previous analysis indicated that some historical and modern haplotypes collected in the Alps clustered with *R. p. pyrenaica,* and one haplotype from the Massif of Chartreuse clustered with *R. p. ornata*, for both the Bayesian analysis (Figures 2.5 and 2.6) and the haplotype network (Figures 2.7 and 2.10), genetic analyses were performed first including these samples in the dataset (Table 2.1 for the complete mtDNA and Table 2.3 for the Cyt b – D-Loop), and then excluding them (Table 2.2 for the complete mtDNA and Table 2.4 for the Cyt b – D-Loop). In addition, considering the strong genetic structuring of chamois populations even on a microgeographic scale, the average geographical distance between samples within each population was calculated.

Complete mtDNA

The analysis of all samples from the Western Alps samples (including those that clustered with *R. p. pyrenaica*: two modern and one historical samples,and R. p. *ornata* one modern sample; Table 2.1), showed that the modern samples have higher levels of nucleotide diversity (π (%) = 1.68) with respect to historical samples (π (%) = 0.51), as well as higher values of Theta S (modern = 237.2, historical = 154.4), Theta π (modern = 247.3, historical = 75), and number of polymorphic sites (modern S = 645, historical S = 531). However, if the allelic richness (Ar) is adjusted for unequal sample size, levels of diversity were lower than historical ones (modern Ar = 5.14, historical Ar = 6.79).

Table 2.1 Diversity indices for the complete mtDNA sequence including the samples from the Western Alps carrying R. p. pyrenaica and R. p. ornata haplotypes. Number of samples (N). Genetic diversity indices: number of haplotypes (K), allelic richness (Ar), number of polymorphic loci (S), nucleotide diversity (π), gene diversity (h), Theta s (θs), Theta π (θπ), average geographic distance between samples (μGD).

Geographic Area	N	K	S	Ar	π %(SD)	h (SD)	θs (SD)	θπ	μGD (km)
Western Alps Modern	9	7	645	5.14	1.68 (0.9)	0.92 (0.09)	237.3 (98.6)	247.3 (132.9)	36.7
Western Alps Historical	18	16	531	6.79	0.51 (0.26)	0.99 (0.02)	154.4 (53.5)	75 (37.9)	104.7
Eastern Alps Modern	20	16	106	7.20	0.14 (0.07)	0.97 (0.03)	29.9 (10.4)	21.1 (10.8)	52.5
Eastern Alps Historical	19	15	92	6.54	0.12 (0.06)	0.96 (0.04)	26.3 (9.3)	18.3 (9.5)	94.4
Apennine Mountains Modern	9	7	7	3.49	0.02 (0.01)	0.94 (0.07)	2.56 (1.4)	3.1 (2.0)	7.8
Apennine Mountains Historical	5	1	0	0.51	n/a	n/a	n/a	n/a	0
Pyrenees Modern	3	3	21	*	0.09 (0.07)	1 (0.27)	14 (8.7)	14 (10.9)	45.2
Pyrenees Historical	2	2	22	*	0.0015 ± 0.0015	1 ± 0.5	22 ± 15.9	22 (22.5)	84.7

When *R. p. pyrenaica* and *ornata* samples were removed from the dataset (Table 2.2), the number of polymorphic sites and the nucleotide diversity in the modern Western Alps samples decreased notably, from 645 to 49 polymorphic sites, and from a nucleotide diversity (%) of 1.68 to 0.13 (Table 2.1). Historical sample diversity also decreased from 531 to 124 polymorphic sites, with nucleotide diversity (%) falling from 0.51 to 0.19 (Table 2.2).

Overall, modern samples from the Eastern Alps showed the highest levels of genetic variability, followed by the Western Alps, the Pyrenees, and the Apennines. Within historical samples, individuals from the Western Alps showed the highest levels of genetic diversity, followed by the Eastern Alps, the Pyrenees and the Apennines.

The historical samples from the Western Alps (Table 2.2) showed a higher variability with respect to modern samples (modern π (%) = 0.13, historical π (%) =

0.19); this result was confirmed even after allelic richness was adjusted for unequal sample sizes (modern Ar = 2.99, historical Ar = 4.84). However, historical samples from these areas showed a higher geographical dispersion with respect to modern samples. The modern samples from the Eastern Alps showed higher levels of genetic variability (π (%) = 0.14) and allelic richness (Ar = 5.20) compared to the historical population (π (%) = 0.12, Ar = 4.85). In the Apennines, modern samples clearly presented higher levels of genetic variability with respect to the historical samples, which only presented one haplotype probably because they were collected from the same geographical nucleus. Given the small sample size, measures of allelic richness were not calculated for the samples from the Pyrenees, but genetic diversity indices show a higher variability in historical samples (modern π (%) =0.09, historical π (%) = 0.15).

Table 2.2 Diversity indices for the complete mtDNA sequence excluding the samples from the Western Alps carrying R. p. pyrenaica and R. p. ornata haplotypes. Number of samples (N). Genetic diversity indices: number of haplotypes (K), allelic richness (Ar), number of polymorphic loci (S), nucleotide diversity (π), gene diversity (h), Theta s (θs), Theta π (θπ), average geographic distance between samples (μGD).

Geographic area	**N**	**K**	**S**	**Ar**	**π % (SD)**	**h (SD)**	**θs (SD)**	**θπ (SD)**	**μGD (km)**
Western Alps Modern	6	4	49	2.99	0.13 (0.08)	0.8 (0.17)	21.5 (10.4)	19.1 (11.4)	30.9
Western Alps Historical	17	15	124	4.84	0.19 (0.1)	0.99 (0.03)	36.7 (13.2)	29.3 (15.1)	120.1
Eastern Alps Modern	20	16	106	5.20	0.14 (0.07)	0.97 (0.03)	29.9 (10.4)	21.1 (10.8)	52.5
Eastern Alps Historical	19	15	92	4.85	0.12 (0.06)	0.96 (0.04)	26.3 (9.3)	18.4 (9.52)	94.4
Apennine Mountains Modern	9	7	7	3.49	0.02 (0.01)	0.94 (0.07)	2.58 (1.39)	3.1 (1.99)	7.8
Apennine Mountains Historical	5	1	0	0.51	n/a	n/a	n/a	n/a	0
Pyrenees Modern	3	3	21	*	0.09 (0.07)	1 (0.27)	14 (8.72)	14 (10.87)	45.2
Pyrenees Hsitorical	2	2	22	*	0.15 (0.15)	1 (0.5)	22 (15.9)	22 (22.49)	84.7

Cyt b – D-Loop

When repeating the analysis for the Cytb – D-Loop sequences with additional samples and including the samples that cluster with *R. p. pyrenaica* and *ornata* (Table 2.3), results showed that the modern samples of the Western Alps have a higher number of polymorphic sites and a higher levels of nucleotide diversity (S = 145, π (%) = 3.72) with respect to the historical samples (S = 60, π (%) = 0.87) , as well as higher values of Theta S (modern = 41.49, historical = 17.17) and Theta π (modern = 55.63, historical = 9.99), while allelic richness was higher in the historical samples (modern Ar = 3.32, historical Ar = 9.73), confirming previous results obtained with the complete mtDNA sequence (Tables 2.1 and 2.2). As expected, the same was observed in the Eastern Alps samples, since it also included samples that cluster with *pyrenaica*.

Table 2.3 Diversity indices for the Cyt b – D-Loop sequence including the samples from the Western and Eastern Alps with R. p. pyrenaica and R. p. ornata haplotypes. Number of samples (N). Genetic diversity indices: number of haplotypes (K), allelic richness (Ar), number of polymorphic loci (S), nucleotide diversity (π), gene diversity (h), Theta s (θs), Theta π (θπ), average geographic distance between samples (μGD).

Geographic area	**N**	**K**	**S**	**Ar**	**π % (SD)**	**h (SD)**	**θs (SD)**	**θπ (SD)**	**μGD (km)**
Western Alps Modern	19	7	145	3.32	3.72 (0.0188)	0.78 (0.07)	41.49 (14.45)	55.63 (28.12)	36.5
Western Alps Historical	19	16	60	9.73	0.87 (0.47)	0.98 (0.02)	17.17 (6.19)	9.99 (5.34)	103.7
Eastern Alps Modern	46	23	141	9.28	2.77 (1.36)	0.94 (0.02)	32.08 (9.3)	41.95 (20.58)	60.2
Eastern Alps Historical	19	15	39	9.17	0.62 (0.34)	0.98 (0.02)	11.16 (4.14)	9.02 (4.86)	94.4
Apennine Mountains Modern	20	2	1	0.58	0.02 (0.02)	0.27 (0.11)	0.28 (0.28)	0.27 (0.34)	7.4
Apennine Mountains Historical	5	1	0	0	n/a	n/a	n/a	n/a	0
Pyrenees Modern	11	7	43	*	0.96 (0.53)	0.91 (0.07)	14.68 (6.1)	15.16 (8.29)	92.4
Pyrenees Historical	2	2	9	*	0.6 (0.63)	1 (0.5)	9 (6.71)	9 (9.49)	84.7

When the samples that cluster with *pyrenaica* and *ornata* were removed from the dataset (Table 2.4), as expected, the number of polymorphic sites and the nucleotide diversity clearly decreased: in the Western Alps the number of polymorphic sites decreased from 145 to 24 and the nucleotide diversity (%) from 3.72 to 0.13 in modern samples, while in historical samples the number of polymorphic sites decreased from 60 to 27 and the nucleotide diversity (%) from 0.87 to 0.54. In the modern samples from the Eastern Alps the number of polymorphic sites decreased from 141 to 71 and the nucleotide diversity (%) from 2.77 to 1.07.

Overall, modern samples from the Eastern Alps showed the highest levels of genetic variability, followed by the Western Alps, the Pyrenees, and the Apennines, confirming previous results obtained with the complete mtDNA sequence (Table 2.2). Within historical samples, individuals from the Eastern Alps showed the highest levels of genetic diversity, followed by the Pyrenees, the Western Alps and the Apennines.

The historical samples from the Western Alps showed slightly lower levels of nucleotide diversity (π (%) = 0.54) with respect to the modern samples (π (%) = 0.58). However, it showed a higher number of haplotypes (modern K = 4, historical K = 15) and a higher level of allelic richness (modern Ar = 2.27, historical Ar = 6.27), confirming previous results obtained with the complete mtDNA sequence (Table

2.2). The modern samples of the Eastern Alps showed higher levels of genetic variability (π (%) = 1.07) with respect to the historical samples (π (%) = 0.62), but it also showed higher geographical dispersion. In the Apennines, modern samples clearly present higher levels of genetic variability (π (%) = 0.58) with respect to the historical samples (π% = 0), but, as for the complete mtDNA sequence, the latter only present one haplotype within five samples.

Table 2.1 Diversity indices for the Cyt b – D-Loop sequence excluding the samples with R. p. pyrenaica and R. p. ornata haplotypes. Number of samples (N). Genetic diversity indices: number of haplotypes (K), allelic richness (Ar), number of polymorphic loci (S), nucleotide diversity (π), gene diversity (h), Theta s (θs), Theta π (θπ), average geographic distance between samples (μGD).

Geographic area	**N**	**K**	**S**	**Ar**	**π% (SD)**	**h (SD)**	**θs (SD)**	**θπ (SD)**	**μGD (km)**
Western Alps Modern	10	4	24	2.27	0.58 (0.33)	0.64 (0.15)	8.48 (3.76)	9.11 (5.18)	23.1
Western Alps Historical	18	15	27	6.27	0.54 (0.30)	0.98 (0.02)	7.85 (3.05)	7.22 (3.97)	114.2
Eastern Alps Modern	37	22	71	7.29	1.07 (0.54)	0.96 (0.02)	17.01 (5.32)	16.78 (8.49)	102.5
Eastern Alps Historical	19	15	39	6.66	0.62 (0.34)	0.98 (0.02)	11.16 (4.14)	9.02 (4.86)	94.4
Apennine Mountains Modern	20	2	1	0.58	0.02 (0.02)	0.27 (0.11)	0.28 (0.28)	0.27 (0.34)	7.4
Apennine Mountains Historical	5	1	0	0	n/a	n/a	n/a	n/a	0
Pyrenees Modern	11	7	43	*	0.96 (0.53)	0.91 (0.07)	14.68 (6.1)	15.16 (8.29)	92.4
Pyrenees Historical	2	2	9	*	0.6 (0.63)	1 (0.5)	9 (6.71)	9 (9.49)	84.7

2.4 DISCUSSION

In this study, I aimed to identify possible differences in the genetic variation and structure between modern and historical chamois samples. The historical samples dated between 1900 and 1915 and can be therefore used as a reference of chamois populations before the major demographic reductions and translocation interventions occurred in the last century. I obtained the complete sequences mtDNA genomes for 36 modern and 61 ancient individuals, and I discuss below the main results in three sections: phylogenetic and phylogeographic patterns, genetic variation levels, and implications for conservation.

Phylogenetic and phylogeographic patterns

The mitochondrial phylogeny (Figure 2.5 and 2.6) and the haplotype network analysis (Figure 2.7 and 2.10) showed the separation into three main groups (mtW, mtC, mtE), consistent with previous studies (Crestanello et al., 2009; Iacolina et al., 2021; Rodríguez et al., 2009, 2010). The ancient samples do not modify the phylogenetic structure inferred using only modern data.

The clade mtW is typical from the Pyrenees but was also found in modern and ancient samples collected in the Western and Eastern Alps. The presence of mtDNA haplotypes from the Alps that falls within the *R. p. pyrenaica* cluster was already documented by Crestanello et al. (2009), who found the *pyrenaica* haplotype in several individuals from Caldonazzo and Velo in the Eastern Alps, and in Gran Paradiso National Park in the Western Alps, and by Rodríguez et al. (2009), who found two additional *pyrenaica* Cyt b haplotypes in Val di Susa in the Western Alps. Crestanello et al. (2009) explained the patchy presence of *R. pyrenaica* haplotypes in the Alpine populations as a consequence of undocumented human-mediated migrations. In fact, the Gran Paradiso National Park was a royal hunting reserve from 1856 to 1922, and animal exchange between kingdoms for hunting purposes were common. Crestanello et al. (2009) therefore suggested that *pyrenaica* genomes in the Western Alps arrived through chamois females donated to Italian king more than one hundred years ago. The presence of such haplotypes in the Eastern Alps could then be a consequence of later documented translocations from the Western to the Eastern Alps in the early 1970s (Toniolo L., personal communication from Crestanello et al., 2009).

Rodríguez et al. (2010) had a different explanation to justify the presence of *pyrenaica* haplotypes in the Alps. Based mainly on the STR markers that do not show *pyrenaica* genotypes in the Alps, they suggest an ancient hybridization among differentiated lineages in the central area of the chamois distribution. The Royal Hunting Reserve in the Western Alps was established in a period prior to the sampling of our historical samples that cluster with *pyrenaica*, meaning that our historical samples cannot discriminate among the two hypotheses. Only the presence of *pyrenaica* haplotypes in Alpine sample predating the likely game species exchanges among kings could support the idea of an ancient and natural haplotype sharing among chamois species from the Alps and the Pyrenees. However, this latter hypothesis was already excluded by genetic simulations in Crestanello et al. (2009).

The Clade mtC included individuals from the Apennine Mountains and the individual already analyzed by Pérez et al. (2014) from the Chartreuse Mountains. The topology of our phylogenetic tree showed the clustering of the haplotype from the Chartreuse Mountains with historical and modern haplotypes from the Apennines, in agreement with previous findings (Iacolina et al., 2021; Pérez et al., 2014; Rodríguez et al., 2009, 2010). However, in our phylogenetic tree the individual from the Chartreuse Mountain forms a well separated branch with respect to the individuals from the Apennine Mountains. Pérez et al. (2014), suggested that the split between *R. p. ornata* and *R. r. cartusiana* occurred during the Pleistocene and explained the mitochondrial similarity between these two groups (classified as two different species based on nuclear markers) as a result of a male migration from Eastern Alps into a small resident Chartreuse population with *ornata*-like mtDNA. Unfortunately, our data cannot clarify this point and the suggested pre-introgression similarity between Apennine and Chartreuse chamois, and more recent translocation events cannot be excluded.

Within the Eastern clade mtE, a genetic separation between Western and Eastern Alps clearly emerges from both the phylogenetic tree (Figure 2.5 and 2.6) and the haplotype network (Figures 2.7 and 2.10). The same topology can be observed in the distribution of both historical (Figure 2.8) and modern haplotypes (Figure 2.9), indicating that recent human interventions did not modify the major feature of this phylogeographic pattern. The geographic structure of genetic variation is not unexpected considering the ecological needs of this species. Indeed, even single populations living in mountain chains separated by valley and rivers are genetically divergent (Crestanello et al., 2009), and we can also see here that haplotype sharing is very limited across the chamois distribution range (Figures 2.7, 2.8, 2.9, 2.10). This pattern was clearly reinforced by the anthropization of the valleys, and global warming reducing mountain peaks habitats will probably further contribute.

Habitat fragmentation and landscape features were already found to be possible drivers of the current genetic structuring in chamois populations by Soglia et al., (2010), who found the Adige valley, the Adda and the Ticino River to be the main topographical barrier to gene flow among the studied chamois populations. In another interesting study, Leugger et al. (2022) examined how habitat and dispersal may have influenced the current genetic structure of chamois populations. Using genomic data (>20 k SNPs) from 449 chamois and a habitat suitability model, they identified two main clades: one in the Eastern part of the Alps and one in the south-western part of the Alps. Additionally, by combining genetic data with topographic and environmental variables, they simulated a model to reconstruct chamois dispersal up to the last glaciation and observed that the Adige Valley, being the last to be colonized, may have caused a greater separation between chamois in the East and West of the valley. The macro-structure between Eastern and Western Alps, was here defined on the basis of the SOIUSA partition system, which consider several aspects including the geology and the phytogeography of the areas (Marazzi, 2005). However, within the Clade mtE, five historical samples, which were located in the Western Alps according to the geographical subdivision of the Alps by SOIUSA, actually clustered within the group of populations from the Eastern Alps. The samples are situated in the eastern part of the Ticino River basin, indicating that the river might serve as a significant impediment to gene flow. This finding supports the conclusions drawn by Soglia et al. (2010) and underscores the importance of the river as a geographic marker for identifying macro-units in chamois management.

Genetic variation levels

The comparison of historical and modern genetic variability may reveal the possible impact of past translocation events on the current levels of genetic diversity. The modern population from the Eastern Alps showed a higher variability and allelic richness with respect to the past for both the complete mtDNA (Table 2.2) (modern π (%) = 0.14, modern Ar = 5.20, historical π (%) = 0.12, historical Ar = 4.85) and especially when the larger Cytb – D-Loop database was analyzed after the removal of samples that falls within the *Pyrenaica* cluster (Table 2.4) (modern π% = 1.07, modern Ar = 7.29, historical π% = 0.62, historical Ar = 6.66). This result cannot be explained by a simple effect of geographic distribution of samples, since historical samples had a wider geographical distribution (average geographic distance: 94.4 km) with respect to modern samples (average geographic distance: 52.2 km). Given that studies on chamois mtDNA have shown a limited gene flow even on a microgeographic scale and a sub-structuring into regional phylogroups (Crestanello et al., 2009; Rodríguez et al., 2010), it is plausible that the higher variability in modern samples is the result of multiple reintroductions and restocking events that occurred after a severe bottleneck event between the two World Wars. Specifically, these reintroductions and restocking events involved animals from different mountain ranges, such as the Gran Paradiso National Park and the Alpi Marittime Natural Park in the Western Alps, which were used as common source for reintroduction and restocking (Crestanello et al., 2009). The translocations of individuals from the Western Alps to the Eastern Alps may have played a role in counterbalancing the observed differences in genetic diversity values between the two areas. Indeed, historical samples from the Western Alps had greater variability than samples from the Eastern Alps, whereas modern samples from the two areas exhibit very similar levels of genetic diversity.

The analysis of samples collected in the Western Alps without the anomalous samples clustering with *R. pyrenaica*, showed a higher variability of historical samples compared to modern samples for both the complete mtDNA (modern Ar = 2.99, historical Ar = 4.84) and the larger Cyt b – D-Loop dataset (modern Ar = 2.27, historical Ar = 6.27). However, this difference may be due to the greater average geographic distance between historical samples (120.1 km) with respect to modern samples (30.9), and not to a loss of variation though time. To improve our understanding of changes in genetic variation over time, it would be useful to collect additional samples with a more uniform geographic distribution and a balanced representation of both modern and historical specimens.

Results obtained for the samples of *R. pyrenaica* from the Pyrenees and the Apennine Mountains do not allow to make consistent considerations on the relationship between ancient and modern variation, due to the very small sample size of the samples from the Pyrenees and to the fact that the five historical *ornata* samples share the same haplotype and all were sampled in the same population. However, when analyzing the complete mtDNA sequence, the *ornata* subspecies showed extremely low levels of nucleotide diversity in modern samples. The species

R. pyrenaica is considered as Least Concern by the IUCN, since its status progressively improved starting from 1990 (Herrero, Lovari, Nores, et al., 2020). However, the *ornata* subspecies, endemic to Italy, is considered as Vulnerable due to the small population size and its reduced range (Herrero, Lovari, Nores, et al., 2020; Lovari et al., 2014). The extremely low levels of genetic diversity observed in these samples can thus be attributed to their reduced population size as a consequence of past events of isolations and bottleneck (Fioravanti et al., 2019; Herrero, Lovari, Nores, et al., 2020; Rodríguez et al., 2010). Although, thanks to a strict protection and to the numerous reintroduction programs, the population is increasing (Herrero, Lovari, Nores, et al., 2020), the low genetic variation observed in this study, highlight the importance of an ongoing monitoring and protection of this subspecies.

Implication for conservation

Even if a simple comparison is not possible due to the different sizes and geographic distribution of the samples, the level of mtDNA genomic variation in the species *R. rupicapra* is similar or higher than the values observed in other non-endangered mammal species (Table 2.5).

Table 2.5 mtDNA genetic diversity measures of three mammal species (Lynx linx, Tupaia montana, Sus scrofa), and of R. rupicapra and R. p. ornata analyzed in this study.

Species	**Extinction risk**	**Number of individuals**	**Segregating sites**	**Nucleotide Diversity**	**Number of haplotypes**
Eurasian lynx (Maria Lucena-Perez et al. 2020)	LC	96	89	0.1	24
Mountain treeshrews (Parker et al. 2020)	LC	58	NA	0.583	34
Wild boar (Niedziałkowska et al. 2021)	LC	101	79	0.06	29
R. rupicapra	LC	37	783	0.6722	27
R. p. ornata	V	9	7	0.0202	7

When modern samples from the Eastern and Westerns Alps are separately analyzed also with indices not affected by the sample size and excluding samples that falls within the *pyrenaica* cluster (Table 2.2), the diversity of course decreases compared to the whole species, but it appears similar in two Alpine areas and much higher than observed in the other two major groups we analyzed in this study, i.e., the Pyrenean and the Apennine chamois. These similar levels of variation in the Alps were likely affected by recent translocations from the Western to the Eastern Alps, considering that historical variation appears higher in the Western Alps.

In general, reintroductions often facilitated the recovery of ungulates populations, and in the chamois it may have reduced the loss of genetic variation in the Eastern Alps. However, besides genetics, several other aspects of species' ecology should be considered when planning animal translocations such as habitat and climate suitability or the potential for diseases transmission (Soorae et al., 2013). Indeed, displacements of animals across vast distances may also produce a loss of local adaptation (Apollonio et al., 2014), leading to a mismatch between the environmental conditions of the reintroduction area and the genetic makeup of the founders, making the newly established population more vulnerable to ecological stresses and unpredictable events (Apollonio et al., 2014). For these reasons, the identification of management units (MUs), is fundamental to establish appropriate conservation objectives and ensure that conservation efforts are accurately directed (Ottewell & Byrne, 2022). Defining each Management Unit (MU) of chamois populations in the Alps accurately, while considering both genetic and ecological differences between them, can potentially lead to a more effective management of the species and an improvement in its genetic health. Considering this, given the genetic distinction between East and West supported by phylogenetic analyses, future management strategies concerning animal translocation should take this distinction into account, and should consider transferring animals from the same management units to ensure the preservation of genetic diversity and the long- term success of the translocation efforts. However, if this is not possible, considering that no visible negative effects of those reintroductions have been highlighted and that the genetic variability has increased in the last few years, translocation of animals between those two areas could be a possible alternative.

Finally, the *R. p. ornata* subspecies clearly shows the lowest values of variation among the different groups considered in this study (Table 2.2), both considering the modern and ancient samples. This subspecies is currently distributed in Abruzzo, Majella, Gran Sasso-Monti della Laga and Monti Sibillini National Parks, as well as in the Sirente-Velino (Dupré et al., 2001; Herrero, Lovari, Nores, et al., 2020). Thanks to numerous reintroductions plans with animals from the original population of the Abruzzo National Park, populations have increased in recent years, but numbers remain small (Dupré et al., 2001; Fioravanti et al., 2019; Herrero, Lovari, Nores, et al., 2020). Considering the small population size and its reduced range, this subspecies is particularly susceptible to climate change and competition with livestock and wildlife (Herrero, Lovari, Nores, et al., 2020; Lovari et al., 2014). In light of this, it is essential to ensure that the genetic diversity of reintroduced populations is maximized by using animals from different sources. The management of the Apennine chamois already involves genetic screening programs aimed at characterizing the genetic variability of existing populations. Despite the challenges posed by the limited number of existing nuclei, reintroduction programs seek to use animals from different locations (Dupré et al., 2001). This can help establish populations that are genetically diverse, reducing the likelihood of detrimental inbreeding and improving their ability to adapt to changing environments in the future (Lutz et al., 2021). Moreover, considering the observed competition between Apennine chamois and the reintroduced red deer, it is essential to carefully evaluate the reintroduction of non-threatened species (F. Ferretti et al., 2015; Lovari et al., 2014).

2.6 SUPPLEMENTARY MATERIAL 2.1

Historical DNA extraction protocol

Bone-cartilage fragments of the nasal cavity were fragmented manually with a scalpel, whereas teeth and bones were pulverized using a freezer mill and genomic DNA was extracted following a phenol-chloroform protocol. After concentration and purification, the DNA was eluted in 45 µl elution buffer. Two negative controls were included for each round of 10 samples extractions. After reducing the bone/teeth in powder, 2.2 volumes of SSC-1X buffer and 0.5% of the final volume of SDS were added and the tubes were placed in a rotary mixer for 5 minutes. After adding 1 volume of saturated phenol in Tris-HCL pH8 the tubes were placed again in a rotary mixer for 45 minutes. Samples were then centrifuged for 15 minutes at 4°C and the supernatant was carefully decanted in a new clean tube. One volume of Isoamylic Phenol Chloroform was added to each sample and after mixing for 5 minutes in a rotary mixer, samples were centrifuged for 10 minutes at 4°C. The supernatant was decanted in a new tube and 1 volume of Isoamilyc chloroform was added. After mixing the samples for 5 minutes in a rotary mixer and centrifuging them at 4000 rpm for 10 minutes at 4°C the samples were ready for concentration. The aqueous layer was transferred in an Amicon Ultra tube and samples were centrifuged at 4000 rpm for 20 minutes at 4°C. The remaining solution was then transferred in a clean microcentrifuge tube and purified following the MERmaid® Spin Kit Bio 101 protocol (MPBiomedicals, Ohio, USA). The DNA was eluted into 45 µl elution buffer.

Library preparation

The library preparation includes the following steps: repair of damage to the extremity of the extracted fragments (Blunt-end repair), ligation of the adapters (Adapter Ligation), repair of any nicks present between adapters and DNA insert (Fill-in), indexing (Indexing PCR) and enrichment (Accuprime PCR). In each step the negative control of the extraction and a negative control of the library were included. The reaction mix for one sample for the blunt end repair step was prepared with the following concentrations: 13.6 µl of water, 5 µl of NEB Buffer 2 10x, 2 µl of BSA 20 mg/ml, 2 µl of dNTP's 2.5 mM, 5 µl of dATP 10 mM, 2 µl of T4 PNK, 0.4 µl of T4 DNA Polymerase 20 µl of DNA template. The thermal cycling profile include 15 minutes at 15° C and 15 minutes at 25 ° C. The product obtained is purified using the MinElute Purification Kit (Qiagen, Inc., Valencia, CA, USA) following these steps:

200 µl of buffer PB are added to the PCR reaction and then all the product is loaded onto the MinElute spin columns. The sample are centrifuged at 14000 rpm for 1 minute and the flow-through discarded. 600 µl of PE buffer are added to each column and centrifuged at 14000 rpm for 30 seconds. The flow-through is discarded again and the columns rotated 180 ° before centrifuging them again for 1 minute at 14,000 rpm, in order to eliminate any residues of PE in all points of the filter of the column. Each MinElute column is placed in a new 1.5 ml collection tube and 18 µl of buffer TET are added to each sample. After waiting 1 minute the samples are centrifugated again at 14000 rpm for 1 minute. At this point the eluate is ready for the next step. The mix for the adapter ligation was prepared with 20 µl of Quick Ligase Buffer 2X (New England Biolabs, USA) and 1 µl of Solexa Adapter Mix (Illumina, USA) and, after adding 1 µl of Quick Ligase Enzime (New England Biolabs, USA) to each sample and living them 20 minutes to room temperature, another purification step was followed, and samples were eluted in 20 µl TET. In the next step (Fill-in), the portion of the adapters are extended by mixing 12 µl of water, 4 µl of Isothermal Buffer 10x, 2 µl of dNTP's 2.5 mM and 2 µl of Bst polymerase 2.0 (New England Biolabs, USA) for a final volume of 40 µl. The next step is the Real-time PCR, used to quantify our library and identify the optimal number of cycles required for final library amplification. The reaction mix is prepared with 7.04 µl of water, 12.5 µl of qBiomarker SYBRgreen, 2.23 of each IS8 forward and IS7 reverse Solexa primer and 1 µl of template for a final volume of 24 µl. A serial dilution of standards of known concentration was used and libraries were diluted 1:10 and 1:100 in order to allow comparison of the starting quantity of DNA in our samples with the standard curve. Thermocycling condition consisted of an initial enzyme activation at 95 °C, followed by 50 cycles of 95°C for 10 s and 60°C for 1 minute and a melting profile that consisted in 95 °C for 154 seconds, 60 °C for 1 minute, 95 °C for 15 seconds and 60 °C for 15 seconds.

In the next step, the libraries are indexed using a different combination of indexes for each sample, in order to discriminate between samples during the sequence analysis. Each sample is split in two and the reaction for one sample was prepared using 10 µl of 10x Pfu Turbo Cx Reaction Buffer (Agilent, USA, CA), 2.5 µl of dNTP 10 mM, 1.5 µl of BSA 20mg/ml, 1 µl of Pfu Turbo Cx DNA Polymerase (Agilent, USA, CA), 67 µl of water and 18 µl of template, for a final volume of 82 µl. 2 µl of each index is then added to each sample and the thermal cycling profile included 2 minutes at 95°C, followed by the number of cycles determined by the real-time PCR at 95°C for 30 seconds, 58°C for 30 seconds and 72°C for 1 minute, with a final step of 72°C for 10 minutes. Upon completion of the real-time PCR, the samples were recombined, and a purification step was carried out. Two dilutions (1:100 and 1:1000) were prepared to perform the Real-Time post-indexing, used to calculate the ratio indexing concentration/library concentration and to decide amplification cycles with Accuprime (Thermo Fisher Scientific, USA). The kit used for this real-time PCR was the Colibrì Quantification Kit (Invitrogen, Vilnius, LT) and the reaction mix was prepared using 16 µl of Colibrì Quantification Master Mix and 4 µl of template for a final volume of 20 µl. Six standards of known concentration are run in triplicate with the samples. In this case, the thermal cycling profile consisted of 95°C for 2 minutes followed by 50 cycles of 95°C for 30 seconds and 60°C for 45 seconds.

For the Accuprime amplification the samples were split in four and the reaction for one sample consisted of 10 µl of 10× AP Pfu run mix 10x, 3 µl of each IS5 and IS6 primers Bridge, 0.8 µl of AccuPrime™ Pfx DNA Polymerase (Invitrogen, CA, USA), 78 µl of water and 5 µl of template, for a final volume of 95 µl. Thermocycling condition consisted of 95°C for 2 minutes, followed by the number of cycles determined by the real-time PCR at 95°C for 15 seconds, 60 °C for 30 seconds and 68 °C for 1 minutes, with a final step at 68 °C for 10 minutes.

The Accuprime PCR products are purified with the MinElute PCR Purification Kit (Qiagen, Inc., Valencia, CA, USA), and analyzed on Agilent Bioanalyzer 2100 (Agilent Technologies, Inc, USA), using the kit DNA1000 (Agilent Technologies, Inc, USA), to estimate the qualitative and qualitative profile of the samples before proceeding with the capture of mitochondrial DNA.

Probe design

For the generation of the probes the quality of DNA of modern samples of chamois was checked and the selected samples were used as template for two PCR-LongRange in order to obtain the entire mitochondrial genome amplified in two partially overlapping portions. Two pair of primers were used with the following sequences:

First primers pair:
Forward: 5’-ATAGGAATTGAACCTACTCC-3’
Reverse: 5’-CATGTTGTTTATTTCTAGGG-3’

Second primers pair:
Forward: 5’-CAAATCTTCATACTAAAACCA-3’
Reverse: 5’-TATGTTATTTATCCGAGGAA-3’

The master-mix for a single sample was prepared with the following concentrations: 27µl of water, 10 µl of 5X Expand LongRange Buffer (Roche Diagnostics, DE) with 12.5 mM MgCl2, 1.5 µl PCR Nucleotide MIX (10mM), 0.15 µl Forward Primer (100 µM), 0.15 µl Reverse Primer (100 µM), 1.5 µl DMSO, 2 µl BSA (10 mg/ml), 0.7 µl Expand Long Range Enzyme mix (5 U/ µl) and 6 µl of template. The thermal cycling profile included an activation step at 92°C for 2 minutes followed by 40 cycles of 92°C for 10 seconds (denaturation), 50°C for 15 seconds (annealing) and 68°C for 9 minutes and 30 seconds (extension). The PCR products were run on 1% agarose gel and then purified using the MinElute Purification Kit (Qiagen, Inc., Valencia, CA, USA) eluting them in 130 µl of TE LOW Buffer. The purified products were then transferred into Covaris microtubes (suitable for ultrasonication) and sonicated with COVARIS S220 to obtain fragments of a length between 150 and 300 bp. The length was checked again by running the fragments on a Bioanalyzer 2100 (Agilent Technologies, Inc, USA) using the DNA 12000 chip assay. The next step was the Blunt-end repair which consists of repairing the single-stranded ends of the sonicated fragments: for a single sample the master-mix was prepared with 10 µl of Blunting Buffer 10X, 10 µl of dNTP’S 1 mM, 4 µl of Blunting Enzime Mix, 16 µl of water and 60 µl of template and the left 30 minutes at room temperature. After purification with MinElute Purification kit (Qiagen, Inc., Valencia, CA, USA) and NanoDrop quantification, the probes were ligated to biotinylated adapters (Adapter Ligation) in a master mix composed, for a single sample, of 20 µl of Quick Ligation Reaction Buffer 2X, 4 µl of Quick T4 DNA Ligase, 1 µl of Adapter Bio-T/B 50 uM and 15 µl of blunt ended DNA, and left 15 minutes at room temperature, in order to catalyze the reaction. After another purification and length check on Bioanalyzer 2100 (Agilent Technologies, Inc, USA), all aliquots were merged and quantified with NanoDrop to obtain probe concentration.

mtDNA capture

The capture of the mtDNA was performed following the protocol developed by Maricic et al. (Maricic et al., 2010), which involves the use of magnetic beads as support for the probes in order to capture the mtDNA by using a magnet. From the check on Bioanalyzer 2100, we calculated sample concentration of the peaks between 300 and 700 bp. Libraries were pooled in equimolar amounts with a total concentration of 2000 ng.

In this step, the baits are subjected to two washes with 200 µl of BWT 1x Buffer, composed by NaCl 2M, Tris-Hcl 10 mM pH8, EDTA 1 mM pH8, Tween 20X 0,1% e H2O. The probes, whose volume was calculated by taking into account a final concentration of 500 ng, were combined with an equal volume of BWT 2x Buffer and after 1 minute at 98 °C in a thermal cycler, they were combined with the baits (after removing the supernatant). Then, the probes and the baits were placed on a rotor and mixed for 20 minutes at 40 rpm at room temperature.

Before the probes-mtDNA hybridization a preliminary phase is required, the preparation of the hybridization mix, which is fundamental to avoid the cross-linking between the adapters of the target molecules of the library we want to capture and those of other non-target molecules. The hybridization mix is prepared with a set of *blocking-oligo*, complementary oligonucleotides to Solexa Illumina adapters (Illumina, USA) P5 and P7 and samples are pooled in a way for which the amount of DNA to be captured equals 2 µl and the final total volume of each pool equals 16.8 µl. The master mix for one samples is then prepared as following: 1 µl di BO4.part1.R (stock conc. 100 µM), 1 µl BO6P7.part2.R (stock conc. 100 µM), 1 µl BO8.P5.part1.R (stock conc. 100 µM), 1 µl BO11.sslib.part2.R (stock conc. 100 µl), 5,2 µl Agilent blocking agent , 26 µl Agilent hybridization buffer (stock conc. 2X) to which 16,8 µl of samples are added, for a total volume of 52 µl. The thermal profile requires 3' at 95°C followed by 30' at 37°C.

At this point the beads previously mixed in the rotor are placed the tubes were briefly centrifuged and placed in the magnet and the supernatant was discarded. The beads were then washed two times with 200 µl of 1x BWT buffer at 50 °C, resuspended with 50 µl of TET buffer and placed at 4°C until the end of the hybridization mix. At this point the beads were placed into the magnet, the TET was removed and the 52 µl of the hybridization mix was added. Samples and beads were then placed in a new tube, closed tightly with parafilm and incubated for 48 hours at 65°C, with the rotation set to 12 rpm. After the two days of incubation, samples and beads were briefly centrifuged and the supernatant was removed. Once the target DNA has hybridized to the probes, three washing steps with 200 µl of BWT 1x buffer steps are performed in order to remove the unhybridized portion of the library. In this phase the supernatant is removed each time using the magnet. Then the beads were resuspended and incubated in 200 µl of Buffer HWT preheated to 60°C for 2 minutes, repeating the operation twice. At this point the beads bound to the target DNA were resuspended in 100 µl of TET and transferred to new 1.5 ml eppendorf tubes to proceed with the elution of the captured library. Using the magnet, the supernatant was removed, and beads were resuspended with 50 µl of NaOH 125 mM in order to allow the breaking of the hydrogen bonds between probe and library strands and releasing only the captured mtDNA into solution, repeating the operation twice. After that the supernatant was transferred in a neutralization solution, previously prepared with 500 µl of PB buffer, 2 µl di ph-Indicator (MinElute Kit Qiagen, Inc., Valencia, CA, USA) and 3,8 µl of 20% acetic acid. This phase is fundamental to avoid DNA degradation. The eluates were then purified with Mini Elute Purification Kit and resuspended in 15 µl of TET buffer.

Quantification and enrichment of captured mtDNA

A Real-Time PCR with the Colibrì Quantification Master Mix (Invitrogen, Vilnius, LT) is performed to obtain information on the concentration values useful to calculate the number of cycles necessary for the subsequent enrichment with *Accuprime PCR* (Invitrogen, CA, USA). The master mix for this last step is prepared in the same way as described in the library preparation step. At this point samples were ready to be sequenced and were sent to the Illumina sequencing platform.

3 DIET AND TROPHIC NICHE OVERLAP BETWEEN CHAMOIS, DOMESTIC SHEEP AND RED DEER

3.1 INTRODUCTION

Understanding the processes underlying interspecific competition is pivotal to determining the ability of animal species to survive in changing environments. Sympatric species can avoid competition by partitioning resources, such as food items, but when these are limited, a potential for competition can occur (R. J. Putman, 1996). Competition may occur into two main forms: interference and exploitation (Lang & Benbow, 2013). Interference competition occurs directly when an individual actively impedes another individual from accessing a resource through aggressive or avoidance behavior (Holdridge et al., 2016; Schoener, 1983). Exploitation is an indirect form of competition and occurs when the use of resources by an individual reduces the amount of those resources for another individual (Case & Gilpin, 1974; Lang & Benbow, 2013). These two mechanisms are classified as real competition, but another mechanism called apparent competition may occur: in this case, the increase in abundance of a predator shared by two species causes competition between the two prey species due to reciprocal negative effects on each other's population densities (Hatcher et al., 2006; Holt, 1977).

While assessing actual competition would require the evaluation of overlap in resource use combined with the analysis of population dynamics (Chirichella et al., 2014), an effective initial step for investigating the potential for interspecific competition and its effect on animal communities is the analysis of the trophic niche of a species (defined as the species' role in a food web represented as an n-dimensional hypervolume), which can provide important insights into the mechanisms of interspecific competition and their effect on animal communities (Pascual-Rico et al., 2020; Rioux et al., 2022; ter Schure et al., 2021). This understanding becomes particularly crucial in mountain areas, where ungulate population dynamics are strongly affected by foraging behavior, especially in summer, when highly nutritious plants are fundamental to sustaining lactation and ensure offspring survival (Lovari et al., 2020; Schweiger et al., 2015).

Starting from the mid-1900s, populations of the Northern chamois (*R. rupicapra*) in the Province of Trento (Central Eastern Alps, Italy), have suffered a numeric decline, with a consistent negative trend (Corlatti et al., 2019). At the same time, red deer numbers have been increasing, which has been hypothesized to impact chamois populations through interspecific competition. Indeed, based on population abundance data from 1984 to 2018 in the Stelvio National Park, Corlatti et al. (2019) suggested a negative impact of red deer on chamois, since chamois densities decreased as red deer densities increased. Several studies reported also a strong dietary overlap between chamois and red deer both for Alpine chamois (Andreoli et al., 2016; Bertolino et al., 2009; Homolka & Heroldová, 2001; Redjadj et al., 2014) and Apennine chamois (Lovari et al., 2014), suggesting the potential for interspecific competition. In addition, in some Alpine areas where livestock is moved to alpine pastures in summer (between July and September), chamois and domestic sheep habitat can overlap during this period. Therefore, domestic sheep could be a potential competitor with regards to food resources, with possible consequences on chamois diet and fitness. For instance, in a study carried out by La Morgia and Bassano (2009), which investigated the summer diet and forage selection of Alpine chamois and domestic sheep in the Western Italian Alps, results showed that sheep grazing negatively affected chamois feeding habits by reducing the consumption of forbs by chamois, potentially impacting their winter fat reserves.

All the above-mentioned studies were conducted by analyzing either rumen contents or faecal pellets using microhistology, which can lead to biased results due to the difficulty in identifying some plant species, since not all food items are identifiable after passing through the ruminant gut. Here, I use a DNA metabarcoding approach, which has shown promising results in providing a taxonomically more detailed picture of the diet in other studies (Goldberg et al., 2020; Littleford-Colquhoun et al., 2022; Nichols et al., 2016; Soininen et al., 2009). In particular, I investigated the summer diet of Alpine chamois in three parks located in the Province of Trento: Stelvio National Park, Paneveggio-Pale di San Martino Natural Park, and Adamello-Brenta Natural Park. Using non-invasive faecal sampling and DNA metabarcoding, the main goal of this study is to investigate differences in diet in different interaction conditions between chamois, red deer and domestic sheep and to investigate whether and to what extent the three species exhibit trophic niche overlap.

3.6 MATERIALS AND METHODS

SAMPLE COLLECTION

Faecal samples of chamois, domestic sheep and red deer were collected in three parks in the Trentino region: Stelvio National Park (PNS), Paneveggio-Pale di San Martino Natural Park (PPPSM) and Adamello-Brenta Natural Park (PAB). Samples were collected in two different seasons: in early summer (mid-June to mid-July 2020) and in late summer (September 2020). In PNS and PPPSM, three areas of interaction between involved species were identified: Area 1) where chamois, red deer and domestic sheep coexist; Area 2) where chamois and red deer graze together; Area 3) where only chamois is present (Table 3.1). In PAB only one area with chamois and no interaction was considered.

Table 3.1 Number of samples in collected in each park, area and period for each species.

Park	Area 1 (Chamois/ Domestic sheep/ Red deer)	Area 2 (Chamois/ Red deer)	Area 3 (Chamois)
PNS			
Early summer	20/20/20	20/20	20
Late summer	20/20/20	20/20	20
PPPSM			
Early summer	20/20/20	20/20	20
Late summer	20/20/20	20/20	20
PAB			
Early summer			20
Late summer			20

In collaboration with wildlife technicians from PNS, PPPSM and PAB, sample collection was carried out in an opportunistic way, by directly observing animal groups. Since female ungulates accompanied by offspring in summer tend to utilize restricted home ranges (Mattiello & Mazzarone, 2010; Mustoni et al., 2017), we attempted to increase the likelihood of sampling individuals feeding within the designated study area by collecting fresh samples from female ungulates only.

Faecal samples were collected in a sterile manner, using gloves and a sterile tongue depressor to collect fecal pellets from the ground, and were then stored in a 50 ml Falcon tube with 90% ethanol. Samples from PNS and PAB were stored at room temperature, while samples from PPPSM were kept at -20°C until laboratory analysis. Location coordinates of each faecal sample was recorded using GPS. As illustrated in Table 3.1, we collected 20 samples for each individual in each area for a total of 520 samples (PNS: 240; PPPSM: 240; PAB: 40; Table 3.1). In PPPSM, ten domestic sheep samples were excluded from following analyses because they were collected outside the sampling area.

STUDY AREAS

Stelvio National Park

The Trentino sector of PNS extends over 17,520 ha between 1300 and 3750 m a.s.l. (Corlatti et al., 2019a). It is characterized by a continental climate with an annual mean precipitation of 900 mm and mean temperatures ranging between -0.4 °C in winter and 13.2 °C in summer (Bonardi et al., 2017a). Over 33% of this sector of the park is covered with mixed coniferous forests consisting of Norway spruce *Picea abies*, European larch *Larix decidua* and Arolla pine *Pinus cembra*, whereas above 2200 m a.s.l., alpine and subalpine meadows of Alpine sedge (*Carex curvula*), Haller's fescue (*Festuca halleri*) and coloured fescue (*Festuca varia*) are found (Bonardi et al., 2017b; Corlatti et al., 2019). Figures 3.1 and 3.2 show the samples distribution in the three different sampling areas for the early summer and late summer sampling season, respectively.

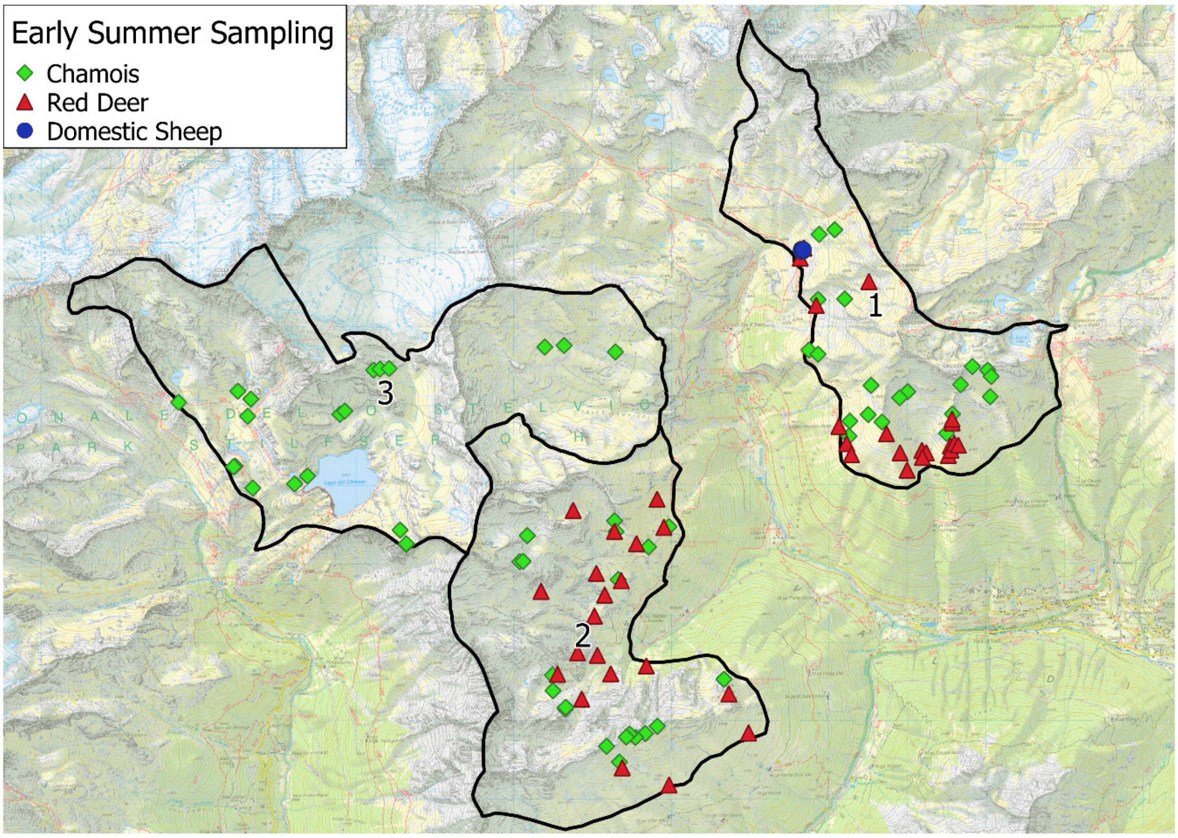

Figure 3.1 Distribution of chamois, red deer and domestic sheep samples in early summer in Stelvio National Park. Area 1) where chamois, red deer and domestic sheep coexist; Area 2) where chamois and red deer graze together; Area 3) where only chamois is present.

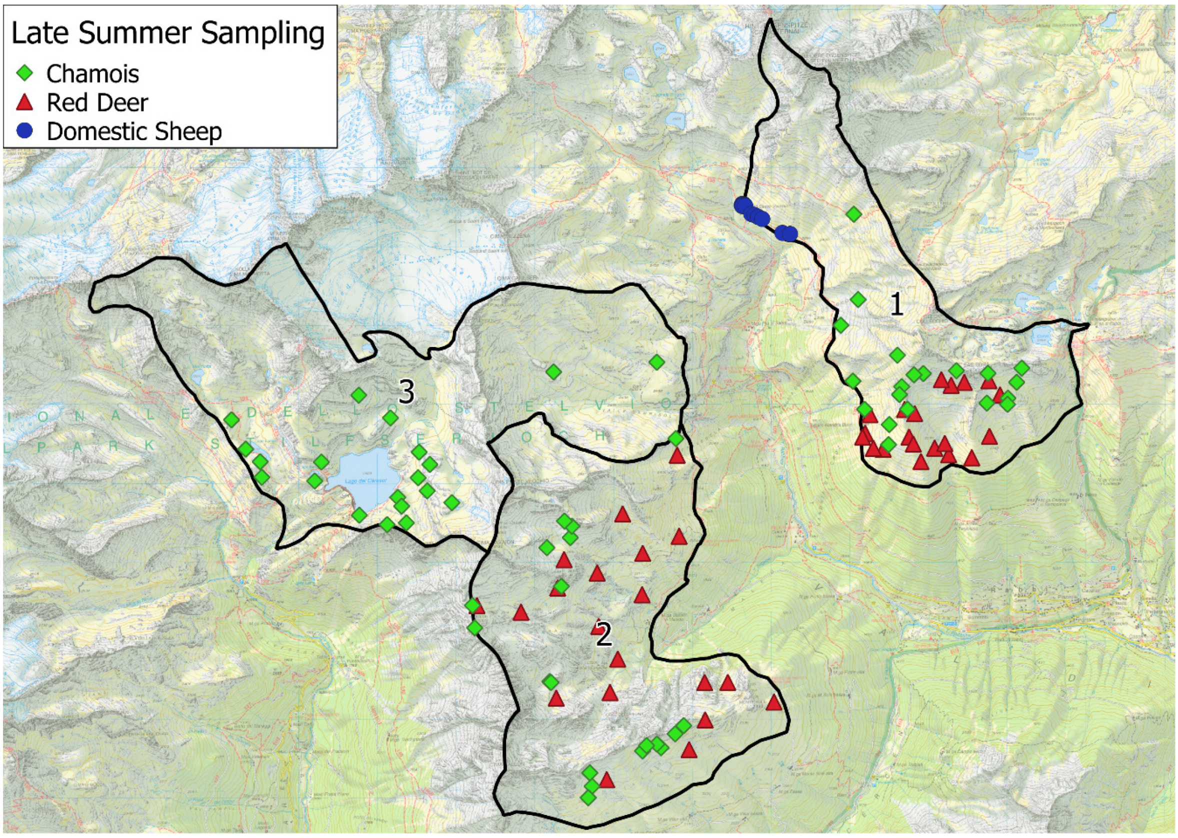

Figure 3.2 Distribution of chamois, red deer and domestic sheep samples in late summer in Stelvio National Park. Area 1) where chamois, red deer and domestic sheep coexist; Area 2) where chamois and red deer graze together; Area 3) where only chamois is present.

In each area, habitat composition was visualized using the habitat maps of Natura 2000 and non-Natura 2000 sites as shown in Figure 3.3.

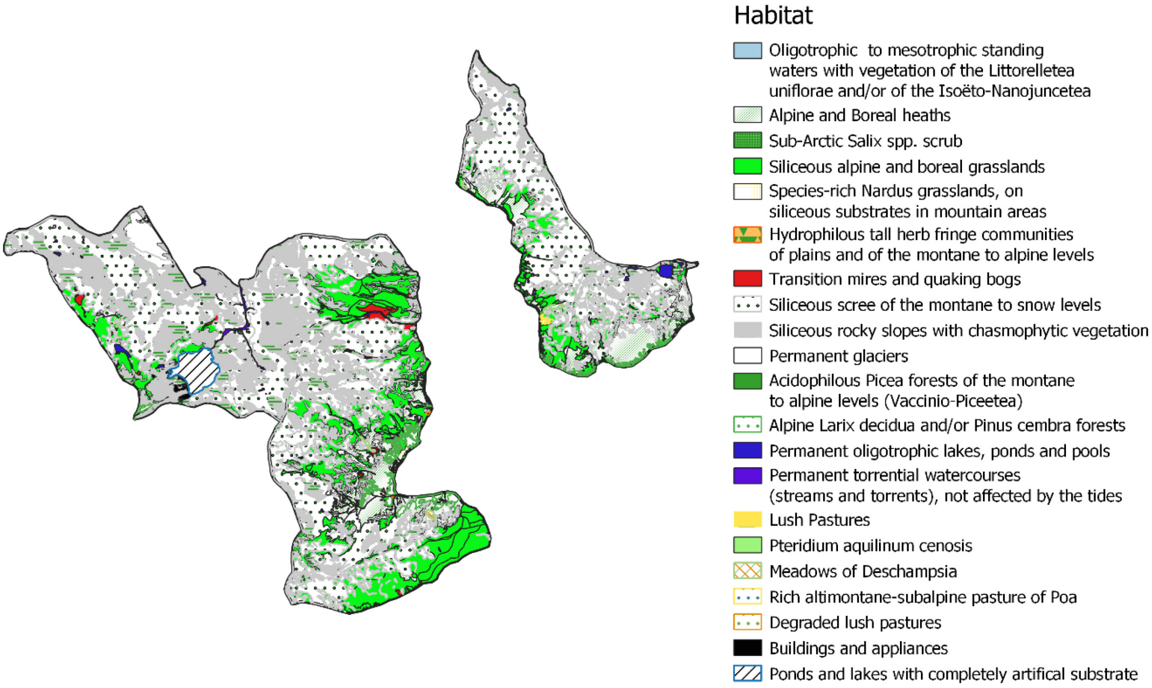

Figure 3.3 Map of the habitats Natura 2000 and non-Natura 2000 for the sampling areas in Stelvio National Park. Data provided by Stelvio National Park.

Table 3.2 shows the relative abundances (in %) of habitat composition in Area 1, Area 2 and Area 3.

Table 3.2 Relative abundances of the most representative habitat Natura 2000 in each area of Stelvio National Park and the relative dominant plant species for each habitat

Habitat	**Dominant plant species**	**Area 1**	**Area 2**	**Area 3**
Siliceous scree of the montane to snow levels	*Androsace alpina, Geum reptans, Luzula alpinopilosa, Oxyria digyna*	50.95%	36.86%	39.13%
Siliceous alpine and boreal grasslands	*Agrostis agrostiflora, Carex curvula subsp. curvula, Festuca halleri, Festuca intercedens, Festuca nigricans, Festuca paniculata, Festuca varia, Juncus trifidus, Luzula alpinopilosa, Salix herbacea*	16.91%	30.20%	13.71%
Siliceous rocky slopes with chasmophytic vegetation	*Asplenium septentrionale, Primula hirsuta*	13.70%	17.58%	38.87%
Alpine and Boreal heaths	*Arctostaphylos uva-ursi, Erica carnea, Genista radiata, Juniperus nana, Juniperus sabina, Loiseleuria procumbens, Rhododendron ferrugineum, Rhododendron hirsutum, Rhodothamnus chamaecistus, Vaccinium gaultherioides*	16.02%	11.52%	0.08%
Alpine Larix decidua and/or Pinus cembra forests	*Calamagrostis villosa, Erica carnea, Larix decidua, Picea abies, Pinus cembra, Rhododendron ferrugineum, Rhododendron hirsutum, Vaccinium myrtillus, Vaccinium vitis-idaea*	0.89%	2.61%	0.11%

Paneveggio Pale di San Martino Natural Park

The PPPSM covers a 19,726 ha in the northeastern portion of the Province of Trento, extending to altitudes between 1100 to 3200 m a.s.l. (Amedei, 2005; D'Aprile et al., 2020). It is characterized by three distinct geographical and landscape units: the Paneveggio Forest in the northern sector includes 2,700 hectares of Norway spruce forest; part of the Dolomite Mountain chain of the Pale di San Martino in the south-eastern sector; and the porphyritic chain of the Lagorai in the western sector. The environment is varied, from rocky cliffs and scree to alpine meadows and pastures, from fir woods to mixed deciduous woods, and from glaciers to peat bogs. As reported by D'Aprile et al. (2020) the tree species that are most commonly found include Norway spruce (*Picea abies* (L.) H.Karst.), European larch (*Larix decidua* Mill.), stone pine (*Pinus cembra* L.), dwarf pine (*Pinus mugo* Turra), silver fir (*Abies alba* Mill.), European beech (*Fagus sylvatica* L.) and European rowan (*Sorbus aucuparia* L.). Other tree species found less frequently are Scots pine (*Pinus sylvestris* L.), mountain maple (*Acer pseudoplatanus* L.), green alder (*Alnus viridis* Chaix), common whitebeam (*Sorbus aria* (L.) Crantz), European aspen (*Populus tremula* L.), European ash (*Fraxinus excelsior* L.), silver birch (*Betula pendula* Roth) and goat willow (*Salix caprea* L.). The climate is alpine, characterized by long, cold winters and rainy summers. Figures 3.4 and 3.5 show the samples distribution in the three different sampling area for the early summer and late summer sampling season, respectively. Also in this Park, habitat composition was analyzed using the habitat maps of Natura 2000 and non-Natura 2000 sites as shown in Figure 3.6.

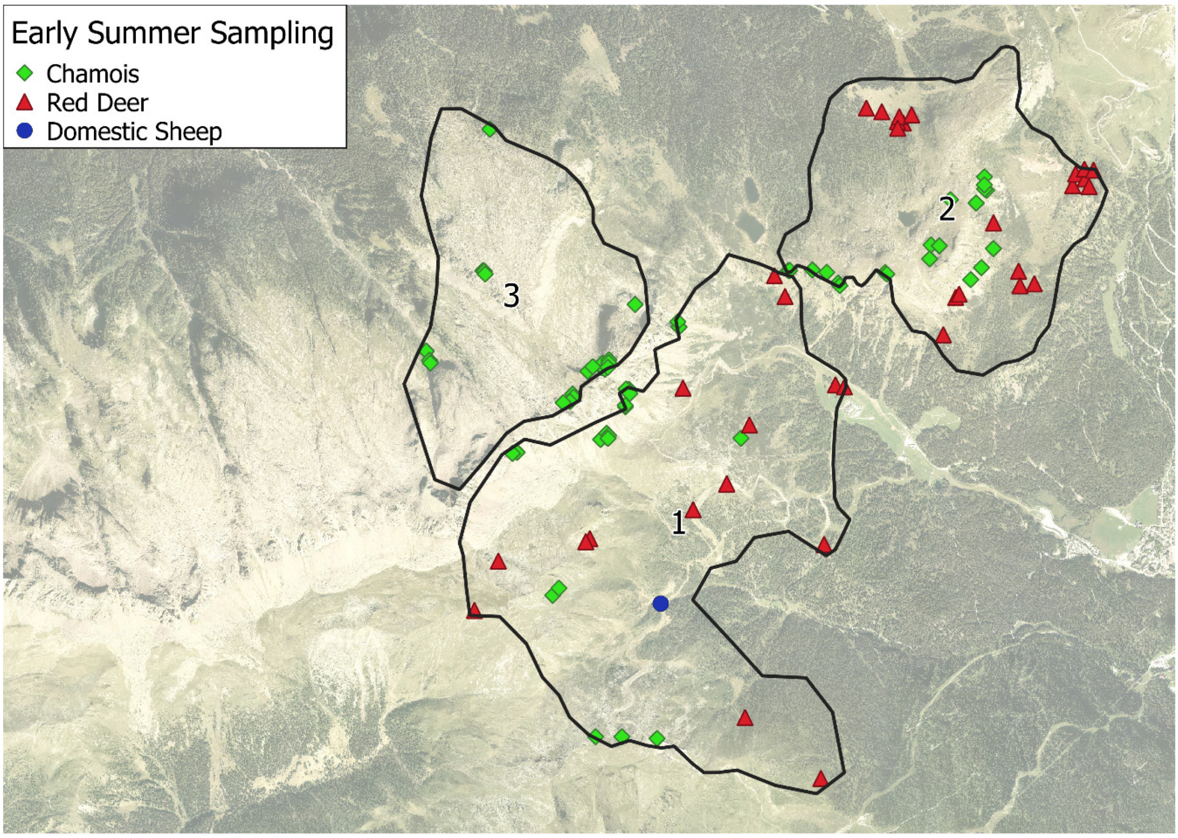

Figure 3.4 Distribution of chamois, domestic sheep and red deer sample in early summer in Paneveggio Pale di San Martino Natural Park. Area 1) where chamois, red deer and domestic sheep coexist; Area 2) where chamois and red deer graze together; Area 3) where only chamois is present.

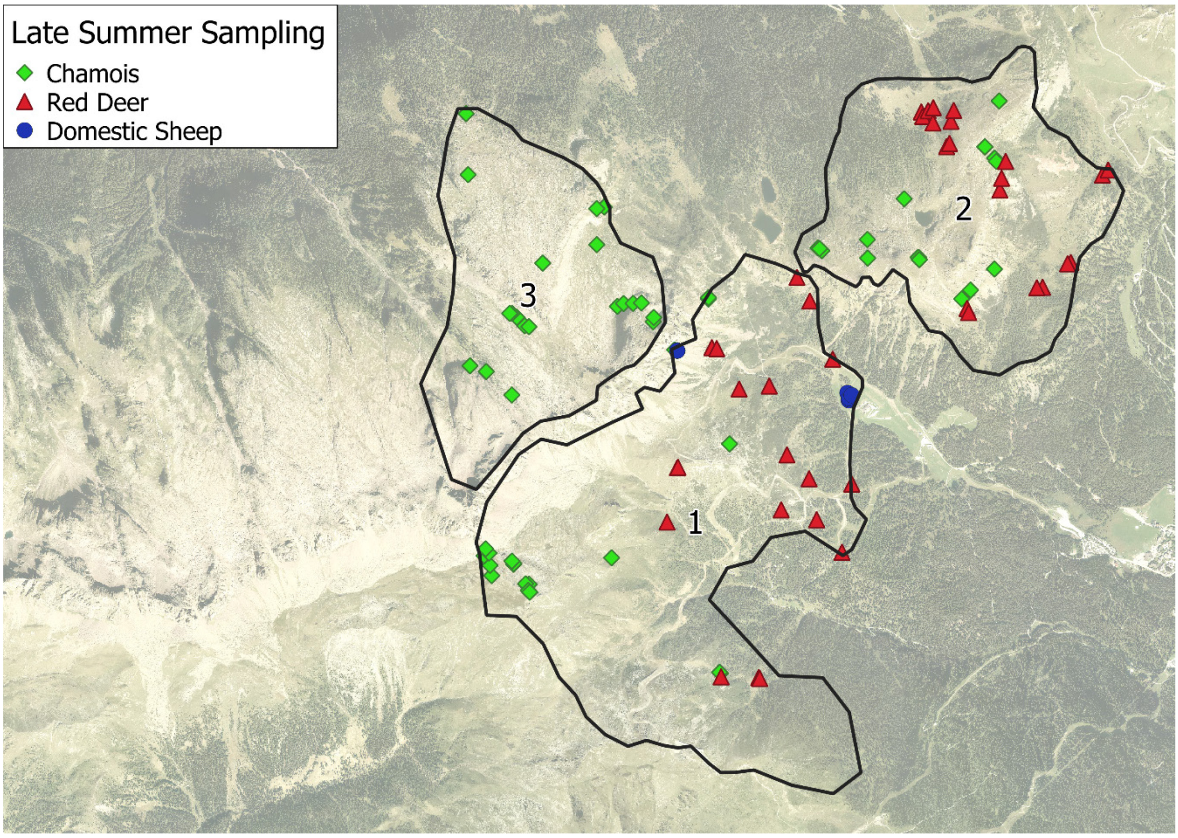

Figure 3.5 Distribution of chamois, domestic sheep and red deer samples in late summer in Paneveggio Pale di San Martino Natural Park. Area 1) where chamois, red deer and domestic sheep coexist; Area 2) where chamois and red deer graze together; Area 3) where only chamois is present.

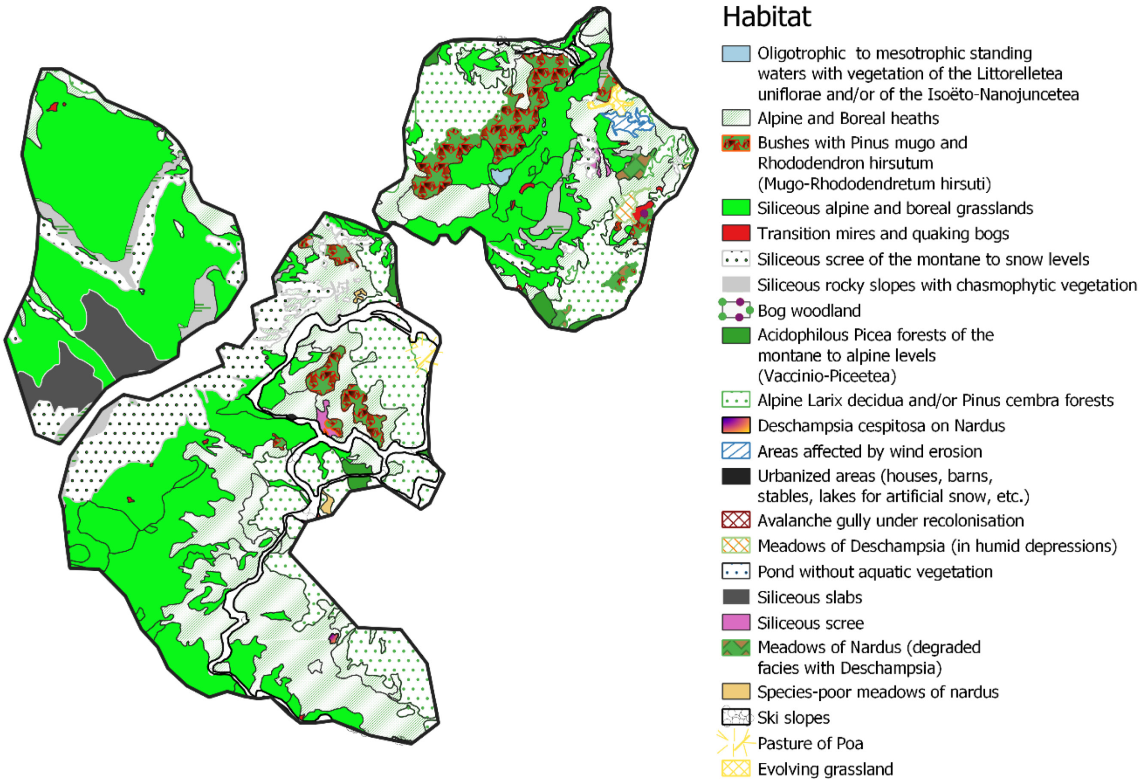

Figure 3.6 Map of the habitats Natura 2000 and non-Natura 2000 for the sampling areas in Paneveggio Pale di San Martino Natural Park. Data provided by Paneveggio Pale di San Martino Natural Park.

Relative abundances of habitat composition in Area 1, Area 2 and Area 3 are shown in Table 3.3.

Table 3.3 Relative abundances of the most representative habitat in each area of Paneveggio Natural Park and the relative dominant plant species for each habitat.

Habitat	**Dominant plant species**	**Area 1**	**Area 2**	**Area 3**
Siliceous alpine and boreal grasslands	*Agrostis agrostiflora, Carex curvula subsp. curvula, Festuca halleri, Festuca intercedens, Festuca nigricans, Festuca paniculata, Festuca varia, Juncus trifidus, Luzula alpinopilosa, Salix herbacea*	27.50%	27.78%	63.80%
Alpine and Boreal heaths	*Arctostaphylos uva-ursi, Erica carnea, Genista radiata, Juniperus nana, Juniperus sabina, Loiseleuria procumbens, Rhododendron ferrugineum, Rhododendron hirsutum, Rhodothamnus chamaecistus, Vaccinium gaultherioides*	32.51%	25.55%	6.37%
Alpine Larix decidua and/or Pinus cembra forests	*Calamagrostis villosa, Erica carnea, Larix decidua, Picea abies, Pinus cembra, Rhododendron ferrugineum, Rhododendron hirsutum, Vaccinium myrtillus, Vaccinium vitis-idaea*	17.21%	21.71%	0.49%
Bushes with Pinus mugo and Rhododendron hirsutum (Mugo-Rhododendretum hirsuti)	*Erica carnea, Pinus mugo, Rhododendron hirsutum, Rhodothamnus chamaecistus, Rhododendron ferrugineum*	2.51%	11.37%	0.00%
Siliceous rocky slopes with chasmophytic vegetation	*Asplenium septentrionale, Primula hirsuta*	0.83%	3.46%	6.85%
Siliceous scree of the montane to snow levels	*Androsace alpina, Geum reptans, Luzula alpinopilosa, Oxyria digyna*	11.09%	1.88%	9.47%
Acidophilous Picea forests of the montane to alpine levels (Vaccinio-Piceetea)	*Abies alba, Calamagrostis villosa, Luzula luzuloides, Luzula nivea, Picea abies, Pinus sylvestris, Vaccinium myrtillus, Vaccinium vitis-idaea*	0.81%	1.55%	0.00%
Ski slopes (non natura 2000)	*Festuca gr. Rubra, Phleum pratense, Trifolium repens, Lolium perenne, Lotus corniculatus, Trifolium hybridum, Dactylis glomerata, Trifolium pratense*	6.33%	1.29%	0.00%

Adamello Brenta Nature Park

The Adamello Brenta Nature Park is located in the western part of the Province of Trento and with 618 km^2 is the largest protected area in the Province. Two geomorphologically distinct areas can be distinguished: the limestone massif of the Brenta Dolomites and the granitic massif of Adamello-Presanella, characterized by the presence of numerous glaciers on the boundary with the Region of Lombardy (Buscaini & Castiglioni, 1977; Farneti et al., 1972; Gavazzi & Massa, 1976). The glacial furrow of the Rendena Valley (about 17 km long) divides the two massifs. The Park extends over altitudes ranging from 400 m a.s.l. in the main valley bottom to 3500 m a.s.l. As shown in Figure 3.7 (early summer sampling period) and Figure 3.8 (late summer sampling period), I chose one area divided in two portions where chamois only was present. Also in this Park, habitat composition was analyzed using the habitat maps of Natura 2000 and non-Natura 2000 sites as shown in Figure 3.9 Relative abundances of habitat composition in this area are listed in Table 3.4.

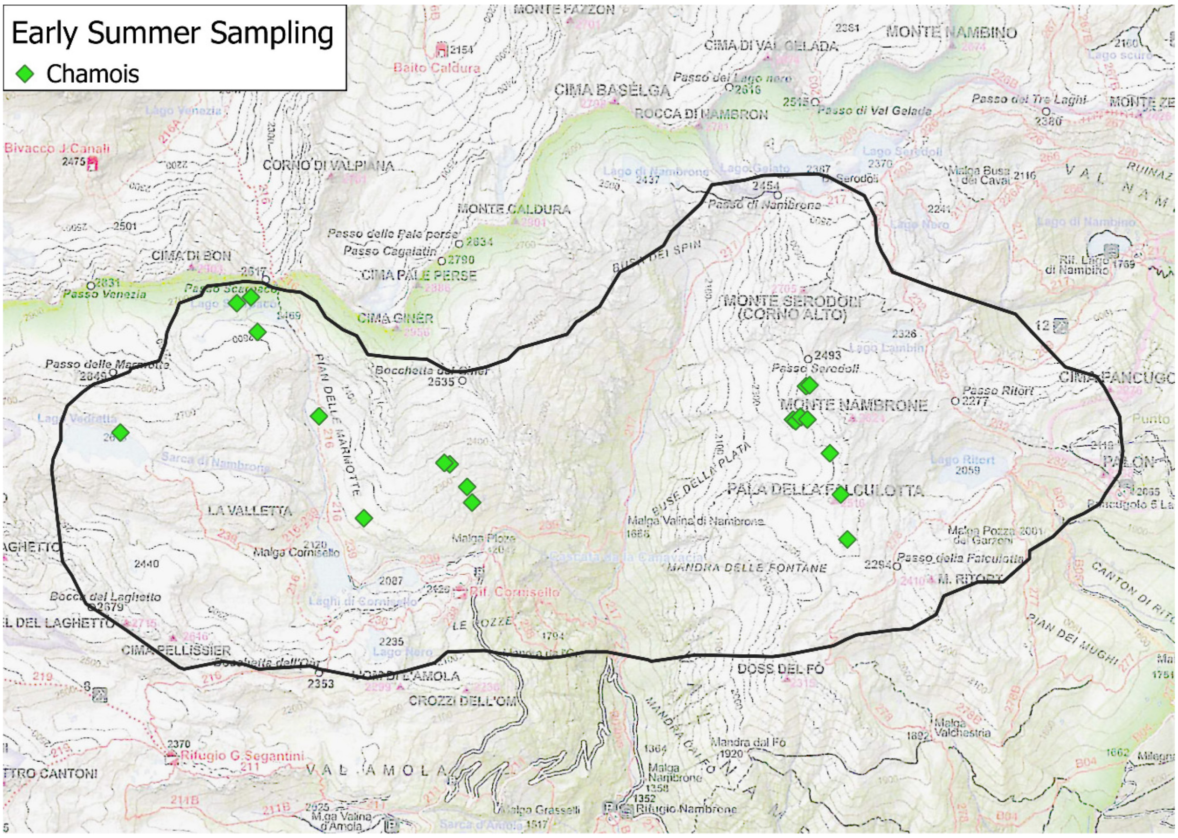

Figure 3.7 Distribution of chamois samples in early summer in Adamello Brenta Nature Park.

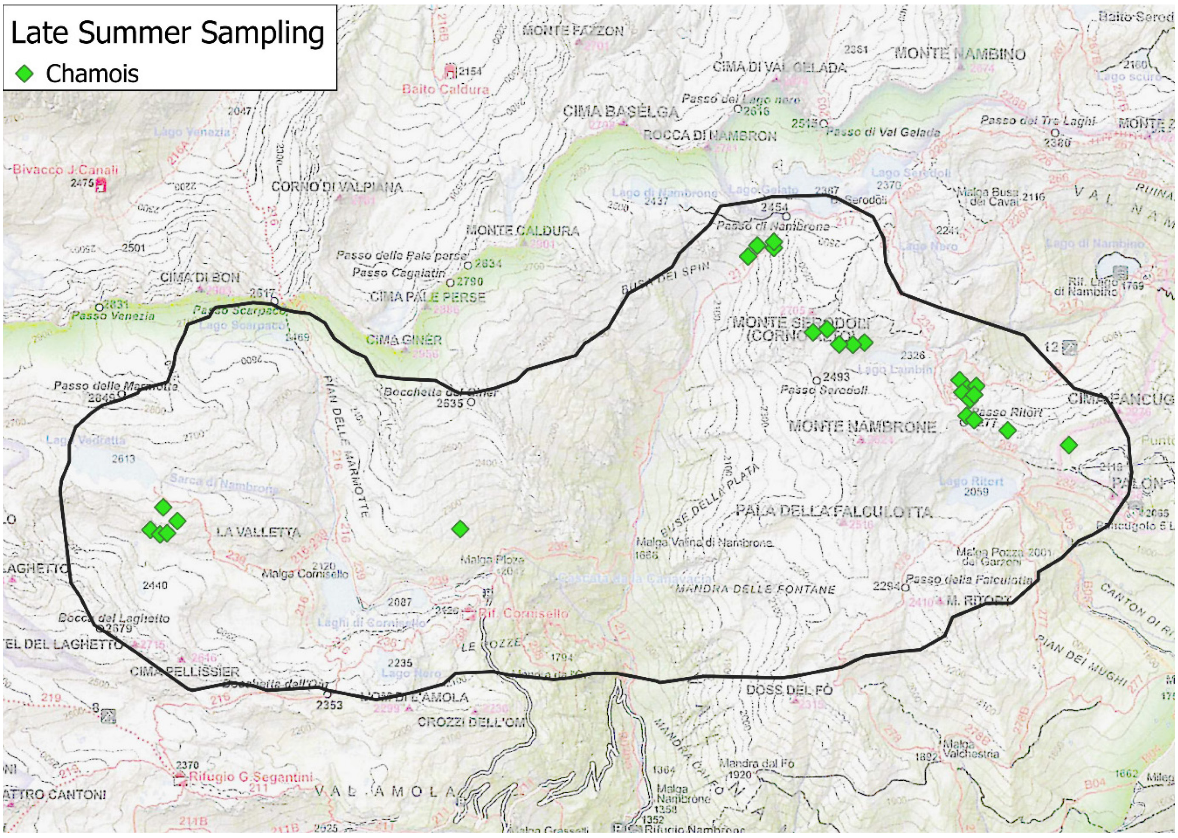

Figure 3.8 Distribution of chamois samples in late summer in Adamello Brenta Nature Park.

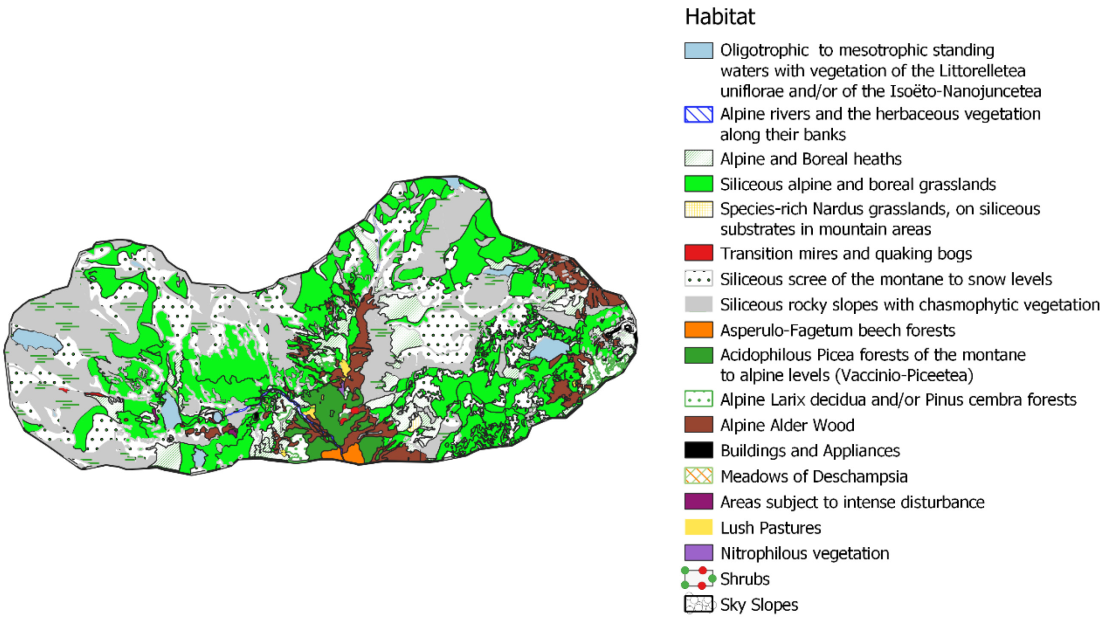

Figure 3.9 Map of the habitats Natura 2000 and non-Natura 2000 for the sampling areas in Adamello Brenta Nature Park. Data provided by Adamello-Brenta Nature Park.

Table 3.4 Relative abundances of the most representative habitat in Adamello Brenta Nature Park area and the relative dominant plant species for each habitat.

Habitat	**Dominant plant species**	**Relative abundance**
Siliceous alpine and boreal grasslands	*Agrostis agrostiflora, Carex curvula subsp. curvula, Festuca halleri, Festuca intercedens, Festuca nigricans, Festuca paniculata, Festuca varia, Juncus trifidus, Luzula alpinopilosa, Salix herbacea*	28.7%
Siliceous rocky slopes with chasmophytic vegetation	*Asplenium septentrionale, Primula hirsuta*	24.7%
Siliceous scree of the montane to snow levels	*Androsace alpina, Geum reptans, Luzula alpinopilosa, Oxyria digyna*	23.9%
Alpine and Boreal heaths	*Arctostaphylos uva-ursi, Erica carnea, Genista radiata, Juniperus nana, Juniperus sabina, Loiseleuria procumbens, Rhododendron ferrugineum, Rhododendron hirsutum, Rhodothamnus chamaecistus, Vaccinium gaultherioides*	7.93%
Acidophilous *Picea* forests of the montane to alpine levels (Vaccinio-Piceetea)	*Abies alba*, *Calamagrostis villosa*, *Luzula luzuloides*, *Luzula nivea*, *Picea abies*, *Pinus sylvestris*, *Vaccinium myrtillus*, *Vaccinium vitis-idaea*	3.71%

LABORATORY ANALYSIS

Plant DNA extraction

Sample distribution in each extraction plate was randomized using a random number generator, in order to avoiding well-to-well effect and thus bias that can confound downstream analysis. Three negative controls and three technical replicates of two samples (one chamois and one red deer) were included and their distribution was also randomized in each plate. The Mag-Bind Stool DNA 96 kit (Omega bio-tek, USA) was used to extract DNA from 40 mg of fresh faecal pellet following the manufacturer's Inhibitor-rich protocol with King Fisher Flex (Thermo Fisher Scientific, Waltham, MA, USA) with the following modifications: 500 µl of SLX-MLUS buffer was added to each sample and then mixed with a lancet in order to further facilitate cell wall rupture. Since the same faecal pellets were analyzed for bacterial microbiota studies (not discussed in this thesis), 2.5 µl and 2.5 µl 1:10 of ZymoBIOMICS™ Spike-in Control I (High Microbial Load; EuroClone, Irvine, CA, USA) mock community were added to the samples stored at -20°C and to the samples stored at room temperature, respectively (see Galla et al., 2022 for details). In addition, to facilitate the lysis of gram+ bacteria, 10 uL of lysozime (50 mg/mL) was added to each sample. To maintain the same concentrations following the increase of SLX-MLUS buffer, 30 µl of proteinase K was added to each sample.

Each sample was eluted in 55 µl of elution buffer, following automated binding and washing on a KingFisher Flex (Thermo Fisher Scientific, Waltham, MA, USA). After checking DNA concentration using a Tecan's Spark® multimode microplate reader (Tecan Group Ltd., Switzerland), each sample was diluted in order to equalize concentration to 2.5 ng/µl.

Plant DNA amplification and sequencing

Primer *c* and *h* of the *trnL* region of the chloroplast were used (Taberlet et al., 2007) for identification of all plant species, and specific primers (*ITS1-Ast*, *ITS1-Cyp*, *ITS1-Poa* and *ITS2-Ros*) for plants of the families *Asteraceae*, *Cyperaceae*, *Poaceae* and *Rosaceae* were used to increase taxonomic resolution (De Barba et al., 2014). For each amplification plate, two extraction negative controls, and one PCR negative control were included in each plate.

The reaction mix for the *trnL* fragment was prepared using GoTaq® G2 Hot Start Polymerase (Promega Corporation, Madison, WI, USA) reagents at the following concentrations: 10 µl of 5X Colorless Flexi Buffer, 1 µl of MgCl2 (25 mM), 0.8 µl of trnL(c) 10 ρmol/µL, 0.8 µl of trnL(h) 10 ρmol/µL, 1 µl of dNTP 10 mM, 0.25 Taq GoTaq 5 U/µL and 8 µL of template, for a final volume of 30 µL. The PCR profile had an initial denaturation step of 2 min and 30 s at 95 °C, followed by 40 cycles of 30 s at 95 °C, 1 min at 55 °C, and no elongation.

The same reagents were used to prepare the 57 µL reaction mix for the amplification step using the primers *ITS1* and *ITS2*: 15 µl of 5X Colorless Flexi Buffer, 50 µl of BSA 1mg/mL, 5.5 µl of MgCl2 (25 mM), 3 µl of Primer *ITS1*-F 10 ρmol/µL, 1.5 µl of Primer *ITS1*-Ast-R 10 ρmol/µL, 1 µl of Primer *ITS1*-Cyp-R 10 ρmol/µL, 1.5 µl of Primer *ITS1*-Poa-R 10 ρmol/µL, 1 µl of Primer *ITS2*-Ros-F 10 ρmol/µL, 1 µl of Primer *ITS2*-Ros-R 10 ρmol/µL, 9 µl of dNTP 10mM, 0.25 Taq GoTaq 5 U/µL, 11.75 µl of H2O and 6 µl of template. The PCR profile had an initial denaturation step of 2 min and 30 s at 95 °C, followed by 50 cycles of 30 s at 95 °C, 1 min at 58 °C, and no elongation.

The amplicons generated with *trnL* and *ITS1-ITS2* primer pairs were quantified and pooled in equimolar quantities. Following this step, amplicons were sequenced using Illumina technology (Illumina, UK) at the Genomics Platform, Fondazione E. Mach with a minimum depth of 30,000 reads per sample.

BIOINFORMATIC ANALYSIS

The analysis of the sequence data was performed using MICCA software pipeline (Albanese et al., 2015), an open-source, GPLv3-licensed software which allow the processing of amplicon sequence data, from raw sequences to OTU tables, taxonomy classification and phylogenetic tree inference, which is commonly used for this type of analysis (Albanese et al., 2015). The first step involved the merging of overlapping paired reads into single reads followed by trimming of both forward and reverse primers. Reads were then quality filtered by discarding sequences shorter than 50 bp for *trnL* and *ITS1* and 80 bp for *ITS2*, as well as reads with more than 0.50% error rate. To characterize the taxonomic structure of the samples, we denoised amplicon sequences using the UNOISE3 protocol, which includes in a single command dereplication, denoising and chimera filtering. For taxonomy assignment, I first constructed a local reference database using the list of plants present in the Province of Trento over 1000 m a.s.l. kindly provided by the Fondazione Museo Civico di Rovereto (Prosser et al., 2019). In order to obtain the gene sequence of interest for each plant and marker, starting from their scientific name, I used the E-Direct (Entrez Direct) command line utility which facilitates programmatic access to the NCBI's suite of interconnected databases from a Unix terminal window. A python script developed by Dr. Diego Micheletti from Fondazione E. Mach, was then used to convert the *fasta* file with the list of plant sequences to a *vsearch* (Rognes et al., 2016) compatible format. The resulting file was then used as a reference database for taxonomy assignment with *vsearch* using a 0.8 cutoff followed by the MUSCLE approach for multiple sequence alignment with a maximum of 2 MUSCLE iterations. After that, the MICCA-processed data (the BIOM file, the phylogenetic tree and the representative sequences) were imported into R-4.1.2 using the phyloseq package (McMurdie & Holmes, 2013) for further filtering and downstream analysis. As the accuracy of taxonomic classification can decrease at deeper taxonomic levels (Matiz-Ceron et al., 2022), taxonomic assignment has been made at the genus level.

The R package *decontam* (Davis et al., 2018) was used to identify and discard all sequences that were more prevalent in negative controls than in positive samples using a prevalence threshold of 0.5. After rarefying samples in order to normalize libraries of differing sizes for each marker, we calculated the number of OTUs that contained less than 10, 20, 30, 40 and 50 reads and which percentage they represented with respect to the whole dataset. Based on this, we further filtered our data by removing OTUs that were in less than 5% of the samples but we kept the ones with at least 10 reads.

To observe the distribution of the replicates among samples, I plotted the PCoA using the Bray-Curtis dissimilarity. Subsequently, to test for differences in OTUs abundances of replicates among plates, a multivariate PERMANOVA on Bray-Curtis dissimilarity was carried out using the *adonis* function from the R *vegan* package (Oksanen et al., 2022).

STATISTICAL ANALYSIS

In order to quantify diet composition, the matrix of read counts of each marker was transformed into relative read abundance (RRA), that is the proportional summaries of counts of each plant in each sample.

A principal Component Analysis (PCA), using the relative abundances of each plant species identified in each sample, was then conducted to visualize dietary niches of the species using the *rda* function from the *vegan* R package (Oksanen et al., 2005) with a covariance matrix. A multivariate analysis of variance (MANOVA) was carried out to determine if significant differences exist between species, sampling periods, presence of competitors (areas), and sampling sites (park). Thereafter, an Analysis of Variance (ANOVA) was carried out on each of the selected PCA axes, followed by a Tukey post-hoc test on the axes that showed significant differences for the pure factor species in order to determine differences in diet between the three species. Finally, food niche hypervolume for each species was conducted along the PCA axes using the R package *hypervolume* (Blonder et al., 2014), a method which quantifies the multidimensional trophic space occupied by each species using a kernel density estimation. I then calculated the overlap between hypervolumes using the Sørensen and Jaccard similarity indexes. For two hypervolumes Sørensen index is calculated as $2*|A \cap B|/(|A| + |B|)$, while for Jaccard index is calculated as $|A \cap B|/ |A \cup B|$ and both index ranges from 0 (complete disjunction between hypervolumes) and 1 (completely identical hypervolumes)

(Blonder et al., 2014; Mammola, 2019). The hypervolume calculation and overlap was then performed for each park separately.

The combined analysis of *trnL* and *ITS1* were performed by transforming the matrix of read counts in presence/absence data. Trophic niches were visualized using non-metric multidimensional scaling (NMDS) with the *metaMDS* function from the *vegan* R package with Jaccard distance (Oksanen et al., 2005). The number of axes (k) to retain depend on the stress value returned from the function: a stress value < 0.2 is considered acceptable (Clarke, 1993).

Data obtained for the *ITS2* fragment were excluded from all analyses since, due to an unacceptably low number of reads, 104 fecal samples would have been excluded from the analysis.

3.3. RESULTS

trnL relative abundances

Sequencing resulted in 6,566,811 filtered reads, of which 55.1% derived from chamois, 31.4% from red deer and 13.6% from domestic sheep fecal pellets.

The final dataset after filtering and refinement of taxonomic assignment contained 499 OTUs, 232 of which could not be assigned to any taxon. However, since the reads associated with these unclassified OTUs represented only 3.83% of total reads, these reads were removed from the dataset. We identified 78 genera from 39 plant Families which were used for subsequent analyses.

The replicate samples used to test for technical replicability, were statistically analyzed to evaluate differences between plates. The multivariate PERMANOVA test on replicate samples showed that there were no differences in OTU abundances between replicates for both chamois (P = 0.197) and red deer (P = 0.211). Indeed, as can be seen from the PCoA plot (Figure 3.10), chamois replicates cluster very closely, as well as red deer replicates.

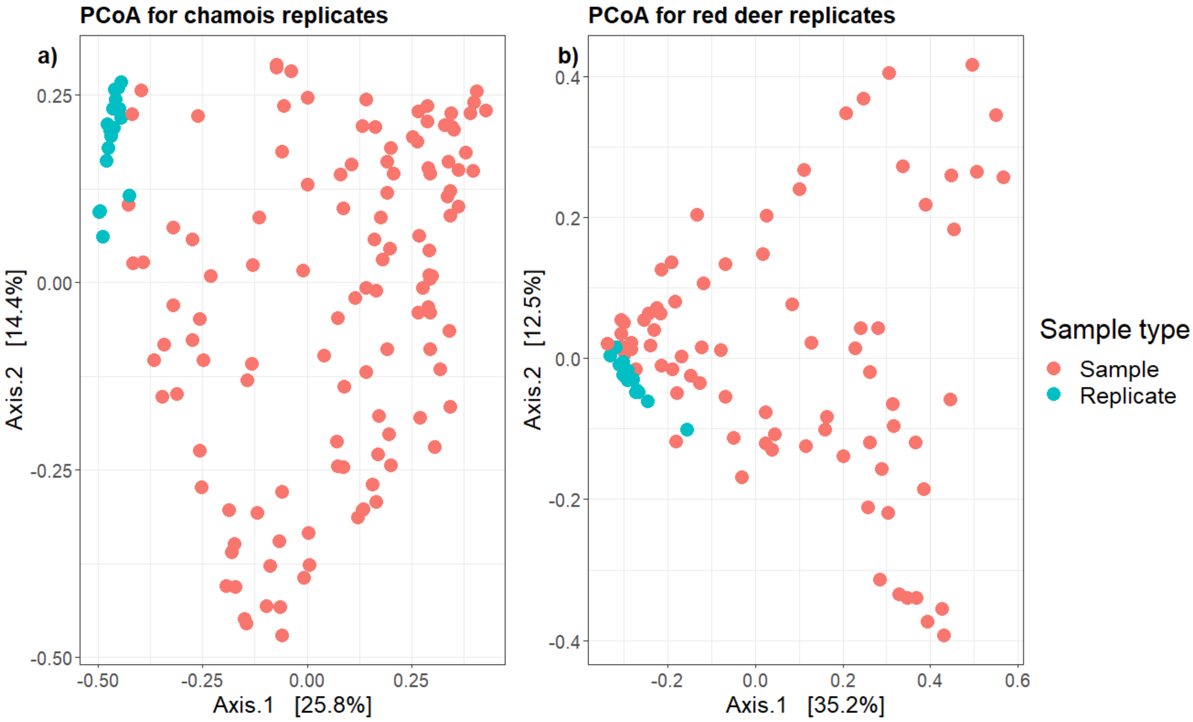

Figure 3.10 Plots of Principal Coordinate Analysis (PCoA) for trnL based on Bray-Curtis distances among replicates and samples of a) chamois and b) red deer.

The ten genera most commonly encountered in the fecal pellets of each of the target species (divided into early summer and late summer and their relative abundances expressed in percentages) are shown in Figure 3.11 and Table 3.5, respectively.

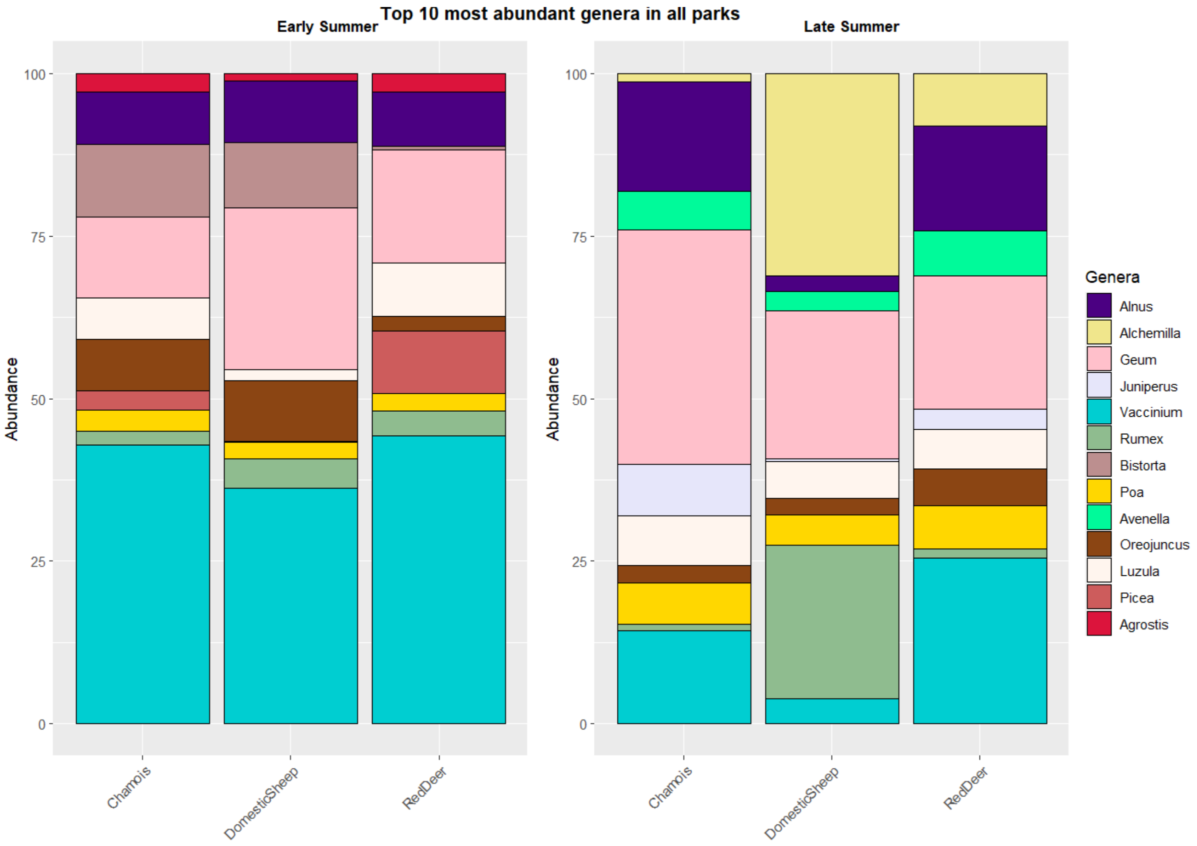

Figure 3.11 Plot bar chart of the top 10 most abundant genera in chamois, domestic sheep and red deer faecal pellets divided by early summer and late summer.

The diet of the three species seems to be more similar in early summer, with a greater consumption of *Vaccinium spp.* (chamois: 42.82%, domestic sheep: 36.24%, red deer: 44.22%) and *Geum spp*. (chamois: 12.49%, domestic sheep: 24.95%, red deer: 17.50%). In addition, chamois and domestic sheep often consume *Bistorta* spp. (chamois: 11.22%, domestic sheep: 10.05%), *Alnus* spp. (chamois: 7.94%, domestic sheep: 9.40%) and *Oreojuncus* spp. (chamois: 7.93%, domestic sheep: 9.32%). Instead, red deer diet seems to be slightly different in early summer, since, in addition to *Alnus spp.* (8.26%), the other most preferred diet items are *Picea spp*. (9.62%) and *Luzula spp*. (8.10%). In late summer, chamois and red deer diets seem to be more similar to each other than to that of domestic sheep with the three most consumed items being *Geum spp.* (chamois: 36.09%, red deer: 20.50%), *Alnus spp.* (chamois: 16.91%, red deer: 16.10%) and *Vaccinium spp.* Chamois: 14.34%, red deer: 25.46%), with only *Geum* being in common with domestic sheep (22.79%). Indeed, domestic sheep diet in late summer is mainly composed of *Alchemilla* (31.12%) and *Rumex spp*. (23.60%).

Table 3.5 Relative abundances of the top 10 most abundant plant genera in chamois, domestic sheep and red deer faecal pellets divided by early summer and late summer

Early summer		**Late summer**	
Chamois diet		**Chamois diet**	
Genus	**Relative abundance (%)**	**Genus**	**Relative abundance (%)**
Vaccinium	42.82	*Geum*	36.09
Geum	12.49	*Alnus*	16.91
Bistorta	11.22	*Vaccinium*	14.34
Alnus	7.94	*Juniperus*	7.91
Oreojuncus	7.93	*Luzula*	7.59
Luzula	6.31	*Poa*	6.29
Poa	3.34	*Avenella*	5.83
Picea	2.94	*Oreojuncus*	2.78
Agrostis	2.90	*Alchemilla*	1.25
Rumex	2.11	*Rumex*	1.00
Domestic sheep diet		**Domestic sheep diet**	
Genus	**Relative abundance (%)**	**Genus**	**Relative abundance (%)**
Vaccinium	36.24	*Alchemilla*	31.12
Geum	24.95	*Rumex*	23.60
Bistorta	10.05	*Geum*	22.79
Alnus	9.40	*Luzula*	5.79
Oreojuncus	9.32	*Poa*	4.68
Rumex	4.45	*Vaccinium*	3.82
Poa	2.65	*Avenella*	2.94
Luzula	1.68	*Oreojuncus*	2.50
Agrostis	1.19	*Alnus*	2.44
Picea	0.08	*Juniperus*	0.33
Red deer diet		**Red deer diet**	
Genus	**Relative abundance (%)**	**Genus**	**Relative abundance (%)**
Vaccinium	44.22	*Vaccinium*	25.46
Geum	17.50	*Geum*	20.50
Picea	9.62	*Alnus*	16.10
Alnus	8.26	*Alchemilla*	8.02
Luzula	8.10	*Avenella*	6.93
Rumex	3.83	*Poa*	6.62
Agrostis	2.88	*Luzula*	5.98
Poa	2.79	*Oreojuncus*	5.73
Oreojuncus	2.25	*Juniperus*	3.24
Bistorta	0.55	*Rumex*	1.41

The PCA to visualize patterns in dietary similarity between species, did not show a clear separation of chamois, domestic sheep and red deer diet (Figure 3.12). However, domestic sheep separate into two clusters on the right and left sides of the plot. In addition, some points in the right cluster do not overlap with points representing chamois and red deer diet. The first axis absorbed 38.36% of the variance, the second axis 16.37% and the third axis 9.57%.

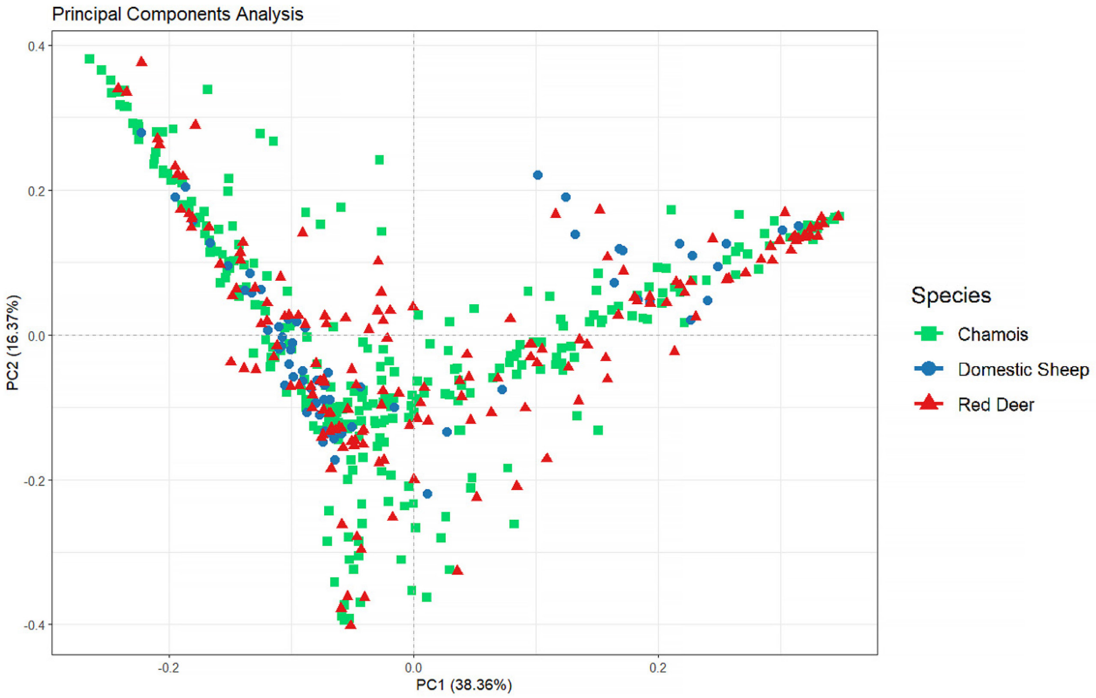

Figure 3.12 Principal component analysis (PCA) based on the relative abundances of all detected plant genera found in chamois, domestic sheep and red deer.

Figure 3.13 shows that the plant genera that contributed significantly to the construction of the PCA were *Geum*, *Vaccinium* and *Alnus*.

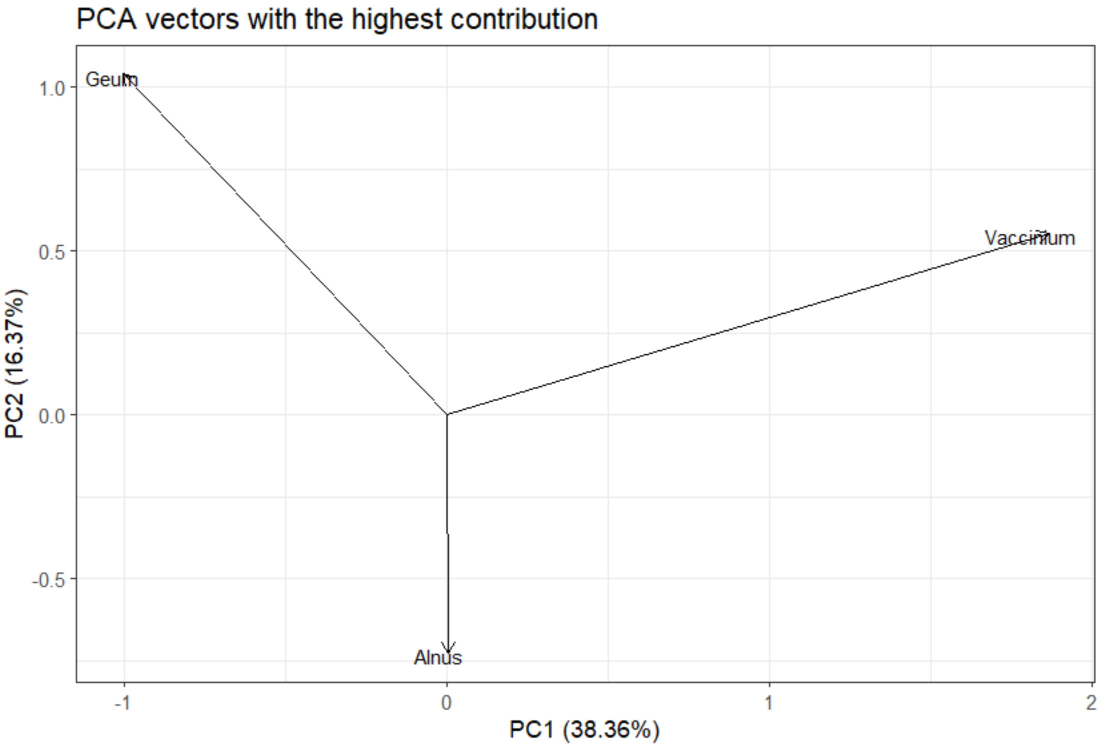

Figure 3.13 PCA vectors with the highest contribution

Geum was negatively correlated with PC1 (-0.66), but positively correlated with PC2 (0.69). *Vaccinium* was positively correlated with PC2 (0.95). Alnus was negatively correlated with both PC2 and PC3 (-0.64 and -0.76, respectively).

The multivariate analysis of variance (MANOVA) performed on the first three axes of the PCA showed significant differences between all the individual variables (species, parks, areas and periods) and their interactions (species and parks; species and areas; parks and areas; species and periods; parks and periods; areas and periods; species, parks and periods; species, parks and areas; parks, areas and periods). Results were not significant only for the interaction between species, areas, and periods and between the interaction of the four variables (species, parks, areas and periods) (Table 3.6).

Table 3.6 Summary statistics of the multivariate analysis of variance (MANOVA) performed on the first 3 PCA axes. Significant results are reported in bold

Multivariate analysis	**Df**	**Pillai**	**approx F**	**num Df**	**den Df**	**Pr(>F)**
Species	2	0.06	5.10	6	966	**<0.001**
Park	2	0.53	58.19	6	966	**<0.001**
Area	2	0.04	3.49	6	966	**0.002**
Period	1	0.27	58.89	3	482	**<0.001**
Species*Park	2	0.11	9.10	6	966	**<0.001**
Species*Area	1	0.02	3.33	3	482	**0.019**
Park*Area	2	0.05	4.05	6	966	**<0.001**
Species*Period	2	0.07	5.86	6	966	**<0.001**
Park*Period	2	0.07	5.90	6	966	**<0.001**
Area*Period	2	0.05	3.71	6	966	**0.001**
Species*Park*Area	1	0.02	2.96	3	482	**0.032**
Species*Park*Period	2	0.11	9.19	6	966	**<0.001**
Species*Area*Period	1	0.01	0.84	3	482	0.474
Park*Area*Period	2	0.04	3.18	6	966	**0.004**
Species*Park*Area*Period	1	0.01	0.94	3	482	0.422
Residuals	484					

Results of the analysis of variance (ANOVA) on the first axis showed that the diet change between species, between parks and periods (Table 3.7). The interaction of various factors was statistically significant (species and parks; parks and areas; species and periods; areas and periods; species, parks and periods, parks, areas and periods) showing statistically different diets between parks and periods, but not between areas.

Table 3.7 Summary statistics of the analysis of variance on the first PCA axis. Significant results are reported in bold.

Analysis of variance on PC1	**Df**	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
Species	2	0.164	0.082	7.952	**< 0.001**
Park	2	3.762	1.881	182.234	**< 0.001**
Area	2	0.050	0.025	2.419	0.0901
Period	1	1.788	1.788	173.248	**< 0.001**
Species*Park	2	0.280	0.140	13.553	**< 0.001**
Species*Area	1	0.003	0.003	0.287	0.5924
Park*Area	2	0.247	0.123	11.959	**< 0.001**
Species*Period	2	0.082	0.041	3.982	**0.0193**
Park*Period	2	0.059	0.030	2.865	0.0579
Area*Period	2	0.075	0.038	3.657	**0.0265**
Species*Park*Area	1	0	0	0.009	0.9265
Species*Park*Period	2	0.267	0.133	12.916	**< 0.001**
Species*Area*Period	1	0.021	0.021	2.004	0.1575
Park*Area*Period	2	0.086	0.043	4.186	**0.0158**
Species*Park*Area*Period	1	0.006	0.005	0.536	0.4645
Residuals	484	4.995	0.010		

The ANOVA on the second axis (Table 3.8) did not show significant differences between species, but found significant differences between parks, areas and periods and the interaction between parks and periods. Additionally, results showed that also for this axis, the diet of the species changes between parks and between periods.

Table 3.8 Summary statistics of the analysis of variance on the second PCA axis. Significant results are reported in bold.

Analysis of variance on PC2	**Df**	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
Species	2	0.007	0.004	0.204	0.8155
Park	2	1.066	0.533	29.314	**< 0.001**
Area	2	0.182	0.091	5.001	**0.0071**
Period	1	0.287	0.287	15.795	**< 0.001**
Species*Park	2	0.648	0.324	17.833	**< 0.001**
Species*Area	1	0.019	0.019	1.038	0.3088
Park*Area	2	0.008	0.004	0.219	0.8030
Species*Period	2	0.114	0.057	3.132	**0.0445**
Park*Period	2	0.550	0.275	15.118	**< 0.001**
Area*Period	2	0.064	0.032	1.753	0.1744
Species*Park*Area	1	0.001	0.001	0.039	0.8429
Species*Park*Period	2	0.033	0.017	0.907	0.4045
Species*Area*Period	1	0.003	0.003	0.184	0.6680
Park*Area*Period	2	0.076	0.038	2.091	0.1247
Species*Park*Area*Period	1	0.029	0.029	1.584	0.2088
Residuals	484	8.798	0.018		

On the third axis, the main result of the ANOVA obtained with the first axis was confirmed (Table 3.9). However, in this case, the period, the interaction between species and parks, and between parks and areas were not significant, while the interaction between species, park and areas was significant.

Table 3.9 Summary statistics of the analysis of variance on the third PCA axis. Significant results are reported in bold.

Analysis of variance on PC3	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Species	2	0.225	0.112	5.806	**0.00322**
Park	2	0.463	0.231	11.951	**< 0.001**
Area	2	0.066	0.033	1.697	0.1843
Period	1	0.053	0.053	2.763	0.0971
Species*Park	2	0.007	0.003	0.178	0.8366
Species*Area	1	0.145	0.145	7.489	**0.0064**
Park*Area	2	0.054	0.027	1.406	0.2462
Species*Period	2	0.456	0.228	11.790	**< 0.001**
Park*Period	2	0.051	0.026	1.328	0.2660
Area*Period	2	0.184	0.092	4.758	**0.0090**
Species*Park*Area	1	0.164	0.164	8.487	**0.0037**
Species*Park*Period	2	0.496	0.248	12.820	**< 0.001**
Species*Area*Period	1	0.000	0.000	0.005	0.9416
Park*Area*Period	2	0.135	0.067	3.482	**0.0315**
Species*Park*Area*Period	1	0.017	0.017	0.868	0.3521
Residuals	484	9.368	0.019		

The Tukey test (Table 3.10) was conducted on the axes that showed significant differences between species, that is axis 1 (Table 3.7) and axis 3 (Table 3.9). Results showed significant differences in the diet between chamois and red deer (PC1), chamois and domestic sheep (PC3) and between red deer and domestic sheep (PC1 and PC3).

Table 3.10 Results of the Tukey post-hoc test on the first and third axes of the PCA to test for differences in the diet of chamois, domestic sheep and red deer. Mean difference (Estimate), its confidence intervals (Conf. Low, Conf. High) and adjusted p-value are reported. Significant values are reported in bold.

Tukey Post-hoc Test	Axis	Estimate	Conf. Low	Conf. High	p-value adj.
Chamois – Red deer	PC1	0.035	0.011	0.059	**0.0016**
Chamois – Domestic sheep	PC1	-0.013	-0.045	0.019	0.6111
Red deer – Domestic sheep	PC1	0.048	0.014	0.082	**0.0031**
Chamois – Red deer	PC3	-0.005	-0.037	0.028	0.9370
Chamois – Domestic sheep	PC3	0.059	0.015	0.103	**0.0045**
Red deer – Domestic sheep	PC3	-0.064	-0.111	-0.017	**0.0042**

The hypervolume analysis (Figure 3.14) showed a high overlap in the trophic niche hypervolume of all three species for their total diet, however, the overlap was more pronounced between chamois and red deer. This is evidenced by a higher portion of shared hypervolume on the left of the PC1-PC2 and PC1-PC3 graph, as well as on the bottom left of the PC2-PC3 graph (Figure 3.13 a). For the preferred diet chamois and red deer shared again a higher overlap with respect to domestic sheep (Figure 3.14 b). In addition, a separate portion of hypervolume is distinguishable for chamois, suggesting some dietary preference for chamois not shared with the other species.

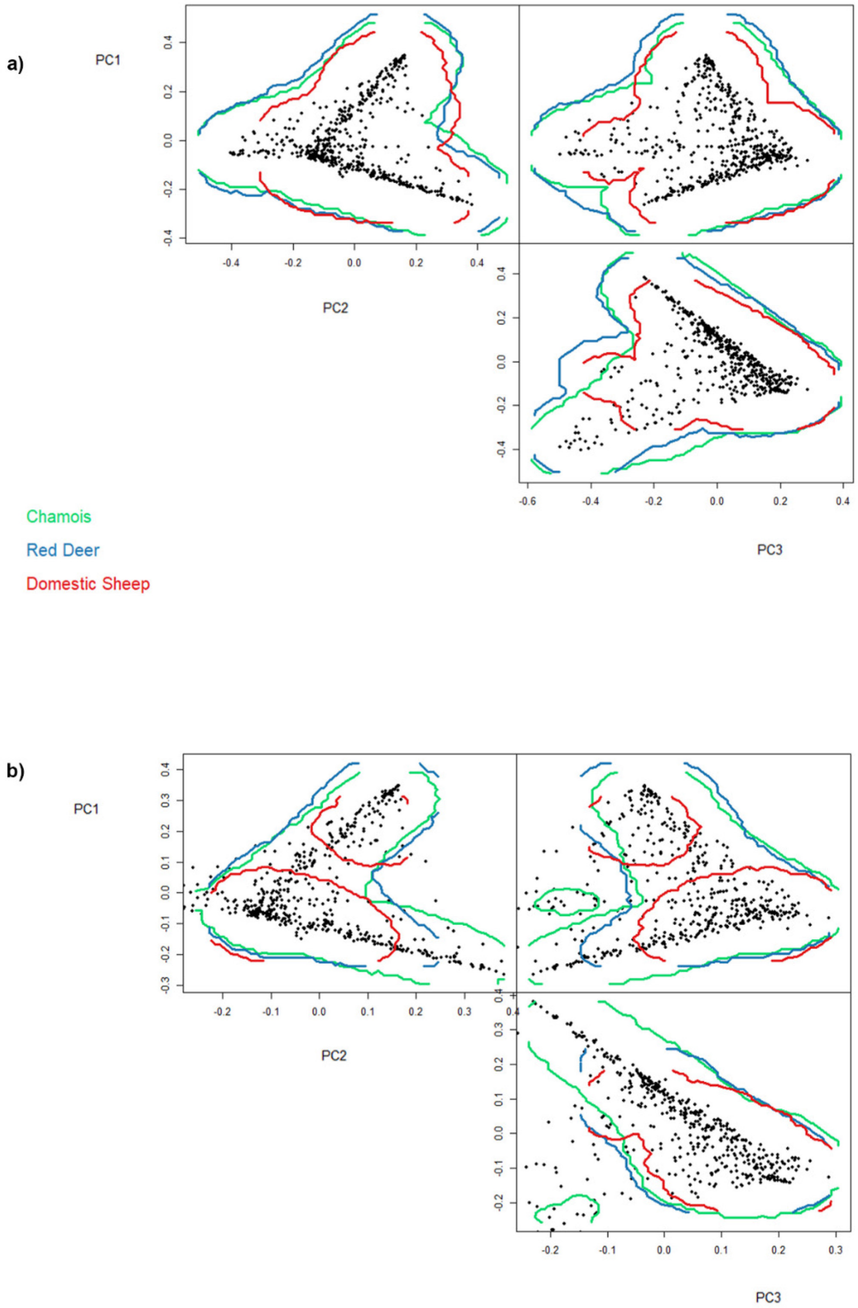

Figure 3.14 Hypervolume plot of a) the total and b) the preferred diet calculated on the first three PCA axes for chamois, red deer and domestic sheep.

In Table 3.11 overlap indexes values are shown. Overlap values were considered low for values below 50%, moderate for values around 55% and high for values above 60%. Sørensen-Dice and Jaccard indices predicted high trophic niche overlap between chamois and red deer both for the total (Sørensen-Dice: 88.9%, Jaccard: 80.9%) and the preferred diet (Sørensen-Dice: 83.2%, Jaccard: 71.3%). Overlap between chamois and domestic sheep for the total diet was high for the Sørensen-Dice index (70.5%) and moderate for the Jaccard index (54.4%), while for the preferred diet decreases considerably, with a moderate value for the Sørensen-Dice index (56.3%) and low value for the Jaccard index (39.2%).

Table 3.11 Sørensen-Dice and Jaccard overlap indexes for chamois - red deer and chamois - domestic sheep total diet and preferred diet based on the PCA scores obtained with relative abundances.

	Chamois - Red deer		**Chamois - Domestic sheep**	
	Total diet	**Preferred diet**	**Total diet**	**Preferred diet**
Sørensen-Dice	88.9%	83.2%	70.5%	56.3%
Jaccard	80.9%	71.3%	54.4%	39.2%

When repeating the analysis for each park separately, (Table 3.12) results showed that chamois-red deer overlap in PNS was high when considering the Sørensen-Dice index for both the total (60.9%) and the preferred diet (61.6%), but low for the Jaccard index (total diet: 44.5%, preferred diet: 44.5%). Chamois-domestic sheep overlap was low with respect to chamois – red deer overlap for both indexes and for both the total (Sørensen-Dice: 14.8%, Jaccard: 8.1%) and the preferred diet (Sørensen-Dice:13.9, Jaccard: 7.2%). In Paneveggio Natural Park the overlap in the total diet between chamois and red deer was high for the Sørensen-Dice index (70.6%), but moderate for the Jaccard index (54.5%), while for the preferred diet was moderate for the Sørensen-Dice index (56%) and low for the Jaccard index (38.9%). In this park as well, overlap between chamois and domestic sheep was low for both the total diet (Sørensen-Dice: 38.3%, Jaccard: 23.7%) and the preferred diet (Sørensen-Dice: 30.1%, Jaccard: 17.7%).

Table 3.12 Sørensen-Dice and Jaccard overlap indexes for chamois - red deer and chamois - domestic sheep total diet and preferred diet in Stelvio National Park and Paneveggio Natural Park.

	Stelvio National Park				**Paneveggio Pale di San Martino Natural Park**			
	Chamois Red deer		**Chamois Domestic sheep**		**Chamois Red deer**		**Chamois Domestic sheep**	
	Total diet	**Preferred diet**	**Total diet**	**Preferred diet**	**Total diet**	**Preferred diet**	**Total diet**	**Preferred diet**
Sørensen-Dice	60.9	61.6	14.8	13.9	70.6	56	38.3	30.1
Jaccard	44.5	44.5	8.1	7.2	54.5	38.9	23.7	17.7

Presence/absence data using a combined analysis of trnL and ITS1

For ITS1, sequencing resulted in 1,003,514 reads after filtering, of which 55% came from chamois samples, 31.1% from red deer samples and 13.4% from domestic sheep samples. Two negative controls of two different plates resulted in positive results, but since they were not recognized as contaminants from the package *decontam*, the identified OTUS were left in the dataset in order to not lose important ecological information.

The PCoA plot (Figure 3.15), showed that chamois replicates cluster closely, as well as red deer replicates. The multivariate PERMANOVA test on replicates showed that there were no differences in OTUs abundances between replicates, both for chamois replicates (P = 0.2) and red deer replicates (P = 0.114).

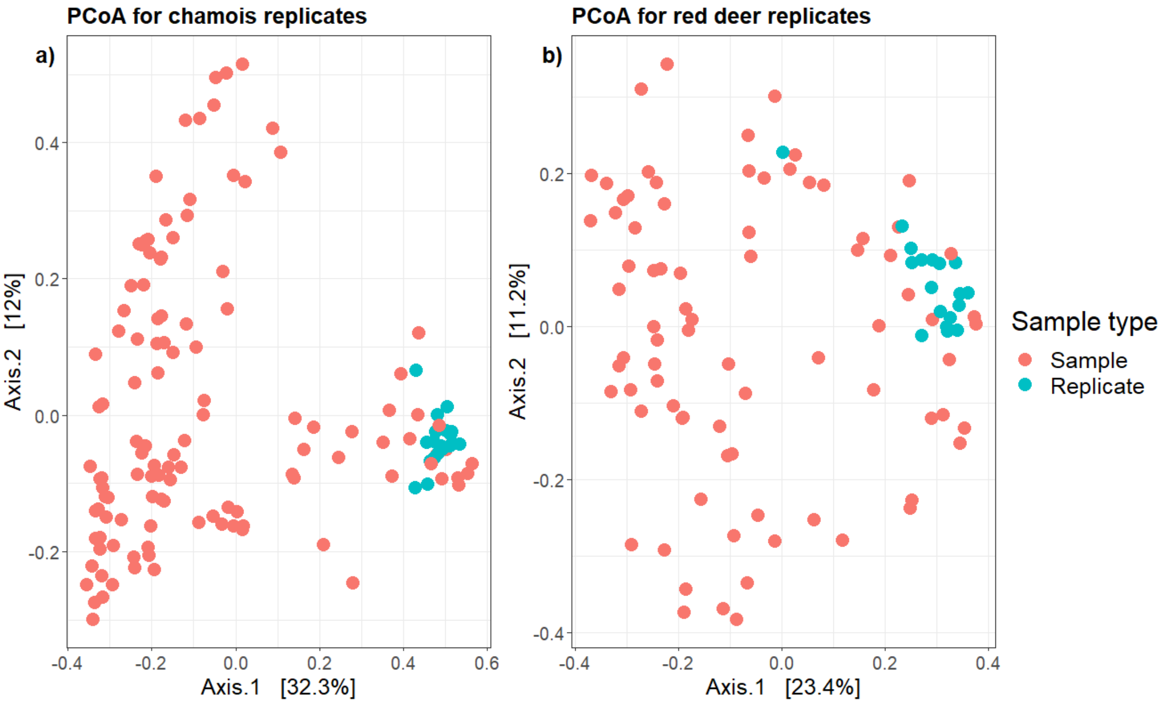

Figure 3.15 Plots of Principal Coordinate Analysis (PCoA) for trnL based on Bray-Curtis distances among replicates and samples of a) chamois and b) red deer.

The final dataset after filtering and refinement of taxonomic assignment contained 144 OTUs, 15 of which could not be assigned to any taxonomic rank. Since the unclassified reads represented only the 4.29% of the total reads, they were removed from the dataset. Within the three families we were able to identify 34 genera. After transforming the read counts in presence absence data, the *trnL* and *ITS1* matrices of presence/absence data were combined in a single matrix, which resulted in a total of 103 genera.

The NMDS analysis resulted in a stress value of 0.168 with four axes. The NMDS plot on the first two dimensions (Figure 3.16) shows a clear separation of chamois diet from that of red deer and domestic sheep, which in turn show a partial diet overlap between them. As can be seen from Figure 3.17 most of the genera contribute to the construction of the NMDS plot.

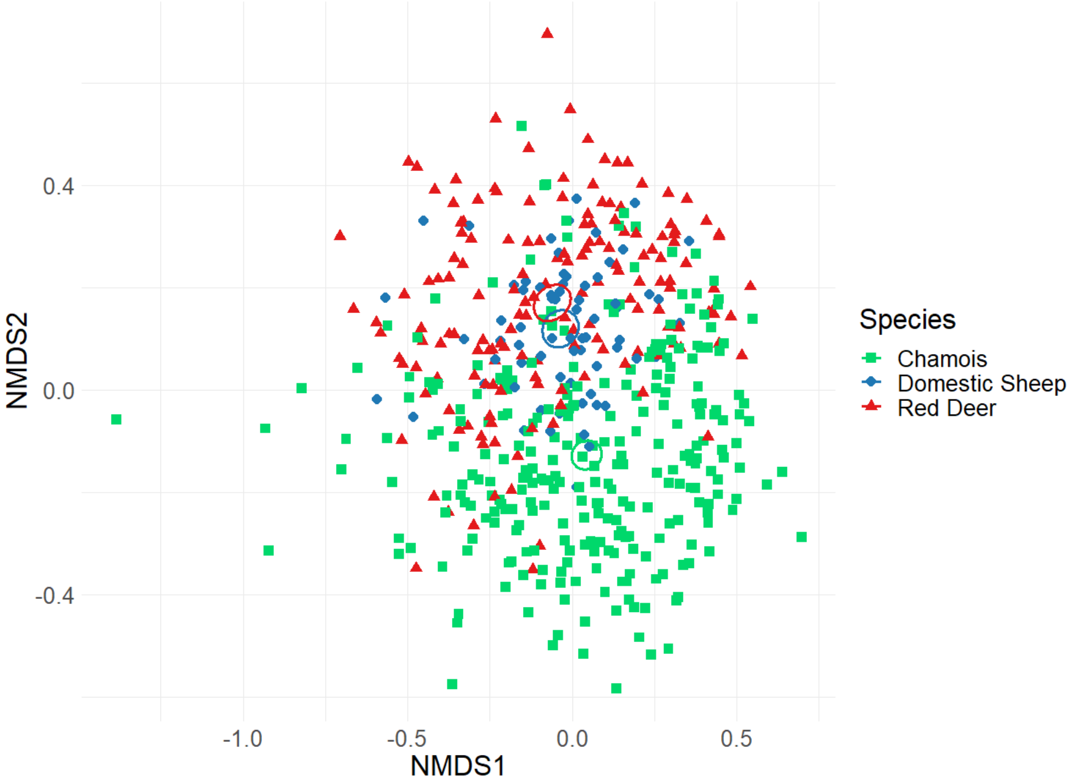

Figure 3.16 Non-metric multidimensional scaling (NMDS) of chamois, domestic sheep and red deer diet composition calculated using the Jaccard index on plants at the genus level identified in the faecal samples and visualized in two-dimensional space (k=4, stress=0.168)

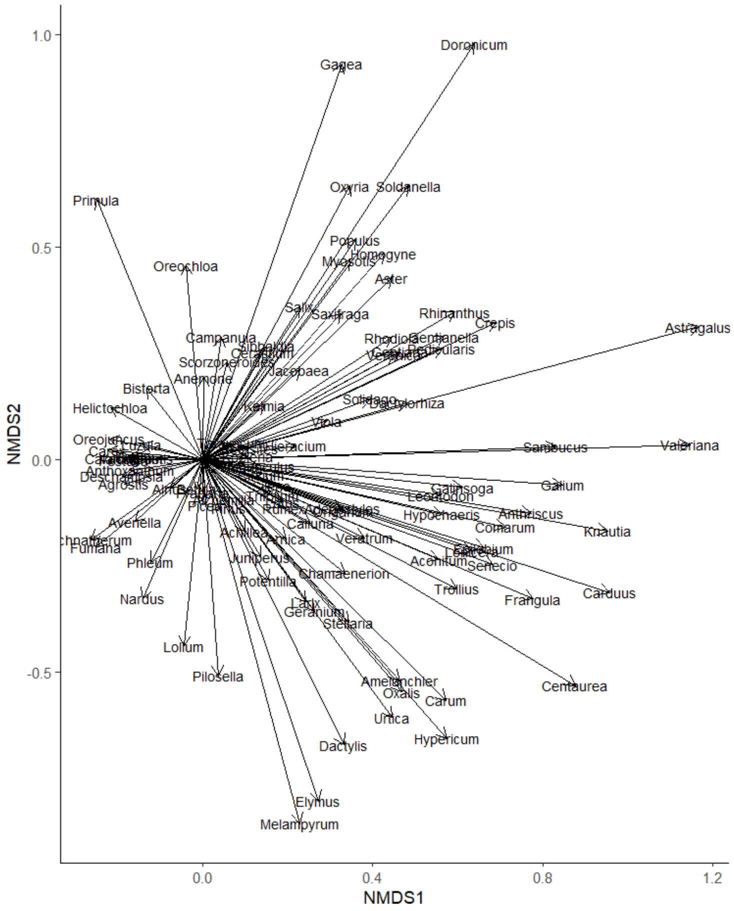

Figure 3.17 Plot showing the scores of the plant genera that contribute to the construction of the NMDS plot.

The multivariate analysis of variance (MANOVA) on the selected four axes showed significant differences between all the variables taken individually and between the interaction between them (Table 3.13). Results were not significant only for the interaction between species, areas, and periods and between the interaction of the four variables, confirming previous results obtained with *trnL* only (Table 3.6).

Table 3.13 Summary statistics of the multivariate analysis of variance (MANOVA). Significant results are reported in bold.

Multivariate analysis	Df	Pillai	approx F	num Df	den Df	Pr(>F)
Species	2	0.60	51.72	8	964	**<0.001**
Park	2	0.79	78.05	8	964	**<0.001**
Area	2	0.15	9.98	8	964	**<0.001**
Period	1	0.26	43.17	4	481	**<0.001**
Species*Park	2	0.11	6.98	8	964	**<0.001**
Species*Area	1	0.06	8.29	4	481	**<0.001**
Park*Area	2	0.19	12.50	8	964	**<0.001**
Species*Period	2	0.17	11.14	8	964	**<0.001**
Park*Period	2	0.09	5.84	8	964	**<0.001**
Area*Period	2	0.04	2.36	8	964	**0.0163**
Species*Park*Area	1	0.03	4.08	4	481	**<0.001**
Species*Park*Period	2	0.19	12.86	8	964	**<0.001**
Species*Area*Period	1	0.00	0.28	4	481	0.8921
Park*Area*Period	2	0.06	3.67	8	964	**<0.001**
Species*Park*Area*Period	1	0.02	2.40	4	481	0.0491
Residuals	484					

The analysis of variance on the first NMDS axis (Table 3.14) showed significant differences in diet composition between species, parks, areas and periods. The interaction of various factors was statistically significant (species and parks; parks and areas; species and periods; parks and periods; areas and periods; species, parks and areas; species, parks and periods) showing thus, that the diet of the species changes between parks, areas and periods.

Table 3.14 Summary statistic of the analysis of variance (ANOVA) on the first NMDS axis. Significant results are reported in bold.

Analysis of variance on NMDS1	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Species	2	1.217	0.609	16.760	**< 0.001**
Park	2	18.907	9.454	260.373	**< 0.001**
Area	2	0.418	0.209	5.758	**0.0034**
Period	1	2.236	2.236	61.591	**< 0.001**
Species*Park	2	1.138	0.569	15.676	**< 0.001**
Species*Area	1	0.138	0.138	3.804	0.0517
Park*Area	2	0.651	0.325	8.962	**< 0.001**
Species*Period	2	0.246	0.123	3.392	**0.0345**
Park*Period	2	0.269	0.135	3.710	**0.0252**
Area*Period	2	0.249	0.125	3.432	**0.0331**
Species*Park*Area	1	0.244	0.244	6.712	**0.0099**
Species*Park*Period	2	0.719	0.360	9.906	**< 0.001**
Species*Area*Period	1	0.008	0.008	0.216	0.6427
Park*Area*Period	2	0.073	0.037	1.012	0.3643
Species*Park*Area*Period	1	0.003	0.003	0.079	0.7789
Residuals	484	17.573	0.036		

The ANOVA on the second NMDS axis (Table 3.15) confirmed most of the results obtained with the first axis. However, the period and the interaction between species and park was not significant as well as the interaction between species, park and period. In addition, in this case, significant results were obtained with the interaction between species and areas, the interaction between parks, areas and periods and the interaction between the four factors (species, parks, areas and periods).

Table 3.15 Summary statistic of the analysis of variance (ANOVA) on the second NMDS axis. Significant results are reported in bold.

Analysis of variance on NMDS2	**Df**	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
Species	2	10.152	5.076	250.363	**< 0.001**
Park	2	2.406	1.203	59.326	**< 0.001**
Area	2	0.842	0.421	20.764	**< 0.001**
Period	1	0.003	0.003	0.139	0.7098
Species*Park	2	0.032	0.016	0.797	0.4514
Species*Area	1	0.480	0.480	23.676	**< 0.001**
Park*Area	2	1.666	0.833	41.086	**< 0.001**
Species*Period	2	0.170	0.085	4.186	**0.0158**
Park*Period	2	0.551	0.275	13.583	**< 0.001**
Area*Period	2	0.217	0.108	5.345	**0.0051**
Species*Park*Area	1	0.115	0.115	5.693	**0.0174**
Species*Park*Period	2	0.065	0.032	1.600	0.2030
Species*Area*Period	1	0.011	0.011	0.535	0.4648
Park*Area*Period	2	0.328	0.164	8.078	**< 0.001**
Species*Park*Area*Period	1	0.181	0.181	8.952	**0.0029**
Residuals	484	9.813	0.02		

The ANOVA on the third NMDS axis (Table 3.16) did not show significant differences between species, but it showed significant differences between parks, areas and periods. In addition, significant differences were found between species and parks, parks and areas, species and periods, the interaction between species, parks and periods and finally between parks, areas and periods.

Table 3.16 Summary statistic of the analysis of variance (ANOVA) on the third NMDS axis. Significant results are reported in bold.

Analysis of variance on NMDS3	**Df**	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
Species	2	0.006	0.003	0.101	0.9042
Park	2	2.667	1.333	48.511	**< 0.001**
Area	2	0.514	0.257	9.351	**< 0.001**
Period	1	1.774	1.774	64.551	**< 0.001**
Species*Park	2	0.476	0.238	8.660	**< 0.001**
Species*Area	1	0.041	0.041	1.478	0.2246
Park*Area	2	0.183	0.092	3.328	**0.0367**
Species*Period	2	0.313	0.157	5.695	**0.0036**
Park*Period	2	0.104	0.052	1.888	0.1525
Area*Period	2	0.082	0.041	1.490	0.2265
Species*Park*Area	1	0.018	0.018	0.654	0.4190
Species*Park*Period	2	1.279	0.640	23.277	**< 0.001**
Species*Area*Period	1	0.006	0.006	0.221	0.6387
Park*Area*Period	2	0.275	0.138	5.010	**< 0.001**
Species*Park*Area*Period	1	0.005	0.005	0.175	0.6757
Residuals	484	13.302	0.028		

The ANOVA on the fourth NMDS axis (Table 3.17), showed significant differences between species, parks and area. Also in this case, many of the interactions showed significant results (species and parks; species and areas; species and periods; parks and periods; species, parks and areas; species parks and periods).

Table 3.17 Summary statistic of the analysis of variance (ANOVA) on the fourth NMDS axis. Significant results are reported in bold.

Analysis of variance on NMDS4	**Df**	**Sum Sq**	**Mean Sq F**	**value**	**Pr(>F)**
Species	2	1.774	0.887	31.040	**< 0.001**
Park	2	0.410	0.205	7.178	**< 0.001**
Area	2	0.368	0.184	6.438	**0.0017**
Period	1	0.002	0.002	0.068	0.7946
Species*Park	2	0.273	0.136	4.774	**0.0088**
Species*Area	1	0.340	0.340	11.895	**< 0.001**
Park*Area	2	0.027	0.014	0.471	0.6246
Species*Period	2	1.381	0.691	24.170	**< 0.001**
Park*Period	2	0.272	0.136	4.763	**0.0089**
Area*Period	2	0.002	0.001	0.027	0.9733
Species*Park*Area	1	0.203	0.203	7.118	**0.0079**
Species*Park*Period	2	0.855	0.428	14.965	**< 0.001**
Species*Area*Period	1	0.002	0.002	0.077	0.7822
Park*Area*Period	2	0.156	0.078	2.730	0.0662
Species*Park*Area*Period	1	0.002	0.002	0.057	0.8109
Residuals	484	13.828	0.029		

The Tukey post-hoc test (Table 3.18) conducted on the axes that found significant differences between species (Tables 3.14, 3.15 and 3.17), showed significant differences between chamois and red deer and chamois and domestic sheep on NMDS1, NMDS2 and NMDS4; between red deer and domestic sheep on NMDS2 and NMDS4.

Table 3.18 Results of the Tukey post-hoc test on the first 3 axes of the PCA to test for differences in the diet of chamois, domestic sheep and red deer diet. Mean difference (Estimate), its confidence intervals (Conf. Low, Conf. High) and adjusted p-value are reported. Significant values are reported in bold.

Tukey Post-hoc Test	**Axes**	**Estimate**	**Conf. Low**	**Conf. High**	**p-value adj.**
Chamois - Red deer	NMDS1	-0.105	-0.149	-0.060	**< 0.001**
Chamois – Domestic sheep	NMDS1	-0.080	-0.139	-0.020	**0.0054**
Red deer - Domestic sheep	NMDS1	-0.025	-0.089	0.039	0.6298
Chamois - Red deer	NMDS2	0.297	0.264	0.330	**< 0.001**
Chamois - Domestic sheep	NMDS2	0.247	0.202	0.291	**< 0.001**
Red deer - Domestic sheep	NMDS2	0.050	0.003	0.098	**0.0362**
Chamois - Red deer	NMDS4	0.089	0.049	0.128	**< 0.001**
Chamois - Domestic sheep	NMDS4	0.160	0.106	0.212	**< 0.001**
Red deer - Domestic sheep	NMDS4	-0.070	-0.127	-0.013	**0.0101**

Trophic niche hypervolume of the total diet (Figure 3.18) showed a clear separation of chamois hypervolume from red deer and domestic sheep hypervolume, especially in the first two NMDS axes, where chamois shows a separate hypervolume portion on the left of the graph suggesting some dietary specialization not shared with the other two species. This can also be observed in almost all NMDS axes: in NMDS1-NMDS4 chamois hypervolume form a separate portion on the left and at the bottom of the graph; in NMDS2-NMDS3 and in NMDS2-NMDS4 on the bottom of the graph. This separation is less pronounced in NMDS1-NMDS3 and NMDS3-NMDS4, although a smaller separate portion of chamois hypervolume can still be observed on the left of both graphs. For the preferred diet (Figure 3.19) this separation is confirmed in all NMDS axes, but not in NMDS3-NMDS4, for which a higher overlap between chamois and red deer can be observed.

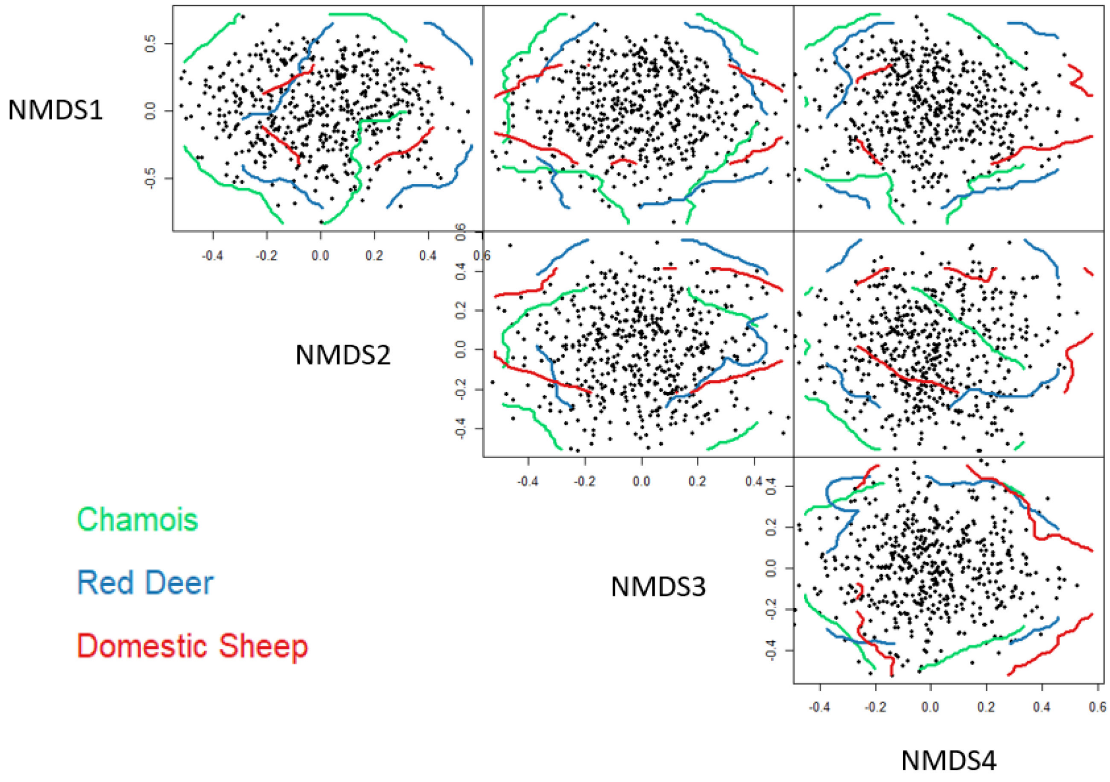

Figure 3.18 Plot of the trophic niche hypervolume of chamois, red deer and domestic sheep for the total diet

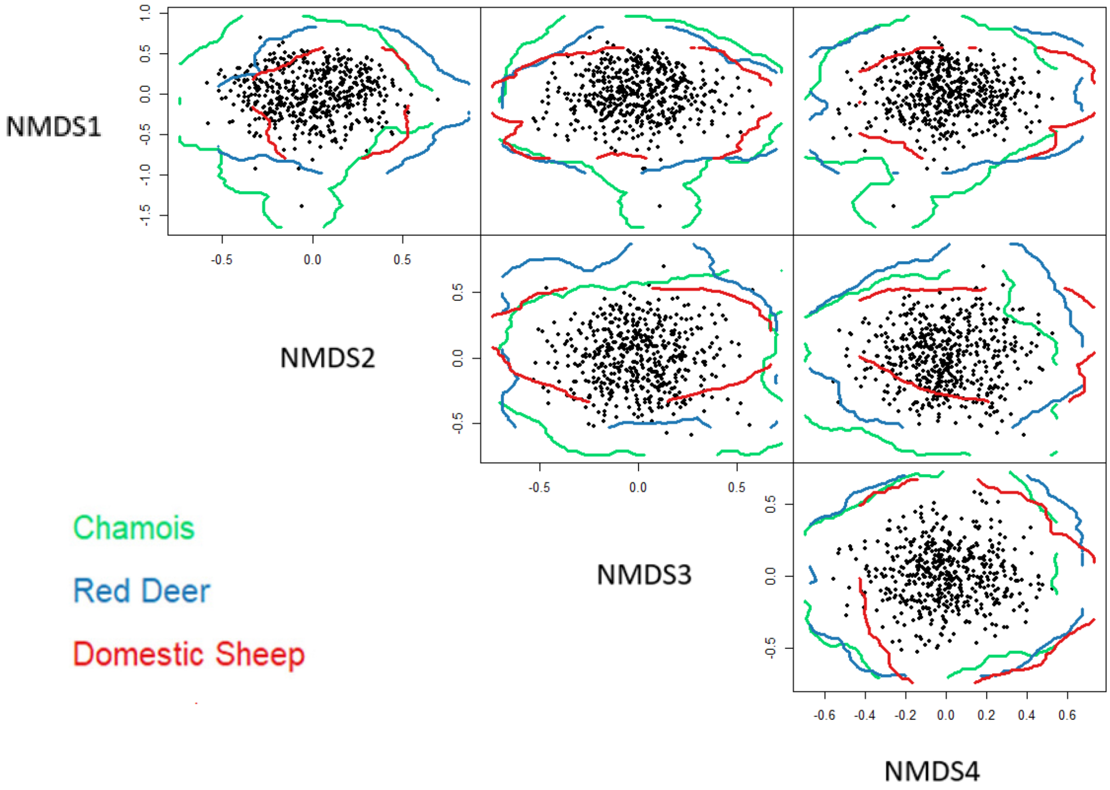

Figure 3.19 Plot of the trophic niche hypervolume of chamois, red deer and domestic sheep for the preferred diet

As shown in Table 3.19, Sørensen-Dice and Jaccard indices predicted a moderate level of trophic niche overlap between chamois and red deer for the total diet (55.2%), while the Jaccard index was low (38.1%). Overlap indexes for the preferred diet were low both for the Sørensen-Dice index (19.5%) and the Jaccard index (10.8%). Overlap between chamois and domestic sheep was low both for the total diet (Sørensen-Dice: 37.9%, Jaccard: 23.4%) and the preferred diet (Sørensen-Dice: 17.5%, Jaccard: 9.6%).

Table 3.19 Overall Sørensen-Dice and Jaccard indices of trophic niche overlap

	Chamois - Red deer		**Chamois - Domestic sheep**	
	Total diet	**Preferred diet**	**Total diet**	**Preferred diet**
Sørensen-Dice	55.2%	19.5%	37.9%	17.5%
Jaccard	38.1%	10.8%	23.4%	9.6%

When looking at the overlap indexes in each park separately (Table 3.20), chamois-red deer overlap in Stelvio National Park for the total diet was low for both the Sørensen-Dice (51.1%) and Jaccard indexes (34.3%). For the preferred diet, both indexes were low (Sørensen-Dice: 29.2%, Jaccard: 17.1%). Overlap with domestic sheep showed low values for the total diet (Sørensen-Dice: 22.1%, Jaccard: 12.4%) and for the preferred diet (Sørensen-Dice: 5.5%, Jaccard: 2.8%). In Paneveggio Natural Park, overlap indexes for chamois and red deer showed low values for the total diet (Sørensen-Dice: 46%, Jaccard: 29.9%) and for the preferred diet (Sørensen-Dice: 14.8%, Jaccard: 8%). Likewise, overlap between chamois and domestic sheep showed low values for the total diet (Sørensen-Dice: 28.5%, Jaccard: 16.6%) and for the preferred diet (Sørensen-Dice: 2.9%, Jaccard: 1.5%).

Table 3.20 Sørensen-Dice and Jaccard indices of trophic niche overlap in Stelvio National Park and Paneveggio Natural Park

	Stelvio National Park				**Paneveggio Pale di San Martino Natural Park**			
	Chamois Red deer		**Chamois Domestic sheep**		**Chamois Red deer**		**Chamois Domestic sheep**	
	Total diet	**Preferred diet**	**Total diet**	**Preferred diet**	**Total diet**	**Preferred diet**	**Total diet**	**Preferred diet**
Sørensen-Dice	51.1%	29.2%	22.1%	5.5%	46%	14.8%	28.5%	2.9%
Jaccard	34.3%	17.1%	12.4%	2.8%	29.9%	8%	16.6%	1.5%

3.4 DISCUSSION

In this study, I used DNA metabarcoding to study the diet of chamois, domestic sheep and red deer, and I summarize and discuss here the main results in three sections: diet and resources utilization in chamois, red deer and domestic sheep, trophic niche overlap between the three species, and implications for conservation. My findings show a higher dietary overlap between chamois and red deer than between chamois and domestic sheep, suggesting a potential for competition for food resources.

Diet and resources utilization in chamois, red deer and domestic sheep

Our study on the diets of chamois, red deer, and domestic sheep reveals the complex and dynamic nature of species interactions and resource utilization. We found that the diet of the three species was more similar in early summer than in late summer and, in this period, was mainly composed by *Vaccinium*, followed by *Geum*. The high consumption of *Vaccinium* in early summer may be related to the fact that many species within this genus in the Alps reach peak maturity, and their berries ripen during this period, representing thus an important food resource to accumulate fat reserves for the winter (Krojerova-Prokesova et al., 2010). During late summer, there was a shift in the diet of the three species, with chamois and red deer consuming a smaller proportion of *Vaccinium* and a greater proportion of *Geum* and *Alnus*, while domestic sheep showed a stronger preference for forbs like *Geum*, *Alchemilla*, and *Rumex*. Therefore, these differences in diet may reflect the different feeding strategies of the species, with domestic sheep relying more heavily on forbs during the late summer season, when the availability of berries and leaves may have decreased, and chamois and red deer exhibiting a mixed feeding strategy, consuming both woody plants and herbaceous species. This observation aligns with the general classification of ungulates in relation to their foraging behavior, which identifies three categories: browsers, grazers, and mixed feeders (Gordon, 2003; Hofmann, 1989; Hofmann & Stewart, 1972; Javier Pérez-Barbería et al., 2001). Browsers mostly feed on woody plants, and their digestive system is adapted to rapidly excrete the highly lignified cell wall fraction (Gordon-Andrew et al., 1994), while grazers, such as domestic sheep, mostly consume grasses and other herbaceous species, and mixed feeders, such as chamois and red deer, include a mixture of browses and grasses/herbaceous plants. In addition, as summer progresses, some plant species begin to senesce and fruit production decreases which may have led the three herbivores to shift their diet toward other plant resources which remain abundant during late summer. Further investigations, exploring food availability in the studied areas and the nutritional quality of the different plant species consumed by the three herbivores in different seasons, would help to further understand their dietary preferences and the factors that influence their foraging behavior.

Trophic niche overlap among chamois, domestic sheep and red deer

The main result of the MANOVA analysis on the overall diet with relative abundances (Table 3.6) showed that the diet of the chamois is different depending on the presence of a potential competitor and on the park. However, this result was confirmed only by the ANOVA on the third axis of the PCA (Table 3.9), which was correlated with the genus *Alnus*, composed of woody plants which could be associated with browsers animals. Therefore, my results suggest that, given that chamois and red deer are considered mixed feeders and domestic sheep is a grazer, dietary differences observed due to the presence of a potential competitor were largely explained only by this axis, which may not be the best representation of the feeding strategy of the species. On the other hand, PCA axis 1, negatively correlated with *Geum* and positively correlated with *Vaccinium*, could be interpreted as a grazing to mixed feeders continuum, while axis 2 (negatively correlated with *Alnus* and positively correlated with *Geum*), as a browser to grazer continuum. These axes better reflect the feeding strategies of the three species and showed no differences between parks and areas, suggesting a high similarity in plant item consumption and thus, a potential for dietary overlap between these three herbivorous species. Despite this, we also detected significant differences between chamois, red deer and domestic sheep diet, suggesting a possible mechanism of resource partitioning in order to limit the effect of interspecific competition (Chesson, 2000; Macarthur & Pianka, 1966).

When considering presence/absence data for *tnrL+ITS1*, however, we obtained somewhat different results: the NMDS showed a higher segregation between chamois, red deer and domestic sheep than the previous analysis, with the latter two species showing closer clustering (Figure 3.16). The overall MANOVA test (Table 3.13) was in agreement with results obtained with the relative abundances of *trnL*, but the ANOVA on each NMDS axes showed that the diet of the species changed between areas and parks in three out of four axes. This discrepancy of the presence/absence data with the relative abundance results could reflect the above-mentioned difference in the number of rare plant items in the diet of the three species. A similar outcome was already observed in a study carried out by Ter Schure et al. (2021), among ten domestic (goat, cattle, water buffalo) and wild (sambar, barking deer, Indian hare, Indian crested porcupine, bonnet macaque, wild boar and Asian elephant) herbivore species, in which they also found contrasting results when using the two methods resulting in larger differentiation in some analyzed herbivore pair species and smaller differentiation in other pairs. Moreover, the additional genera detected with ITS1 may have contributed to the higher differentiation observed between the species by capturing rare food items that were missed with the *trnL* only approach.

The analysis of the hypervolume calculation with overall relative abundances suggests a higher overlap between chamois and red deer than between chamois and domestic sheep. Diet overlap between chamois and red deer was very high for both the total diet (Sørensen-Dice index: 88.9%, Jaccard index: 80.9%) and the preferred diet (Sørensen-Dice: 83.2%, Jaccard index: 71.3%). Previous studies reported a high similarity in the diet of chamois and red deer in the Central Italian Alps (Andreoli et al., 2016), in the Western Alps (Bertolino et al., 2009), in the French Alps (Redjadj et al., 2014), and in the Jeseniky Mountains (Czech Republic) (Homolka & Heroldová, 2001). A high degree of spatial and trophic overlap with red deer was also recorded by (Lovari et al., 2014) and (F. Ferretti et al., 2015) for the Apennine chamois. The hypervolume calculation with relative abundances between chamois and domestic sheep for the total diet showed a high overlap for the Sørensen-Dice index (70.5%) and a moderate overlap for the Jaccard index (54.4%). For the preferred diet the Sørensen-Dice index reported a moderate value of overlap (56.3%), while for Jaccard was low (39.2%). The two metrics both express the similarity between two communities, however, the Jaccard index calculates the proportion of unique species in the two communities compared to the total number of species recorded, while the Sørensen index gives double weight to the species that are shared between the two communities (Hao et al., 2019). The high Sørensen-Dice index and the low Jaccard index for the preferred diet, could indicate a high similarity in the diet between chamois and domestic sheep, but possibly, one of the two species is consuming a larger proportion of the shared food items. Previous research has analyzed patterns of dietary overlap between chamois and domestic sheep to evaluate potential competition and interspecific interactions. For instance, La Morgia and Bassano (2009), who studied the summer diet of male alpine chamois in the Italian Alps, found a high degree of overlap and a reduction in the niche breadth of Alpine chamois in the presence of domestic sheep grazing in the same range. The overlap was higher when the two species were spatially segregated, while when domestic sheep arrived in the same range of Alpine chamois, the overlap was lower and they found a change in the dietary composition of chamois, resulting in a reduction in their consumption of forbs. A moderate to high overlap was also observed by Espunyes et al. (2019) between the Pyrenean chamois and domestic sheep even if they could not detect a specific effect of sheep flock on chamois diet.

Importantly, results conducted separately on each park with relative abundances confirmed a higher overlap between chamois and red deer than between chamois and domestic sheep (Table 3.12), with which overlap was low in both parks and for both the total and the preferred diet. The differences observed for the two indices in the case of chamois and red deer overlap in both PNS and PPPSM, reflect again a high similarity in the diet of the two species, but with differences in their relative contribution to the diet.

Presence/absence data also confirmed a higher overlap between chamois and red deer than between chamois and domestic sheep, especially for the total diet (Table 3.19). This observation was consistent across each park (Table 3.20), although, in this case, none of the overlap values reached high levels (no more than 51%), indicating the potential for trophic segregation among these species.

In conclusion, compared with presence/absence data, our results based on relative abundances gave a higher degree of overlap between the studied species. Deagle et al. (2019) showed that presence/absence data tend to overestimate low frequency abundances and its accuracy can be affected by the data filtering threshold, leading to inaccurate conclusions especially in trophic niche studies, when results show that species are segregating. This is because the distinction may only be based on the partitioning of rare food items that are given equal weight as common food items. Relative abundances, on the other side, are less susceptible to misinterpretation of rare food items, such as low abundance reads that may come from sources such as PCR errors or sequencing errors or contamination as well as from accidental ingestion (Deagle et al., 2019). In fact, when converting data to presence/absence the information on the relative abundance of each food item is removed and each of them is equally present in each sample. Moreover, Deagle et al. (2019) reported a more accurate representation of population-level diet even with moderate recovery bias incorporated. Therefore, I conclude that relative abundance-based data, by considering the relative contribution of the different food items, provided a more comprehensive understanding of the species' feeding habits and the trophic interactions.

Implications for conservation

Resource partitioning is typically employed by coexisting species to reduce interspecific competition (Chesson, 2000; Macarthur & Pianka, 1966). Our findings reveal a higher degree of trophic overlap between chamois and red deer than between chamois and domestic sheep, suggesting possible interspecific competition between wild species. It is important to note however, that the high level of observed overlap could also be attributed to an abundance of resources in the area. Indeed, a high degree of overlap between two or more organisms does not always indicate competition, since they can utilize plenty of resources without adversely affecting each other (Pianka, 1974). Nevertheless, given the documented negative effect of increasing red deer densities on chamois growth rate (Corlatti et al., 2019a) and the already observed high similarity of dietary item consumption between chamois and red deer in other areas (Andreoli et al., 2016; Bertolino et al., 2009; Homolka & Heroldová, 2001; Redjadj et al., 2014), we cannot exclude a potential for interspecific competition among these species. Given these results, it is vital to develop management plans that consider the potential impact of competition between these species. Studying the effect of reduced red deer densities (where possible, for example during culling), and monitoring chamois populations continuously, particularly where populations have declined, is crucial.

Conversely, chamois and domestic sheep exhibited a lower trophic overlap with respect to chamois and red deer, and within each park, the overlap was minimal, implying trophic segregation and coexistence through resource partitioning. While sheep grazing can have positive effects for the conservation of grassland ecosystem by promoting biodiversity, forage production, improving soil fertility and positively influencing vegetation composition through a high species turnover rate (Benthien et al., 2018; Fernández-Guisuraga et al., 2022), potential competition between these species could still arise over time with possible negative consequence on resource availability for chamois (Rüttimann et al., 2008). For example, sheep grazing in the summer could diminish food availability for chamois in other seasons, potentially resulting in lower survival rates for chamois offspring and slower population growth (Mysterud, 2000; R. J. Putman, 1996; Rüttimann et al., 2008). In addition, diseases such as brucellosis (Ferroglio et al., 2000; Garin-Bastuji et al., 1990) and infectious keratoconjunctivitis (Belloy et al., 2001; Giacometti et al., 2002; Nicolet & Freundt, 1975) can be transmitted from livestock to wildlife, and domestic sheep can represent a potential source of infection for chamois (Gaffuri et al., 2006). Therefore, monitoring the chamois-domestic sheep interaction continually would be desirable to observe any changes in resource utilization in other periods of the year, as well as to assess the potential impacts of grazing on chamois health and disease transmission. For instance, further investigation, including data on the winter diets, could be useful to study changes in chamois feeding habits during periods of scarcity of resources and to assess the long-term impacts of domestic sheep summer grazing on chamois populations. This would provide a more comprehensive understanding of the ways in which species interact with their environments and one another and could have important implications for wildlife management and conservation efforts.

4 A MULTI-CRITERIA DECISION MAKING APPROACH FOR CHAMOIS MANAGEMENT IN THE ALPS

4.1 INTRODUCTION

Preserving the function of natural ecosystems and their species communities is fundamental for ethical and biological reasons; indeed, as human activities have contributed significantly to the damages caused to biodiversity, it is our responsibility to take action to restore what has been lost (Smith & Smith, 2007). Conservation measures are thus necessary for recovering biodiversity losses. Very often, management interventions have led to social conflicts as a result of the diverse objectives or interests of stakeholders (Andonegi et al., 2021; Guaita Martínez et al., 2019), who are seldom involved in the decision-making process. Indeed, wildlife management requires knowledge of species biology and ecology, but must not ignore the human dimension, including engagement of stakeholders and economic evaluations (Riley et al., 1973). In this context, participatory management approaches can lead to better conservation outcomes, since they allow for a better understanding of the problem from different perspectives and resulting in more effective long-term solutions (Dick et al., 2016). For example, gaining insight into people's perceptions and attitudes towards conservation efforts can aid in the identification of more targeted management strategies that better address the underlying issues and promote a greater level of social acceptance (Klich et al., 2018; Tosi et al., 2015).

A powerful method widely applied to natural resources management including forest management (e.g. (Diaz-Balteiro & Romero, 2004; Laukkanen et al., 2002; Martin et al., 2000), conservation planning (e.g. (Bayliss et al., 2003; Moffett & Sarkar, 2006) or wildlife management (e.g Berbel & Zamora, 1995) is the Multi-Criteria Decision Making Analysis (MCDA) (Adem Esmail & Geneletti, 2018). This approach can provide a comprehensive framework for assessing alternative options and facilitating communication between groups with differing interests (Davies et al., 2013), support a more informed and participatory decision- making process and includes quantitative and/or qualitative aspects of the problem (Mendoza et al., 1999). In this study MCDA has been applied to evaluate the trade-offs of several management strategies aimed at minimizing the potential for interspecific competition between chamois and red deer or domestic sheep. Indeed, in some Alpine areas, chamois population densities have experienced considerable decreases in the last decade; at the same time, red deer has reached higher densities, increasing its range and its consistency and suggesting the possibility of competition effects between the two species (Corlatti et al., 2019). In addition to this, as domestic sheep are moved to Alpine pastures in summer, and chamois share the same habitat with this livestock during this season, there is the potential for multispecific competition with possible consequences on chamois population growth, survival, and reproduction (Begon et al., 2006).

The purpose of this study was thus to apply MCDA to evaluate the trade-offs of several management strategies aimed at minimizing the possible effects of interspecific competition by considering the impact of each strategy on the local economy, social-cultural aspects, and the environment based on the opinions of the stakeholders involved in the study.

4.6 MATERIALS AND METHODS

Methodological background

MCDA is a decision-making approach used to support decision-makers in selecting the most appropriate alternative by considering the trade-offs associated with each option (Adem Esmail & Geneletti, 2018) and includes quantitative and/or qualitative aspects of the problem (Mendoza et al., 1999). According to Silva & Belderrain (2019) the MCDA approaches can be classified as aggregation methods or outranking methods.

Aggregation methods use additive models and assume that the decision maker is capable of identifying several discrete alternatives to be compared, and of setting up hierarchical criteria for evaluating them (Gomes et al., 2004; Silva & Belderrain, 2019).This group includes several methods such as Multi-Attri-bute Value Theory (MAVT), Multi-attribute Utility Theory (MAUT), Measuring Attractiveness by a Categorical Based Evaluation Technique (Macbeth) and Analytic Hierarchy Process (AHP) (Silva & Belderrain, 2019).

Instead, by avoiding the comparison of the alternatives and not requiring the structuring of the criteria in a hierarchical manner, outranking methods are more flexible. The most commonly used techniques classified within this method are PROMÉTHÉE (Preference Ranking Organization Method for Enrichment Evaluations) (Mareschal et al., 1984) and ELECTRE (Elimination et Choix Traduisant la Réalité) (Roy, 1968; Silva & Belderrain, 2019).

As shown in Figure 4.1, the overall process involved in structuring an MCDA is the same, regardless of the specific technique used and can be summarized in five steps:(i) the identification of the problem and the decision context; (ii) the structuring of the problem through the definition of the values, objectives, and uncertainties; (iii) the building of the model through the identification of the alternatives and the definition of the criteria with the decision makers; (iv) the evaluation and selection of the alternatives using an analytical model and (v) the development of an action plan which is fundamental to support decision making through the ranking of the alternatives.

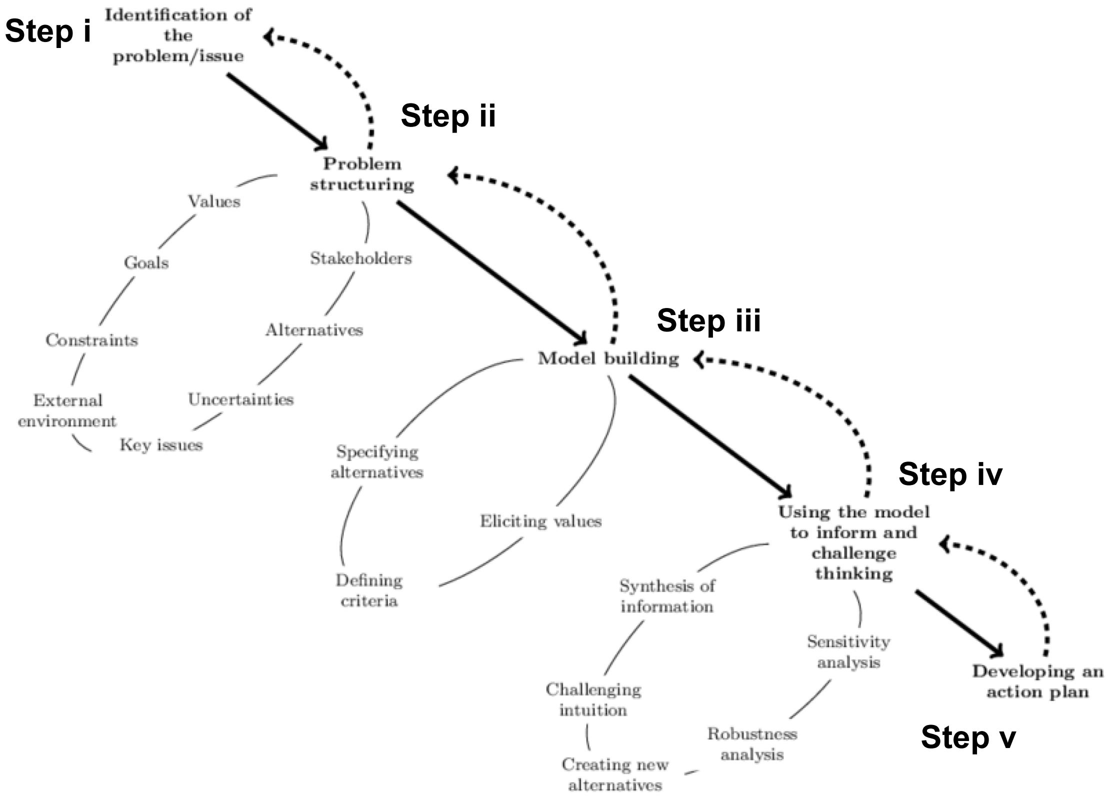

Figure 4.1 Steps in MCDA. From Belton and Stewart, 2002.

As can be seen from the diagram, the process is iterative which means it is possible to reverse at each step and modify the model if necessary (Silva & Belderrain, 2019).

The methodological steps followed in this study, consistent with the overall MCDA process, can be summarized as follows. In the first step, the problem was identified, which involves the identification and structuring of the attributes to measure how effectively the objectives are met (V. Ferretti & Comino, 2015). The second step involved the identification of the alternative options to solve the problem and of the criteria and sub-criteria that characterize each attribute of the options. This step involved the definition of a panel of experts in order to validate the identified criteria and eventually identify new criteria. In addition, during this phase the weighting of each criteria and sub-criteria was identified; that is, in order to consider all the aspects of sustainability on the same level, 1/3 of the weight was assigned to each dimension (economic impact, socio-cultural impact and environmental impact) and the weight of ((1/3)/n) was assigned to each criteria, with n being the number of criteria for one dimension. The third step involved the elicitation of stakeholder preferences for criteria scoring. The criteria scoring was then performed by sending out a questionnaire in which each participant was asked to assign a value from 0 to 5 to each identified criterion and sub-criterion, where 0 represents an impact close to zero and 5 represents a very strong positive impact. Once all criteria and sub-criteria were scored and weighted, the next step involved the analysis of the results for aggregating all the selected criteria to assign a final comparable score to each alternative. To this end, the weighted sum method (WSM) (Belton & Stewart, 2002; V. Ferretti & Comino, 2015) was used, which involve the application of the following equation:

$$V(a) = \sum w_i \times v_i(a_i)$$

where $V(a)$ represents the overall value of the alternative a, w_i is the weight assigned to the attribute i and $v_i(a_i)$ is the score assigned to the attribute i in relation to alternative a. Given that each criterion and sub-criterion in the three dimensions is rated on a scale of 1 to 5 and that the three dimensions have a weight of 1/3, the highest Weighted Sum Model (WSM) value that can be achieved for each alternative in our study is 5.

Case study and development of the analysis

The study area was located in the Province of Trento in Italy, where a potential for interspecific competition between chamois and red deer or domestic sheep was hypothesized. The research problem was defined as: “How can we manage wild and domestic species in order to reduce the competition between chamois, red deer and domestic sheep?”.

Structuring the decision problem

In order to reduce competition between chamois and red deer or sheep we evaluated the economic, socio-cultural and environmental trade-offs of four management strategies: i) regulating red deer densities by increasing culling quotas for red deer; ii) where chamois is at high density, translocating red deer to other areas; iii) limiting sheep grazing in areas with high densities of chamois; iv) maintaining the status-quo. The analysis of relevant literature allowed the identification of the criteria and sub-criteria within each dimension (economic, socio-cultural and environmental). The following are the sources used for the analysis:

a) Environmental impact dimension

- Impact of high red deer densities on biodiversity in relation to forest renewal, other autochthonous species and chamois:
  (Andreoli et al., 2016; Bertolino et al., 2009; Bonardi, 2015; Borowski et al., 2021; Corlatti et al., 2019a; D'Aprile et al., 2020; Homolka & Heroldová, 2001; Motta & Franzoi, 1997; Motta & Nola, 1996; Pedrotti & Bragalanti, 2008; Redjadj et al., 2014b; Richard et al., 2010; Rooney & Waller, 2003)
- Impact of sheep grazing on biodiversity in relation to forest renewal, other autochthonous species and chamois:
  (Fankhauser et al., 2008; la Morgia & Bassano, 2009; Mattiello et al., 2007; Molle et al., 2005; Rüttimann et al., 2008; Sharrow et al., 1992).

b) Economic impact dimension

- Evaluation of costs and benefits related to the culling activity:
  (Goio et al., 2008; Grilli et al., 2013; Scolozzi, 2012)
- Potential financial support for grazing activity through Common Agricultural Policy (CAP) subsidies and Rural Development Program (RDP) funding programs
  (*Italy CAP Strategic Plan 1.2*, 2022; *Italy-Rural Development Programme (Regional)-Trento*, 2021)

c) Socio-cultural impact dimension

- Impact of high red deer densities on the social dimension in relation to the damages caused to local economic activities.
  (Corgatelli et al., 2019; Pedrotti & Bragalanti, 2008; Valente et al., 2020)
- Impact of sheep grazing on local economic activities. No relevant literature was found for this aspect; however, L'Adige and Il Nuovo Trentino, reports several cases in which grazing sheep have caused damage to crops, defining the situation as an ancient "war" between shepherd and farmers[1]. Hunters refers to the acceptability of the considered alternatives by local hunters as a result of a greater availability of grazing for wildlife.

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[1] https://www.ladige.it/cronaca/2020/07/01/pecore-sui-terreni-privati-arriva-il-conto-al-pastore-che-dovra-pagare-i-danni-1.2539008
https://www.giornaletrentino.it/cronaca/trento/le-pecore-danneggiano-il-pastore-paga-1.2375569
https://www.giornaletrentino.it/cronaca/vallagarina/besenello-guerra-contro-le-pecore-1.1534366
https://www.giornaletrentino.it/cronaca/alto-garda-e-ledro/con-le-pecore-devasta-un-orto-1.2022670

By doing this, for each alternative, three dimensions, seven indicators and 14 sub-indicators were identified. The validation of the identified criteria and sub-criteria was performed by sending a questionnaire (Supplementary material 4.1) to a panel of experts balanced between stakeholder types: researchers from the field of biology/ecology (n=4), administrators (n=2), economists (n=1), technologist (n=2). Following analysis of their evaluations, the three dimensions (economic impact, socio-cultural impact and environmental impact) were reduced to 6 indicators and 13 sub-indicators, as described in the next paragraph.

Criteria and sub-criteria of each dimension

Dimension 1: Economic impact

The economic impact dimension was divided in two indicators, costs, and benefits with two sub-indicators each. Private and public and refers to the costs and benefits generated by the application of each alternative to private or public stakeholders (Table 4.1).

Table 4.1 Criteria and sub-criteria of the economic impact dimension

Dimension	Criteria	Sub-criteria
Economic impact	Benefits	Privates
		Public
	Costs	Privates
		Public

Dimension 2. Socio-cultural impact

This dimension includes one indicator: ‘social acceptance’, and four sub-indicators (local populations, hunters, farms/breeders and non-local populations) and refers in general to the acceptance of each alternative by the four groups identified as being involved in the decision-making process (Table 4.2).

Table 4.2 Criteria and sub-criteria of the socio-cultural impact dimension

Dimension	Criteria	Sub-criteria
Socio-cultural impact	Social acceptance	Local populations
		Hunters
		Farms/breeders
		Nonlocal populations

Dimension 3. Environmental impact

This criterion included three indicators (flora, fauna and chamois), and 7 sub-indicators (impact on the composition and distribution of forests, herbaceous and shrubby vegetation and biodiversity, pasture maintenance, impact on other autochthonous species, impact on diseases transmission, impact on chamois demography and growth rates; Table 4.3).

Table 4.3 Criteria and sub-criteria of the environmental impact dimension

Dimension	Criteria	Sub-criteria
Environmental impact	Flora	Impact on the composition and distribution of forests, herbaceous and shrubby vegetation, and biodiversity
		Pasture maintenance
	Fauna	Impact on other autochthonous species
		Impact on diseases transmission
	Chamois	Impact on chamois demography and growth rate

Description of each criteria and sub-criteria within each alternative

In order to reduce competition for food resources between chamois, red deer and domestic sheep, four alternative options were identified as follow:

1) Regulating red deer densities by increasing culling quotas for this species: the first option aims at reducing red deer consistencies by increasing the number of animals to be hunted through a specific hunting control program.

   Within the **economic impact** dimension, ‘private costs’ refer to the costs that private individuals should bear if this alternative is applied, such as the purchase or maintenance of hunting equipment or dogs, while ‘public costs’ involve the costs to be allocated for funding the hunting control plan and/or the costs of bureaucracy.

   ‘Private benefits’ consider the economic value of the slaughtered animals in the meat processing chains as well as to the economic benefits generated by the possible reduction of damages that red deer cause to crops of local populations as a consequence of reduced red deer densities. ‘Public benefits’ refer in general to the economic benefits which the community would enjoy directly or indirectly generated for example by a reduction in road collisions with wildlife and/or by a greater repopulation of chamois with effects on the tourist attraction, but also of a greater tourist flow determined by the hunting activity.

   Regarding the **socio-cultural impact** dimension, local populations refer to the acceptability of this alternative by owners of vegetable gardens for private use in relation to any damages caused by grazing and trampling of red deer and domestic sheep. Indeed, even being partially a density independent problem, red deer damage to private crops represents a problem of great weight in terms of social acceptance rather than an economic problem (Pedrotti & Bragalanti, 2008; Pedrotti & Gandolfi, 2022). Hunters refers to the acceptability of the considered alternatives by local hunters, following the increase of the huntable animals. Farms/breeders refers to the acceptability of this alternative by farms and/or breeders following, for example, a greater availability of the herbaceous component in meadows-pastures due to the reduction of grazing impacts. Non-local populations refer to the acceptability of this alternative by citizens, environmental associations and any other stakeholder interested in the topic for ethical or ideological reasons.

Within the **environmental impact** dimension, the ‘impact on the composition and distribution of forests, herbaceous and shrubby vegetation and biodiversity’ refers to the positive impact that the application of this alternative would have in terms of forest renewal and on the shrub layers of the undergrowth, with consequences on the specific diversity and ecological stability of the forests, as well as on the competitive relationships between plant species (in relation to trampling and grazing). ‘Pasture maintenance’ refers to the positive impact that the application of this alternative would have in terms of maintaining open areas of secondary grazing with consequences on the recolonization times of these areas by shrub species and therefore on the trophic availability for other species (in relation to trampling and grazing). ‘Impact on other autochthonous species’ refers to the positive impact that the application of this alternative would generate on the other native species present in the area, with consequences therefore on the availability of resources and/or on the possible reduction of the disturbance. ‘Impact on disease transmission’ refers to the positive impact that the application of this alternative would have on the transmission of pathogens in the wild-wild and wild-domestic interfaces. ‘Impact on chamois demography and growth rates’ refers to the impact that this alternative would have on the demographic component and on the growth rate of the chamois, thus influencing the population densities and annual growth rates.

2) <u>Red deer translocation in other areas</u>: the second option aims at reducing red deer densities by translocating red deer from areas of preferred chamois habitat to other less important areas, possibly based on genetic studies for the choice of the appropriate red deer population to translocate. Criteria and sub-criteria were defined as follows.

Within the **economic impact** dimension, ’private costs’ refer to the costs that private individuals must bear in order to carry out red deer translocation activities (for example, for the purchase of equipment and clothing), while ‘public costs’ involve the costs related to the construction of corral traps, animal transfer costs, personnel recruitment, and bureaucracy costs. ‘Private benefits’ refer to the benefits generated by the possible reduction of damages that red deer cause to crops of local populations. ‘Public benefits’ refer in general to the economic benefits which the community would enjoy directly or indirectly from the increased chamois population as a result of the reduced deer density, for example the effects on the tourist attraction.

In the **socio-cultural impact** dimension, the local populations, farms/breeders, and non-local populations are evaluated in the same way as the previous alternative, while ‘hunters’ refers to the acceptability of the considered alternatives by local hunters, following the reduction in the number of huntable animals resulting from translocating them.

Regarding the **environmental impact** dimension, the description for each criteria and sub-criteria is the same as the previous alternative.

3) <u>Limiting sheep grazing in areas with high densities of chamois</u>: the aim of this alternative is to reduce the dietary competition between chamois and domestic sheep by limiting sheep grazing in those areas where chamois densities are higher, or areas where the availability of resources is lower.

Within the **economic impac**t dimension, ‘private costs’ refer to the costs that private individuals have to bear generated by loss of incomes for the CAP and RDP funds and/or by the purchase of lost forage and/or by the rental of new land. ‘Public costs’ involve the costs related to bureaucracy and with permanent monitoring for rules compliance. Private benefits refer to the economic benefits that private individuals would benefit from renting new pastures and/or selling forage by farmers. Public benefits refer in general to the economic benefits which the community would enjoy directly or indirectly generated for example by a greater repopulation of chamois with effects on the tourist attraction.

Regarding **socio-cultural impact**, ‘local populations’ refer to the acceptability of the considered alternative by owners of orchards, vineyards or vegetable gardens for private use in relation to any damages caused by grazing and trampling of domestic sheep. Farms/breeders refers to the acceptability of this alternative by farms and/or breeders as a result, for example, a reduced grazing availability with consequent revision of the pasture plans. Non-local populations refer again to the acceptability of this alternative by citizens, environmental associations and any other stakeholder interested in the topic for ethical or ideological reasons.

Regarding the **environmental impact** dimension, the impact on the composition of forests, of herbaceous and shrubby vegetation, the impact on other autochthonous species and the impact on disease transmission, the description is the same as the previous alternatives. Pasture maintenance refers to the positive impact that the application of this alternative would have in terms of maintaining open areas of secondary pasture. Through the selection of food essences, trampling and the release of manure, grazing can bring significant changes in the structure of the vegetation and in the composition and variety of plant species. The criteria ‘chamois’ had the same definition as above.

4) Maintaining the status-quo: this alternative involves maintaining the current management system for domestic and wild populations. Criteria and sub-criteria are the same as for the other alternatives, but all refer to the cost and benefit of maintaining the current state of management of the species.

4.7 RESULTS

The questionnaire received a total of 51 replies, 24 from researchers, four from environmental associations, nine from hunters, four from farms/breeders and 10 from research institutions or parks. Since missing data represent only 0.9% of the total data, they were left in the dataset.

Overall ranking of the alternative options

As shown in Table 4.4, overall, between the different strategies identified to reduce competition between chamois and red deer or domestic sheep, alternative 1 (increasing red deer culling quotas) was ranked the highest among all four alternatives. It should be noted that the scores acquired by the various alternatives, even identifying a precise classification, do not show particularly evident score differences, especially for the alternative 1 (increasing red deer hunting quota) and the alternative 4 (maintaining the status quo). Alternative 1 obtained the highest score in the socio-cultural impact dimension (1.05), followed by the environmental impact (0.99) and the economic impact dimension (0.81). The second ranked alternative option (maintaining the status quo), obtained the highest score in the environmental impact dimension (1.04), followed by socio-cultural impact (0.95) and economic impact (0.70). Limitation of sheep grazing was ranked third and received the highest score in the environmental impact dimension (1.01), while the socio-cultural and the economic impact dimension were comparable (0.79 and 0.78, respectively).

The lowest ranked alternative option was the translocation of red deer in other areas, which obtained the highest score for economic impact (0.86), while the socio-cultural and the environmental impact dimension received a similar score (0.83 and 0.82, respectively).

Table 4.4 Standardized scores of the alternatives ranked in a descending order, from the alternative that received the highest score, to the alternative that received the lowest score.

Alternative options	Economic impact	Socio-cultural impact	Environmental impact	Total score
Increasing red deer culling quotas	0.81	1.05	0.99	2.84
Maintaining the status quo	0.70	0.95	1.04	2.70
Limiting sheep grazing in certain areas	0.78	0.79	1.01	2.58
Red deer translocation in other areas	0.86	0.83	0.82	2.51

Ranking of the alternative options for each stakeholder group

As can be seen from Figure 4.2 the scores given to each dimension of each alternatives varies considerably when considering the different stakeholder groups, resulting in different preference scores for each dimension and reflecting, therefore, the different individual values and perception of the problem by each individual.

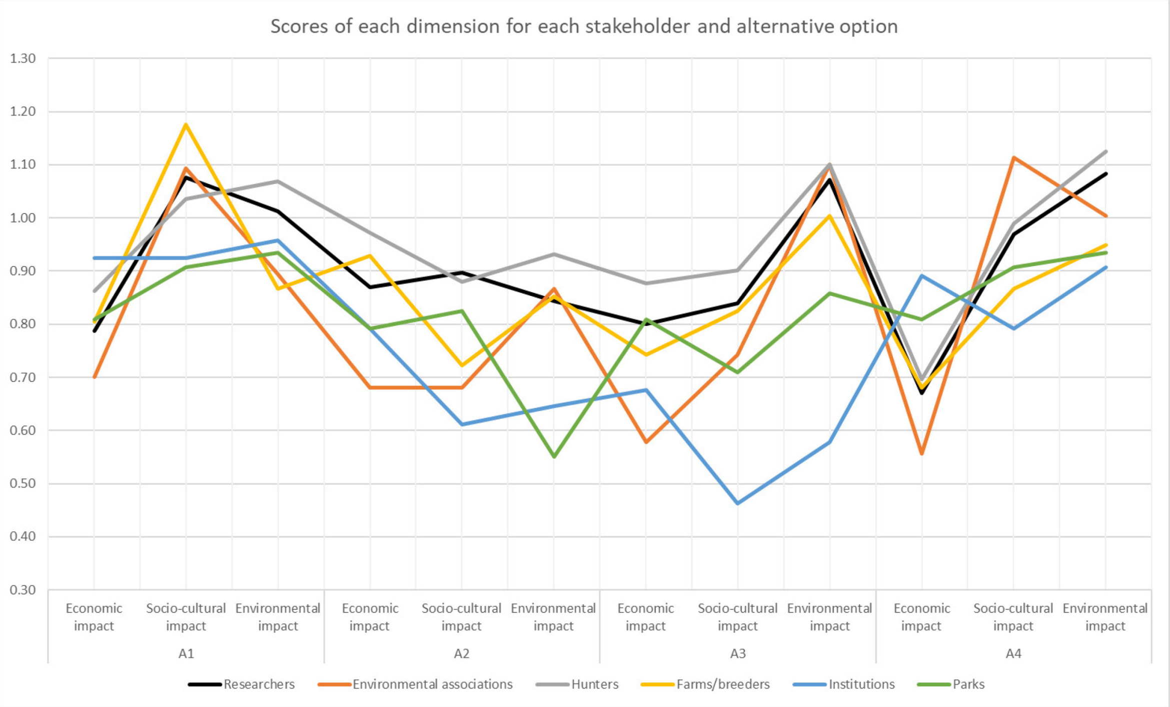

Figure 4.2 Scores of each dimension for each stakeholder and alternative option. Alternatives: increasing red deer culling quotas (A1), red deer translocation in other areas (A2), limitation of sheep grazing in certain areas (A3), maintaining the status quo (A4)

Researchers

When looking at the results for each group of responders separately, for the researcher's group, within the different strategies aiming at reducing the possible consequences of the potential interspecific competition between chamois and red deer or domestic sheep, increasing red deer hunting quotas was still ranked first, followed by maintaining the status quo, limitation of sheep grazing in certain areas and red deer translocation in other areas (Table 4.5). In this case, the ranking was comparable to the overall ranking, likely due to the fact that the research group comprised half of the total stakeholder group dimension.

Table 4.5 Standardized scores of the alternatives for the researchers group ranked in a descending order, from the alternative that received the highest score, to the alternative that received the lowest score.

Alternative options	Economic impact	Socio-cultural impact	Environmental impact	Total score
Increasing red deer culling quotas	0.79	1.08	1.01	2.88
Maintaining the status quo	0.67	0.97	1.08	2.72
Limiting sheep grazing in certain areas	0.80	0.84	1.07	2.71
Red deer translocation in other areas	0.87	0.90	0.84	2.61

Environmental associations

The ranking positions obtained from the group of the environmental associations is the same as the group of researchers, even if a lower score was obtained for each alternative (Table 4.6). In addition, increasing red deer hunting and maintaining the status quo obtained almost the same results (2.69 and 2.67, respectively), therefore, for this group both could represent adequate solutions.

Table 4.6 Standardized scores of the alternatives for the environmental association group ranked in a descending order, from the alternative that received the highest score, to the alternative that received the lowest score.

Alternative options	Economic impact	Socio-cultural impact	Environmental impact	Total score
Increasing red deer culling quotas	0.70	1.09	0.89	2.69
Maintaining the status quo	0.56	1.11	1.00	2.67
Limiting sheep grazing in certain areas	0.58	0.74	1.10	2.42
Red deer translocation in other areas	0.68	0.68	0.87	2.23

Hunter group

Unsurprisingly, increasing the hunting of red deer quotas ranked first by the hunter group, which obtained the highest score among all groups considered (Table 4.7). Limitation of sheep grazing in certain areas was ranked second, followed by maintaining the status quo and red deer translocation in other areas.

Table 4.7 Standardized scores of the alternatives for the hunter group ranked in a descending order, from the alternative that received the highest score, to the alternative that received the lowest score.

Alternative options	Economic impact	Socio-cultural impact	Environmental impact	Total score
Increasing red deer hunting quotas	0.86	1.04	1.07	2.97
Maintaining the status quo	0.88	0.90	1.10	2.88
Limiting sheep grazing in certain areas	0.70	0.99	1.12	2.81
Red deer translocation in other areas	0.97	0.88	0.93	2.78

Farmers/breeders group

The farmers/breeders group, again ranked increased deer hunting in first position, followed (interestingly) by limitation of sheep grazing in certain areas, red deer translocation in other areas and maintaining the status quo (Table 4.8).

Table 4.8 Standardized scores of the alternatives for the farmers/breeders group ranked in a descending order, from the alternative that received the highest score, to the alternative that received the lowest score.

Alternative options	**Economic impact**	**Socio-cultural impact**	**Environmental impact**	**Total score**
Increasing red deer culling quotas	0.80	1.18	0.87	2.85
Limiting sheep grazing in certain areas	0.74	0.83	1.00	2.57
Red deer translocation in other areas	0.93	0.72	0.85	2.50
Maintaining the status quo	0.68	0.87	0.95	2.50

Institutions group

Increasing red deer hunting was ranked first also for the institution group. This alternative was followed by maintaining the status quo, red deer translocation in other areas and limitation of sheep grazing in certain areas (Table 4.9).

Table 4.9 Standardized scores of the alternatives for the institution group ranked in a descending order, from the alternative that received the highest score, to the alternative that received the lowest score.

Alternative options	**Economic impact**	**Socio-cultural impact**	**Environmental impact**	**Total score**
Increasing red deer culling quotas	0.92	0.92	0.96	2.81
Maintaining the status quo	0.89	0.79	0.91	2.59
Red deer translocation in other areas	0.79	0.61	0.65	2.05
Limiting sheep grazing in certain areas	0.68	0.46	0.58	1.72

Parks group

Finally, the only group that showed a different first ranked position, was the parks group, although the score difference with the alternative 1 was very low, showing thus similar results as the environmental associations group and thus, both representing adequate solutions (Table 4.10).

Table 4.10 Standardized scores of the alternatives for the parks group ranked in a descending order, from the alternative that received the highest score, to the alternative that received the lowest score.

Alternative options	Economic impact	Socio-cultural impact	Environmental impact	Total score
Maintaining the status quo	0.81	0.91	0.94	2.65
Increasing red deer culling quotas	0.76	0.94	0.91	2.61
Limiting sheep grazing in certain areas	0.81	0.71	0.86	2.38
Red deer translocation in other areas	0.79	0.83	0.55	2.17

When looking at each alternative separately, for the alternative 1, three stakeholders out of six (researchers, environmental associations and farms/breeders) agreed that the socio-cultural dimension was the most important aspect when evaluating the best strategy to reduce potentially harmful consequences on chamois populations, followed by the environmental impact. Within this alternative, the economic impact received the lowest score among all the stakeholder groups (Figure 4.3).

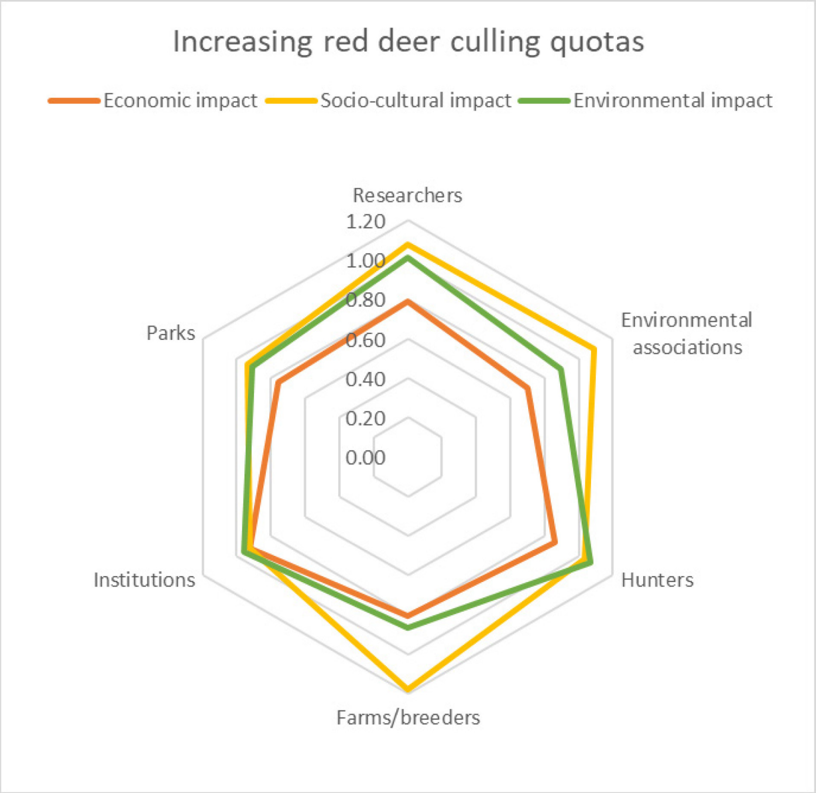

Figure 4.3 Radar chart representing the different perspectives of all the stakeholders involved in the study in the scoring of each dimension for the alternative 1 (increasing red deer culling quotas). The orange line represents the economic impact dimension, the yellow line the socio-cultural impact dimension and the green line represents the environmental impact dimension.

For the second alternative, red deer translocation in other areas, the economic impact dimension was considered the most important by three stakeholder groups out of six (institutions, farms/breeders, hunters). Instead, parks and researchers considered the socio-cultural and economic impact as the most important. The environmental impact dimension was considered as the most important only for environmental associations, while the other groups placed second (hunters, farms/breeders, institutions) or third (parks, researchers) position (Figure 4.4).

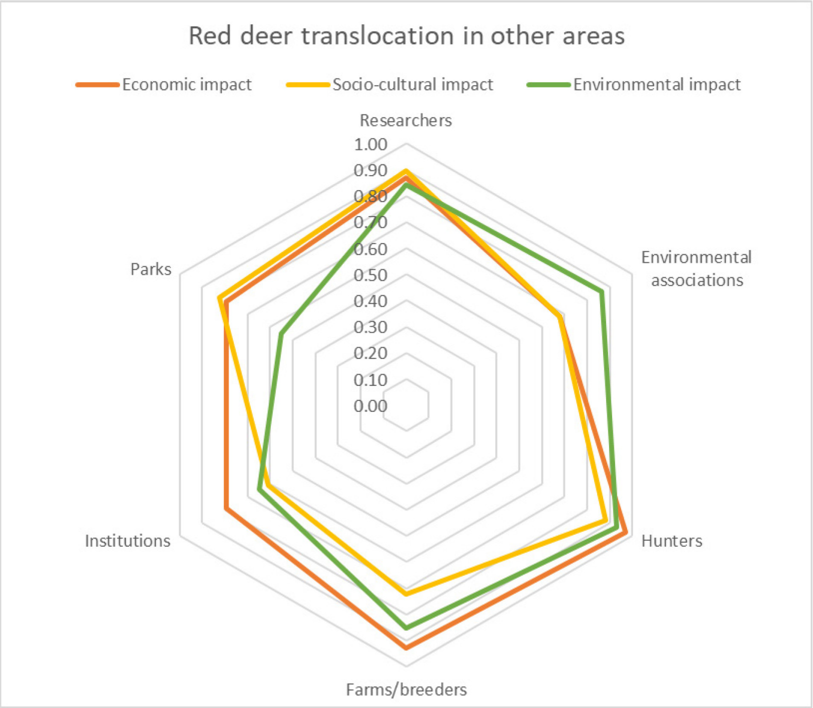

Figure 4.4 Radar chart representing the different perspectives of all the stakeholders involved in the study in the scoring of each dimension for the alternative 2 (Red deer translocation in other areas). The orange line represents the economic impact dimension, the yellow line the socio-cultural impact dimension and the green line represents the environmental impact dimension.

When scoring alternative 3, limitation of sheep grazing in certain areas, five stakeholders out of six (researchers, environmental associations, hunters, parks, and farm/breeders), considered the environmental impact dimension as the most important and all of them but the parks and the institutions considered the socio-cultural impact as the second most important dimension. The only group that differs more from the other stakeholders is the institution group, which considered the economic impact dimension as the most important, followed by the environmental impact and the socio-cultural impact (Figure 4.5).

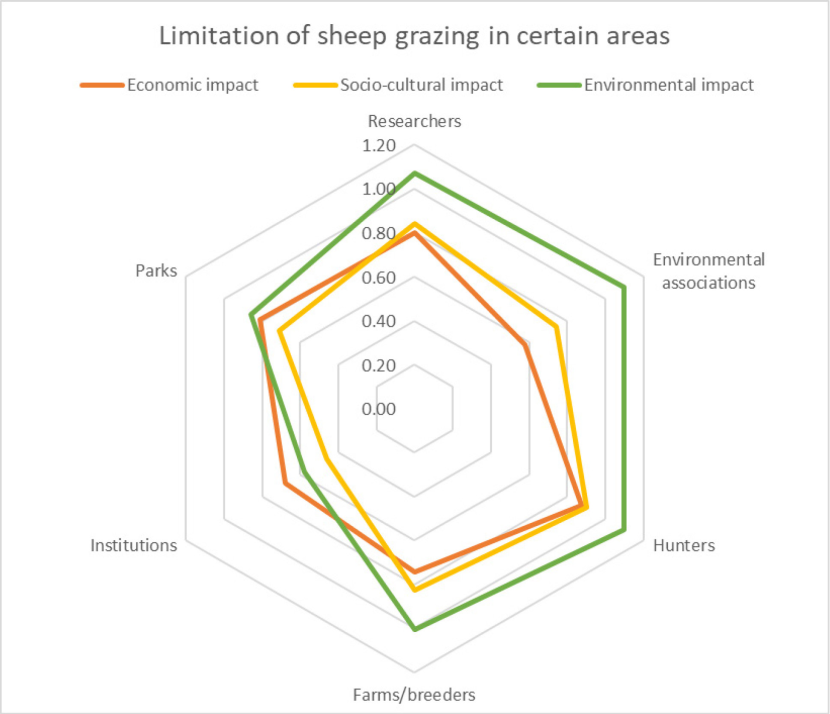

Figure 4.5 Radar chart representing the different perspectives of all the stakeholders involved in the study in the scoring of each dimension for the alternative 1 (limitation of sheep grazing in certain areas). The orange line represents the economic impact dimension, the yellow line the socio-cultural impact dimension and the green line represents the environmental impact dimension.

For alternative 4 (maintaining the status quo), all the stakeholders except one (environmental associations), agreed in considering the environmental impact dimension as the most important, followed by the socio-cultural dimension, for all of them except the institution group. Indeed, except for the latter which ranked it as the second most important dimension, economic impact was considered as the least important by all the stakeholders. Finally, the socio-cultural impact was considered as the most important only for environmental associations (Figure 4.6).

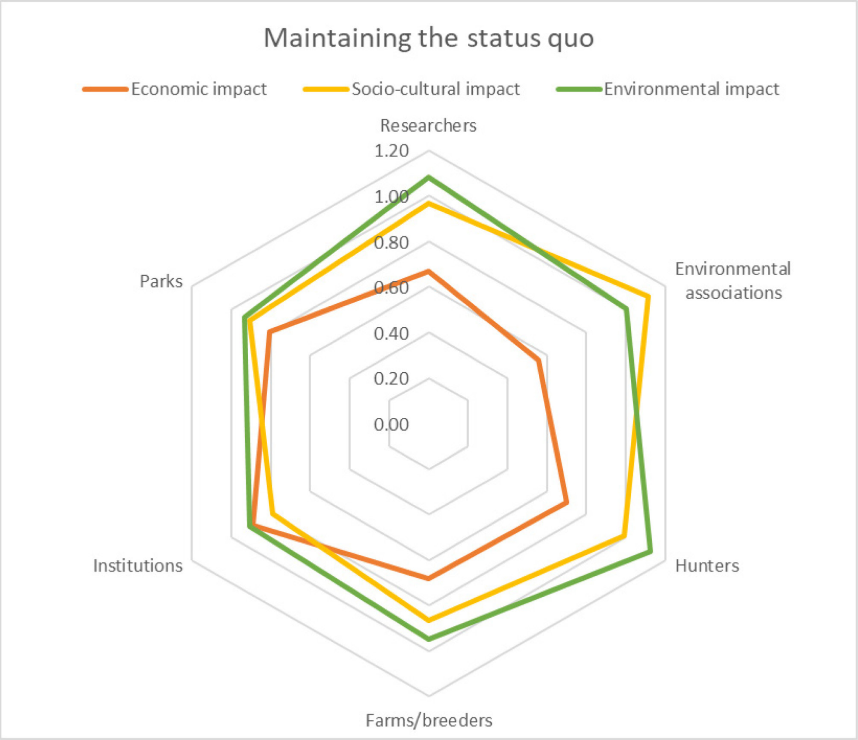

Figure 4.6 Radar chart representing the different perspectives of all the stakeholders involved in the study in the scoring of each dimension for the alternative 1 (maintaining the status quo). The orange line represents the economic impact dimension, the yellow line the socio-cultural impact dimension and the green line represents the environmental impact dimension.

4.8 DISCUSSION

In this study, I examined four alternative options designed to reduce competition between chamois, red deer, and domestic sheep. My findings show that increasing red deer culling quotas was selected as the best alternative option by all stakeholder groups and highlight the importance of considering multiple perspectives in the decision-making process for wildlife management and species conservation.

Final ranking of the alternative options

The MCDA applied in this study allowed us to rank the four considered options for chamois management and to investigate the different perceptions of the economic, socio-cultural, and environmental dimensions of local stakeholders.

Overall, my results showed that increasing red deer culling quotas was the preferred management solution (Table 4.4), with the socio-cultural dimension considered the most important in this context (score = 1.05). Reducing red deer densities by increasing red deer culling quotas would thus have a positive impact on social acceptance: this result reflects how heavily high densities of red deer affect human activities in some areas of the Province of Trento, due to the damage caused to pastures, hay meadows, vegetable gardens and orchards through excessive trampling and grazing (Pedrotti & Gandolfi, 2022) and highlight the need of considering the social dimension for ensuring the long-term success of sustainable and socially acceptable management strategies.

The environmental impact was considered the second most important dimension to be taken into account during the decision-making process (score = 0.99). This result likely reflects a growing awareness of the ecological consequences of high red deer densities and highlight the need to prioritize conservation efforts that address these impacts. Indeed, it has been observed that high red deer densities can have negative impacts on the composition and distribution of forests, herbaceous and shrubby vegetation (Bonardi, 2015; D'Aprile et al., 2020; Motta & Franzoi, 1997; Motta & Nola, 1996; Pedrotti & Gandolfi, 2022 Forest et al., 2005; Gill & Beardall, 2001; Rooney & Waller, 2003). In addition, high red deer densities can also have effects on the dynamics of other species (Pedrotti & Gandolfi, 2022b). For example, roe deer counts carried out in recent years have demonstrated a decrease in the numbers of surveyed individuals and a negative correlation between the number of red deer and roe deer. Other studies demonstrated a negative effect of high red deer densities on roe deer body mass (Richard et al., 2010) and population density (Borkowski et al., 2021). The effect was also evident for capercaillie, as the high densities of red deer was associated with a strong reduction in the resources necessary for this bird in the wintering areas of the species. In these areas the undergrowth component is particularly grazed by red deer, reducing the availability of the shrub layer and consequently of the shelter and feeding areas for capercaillie chicks (Pedrotti & Gandolfi, 2022).

Somewhat surprisingly, the dimension that received the lowest score was the economic impact (score = 0.81). However, this represents an important dimension during the decision-making process since the applicability of a management practice strongly depends on its economic feasibility.

The alternative that received the lowest score was ‘red deer translocation in other areas. In this case, the economic impact received the highest score (score = 0.86), followed by the sociocultural (score = 0.83) and the environmental impact (score = 0.82) dimensions. Nevertheless, the economic dimension is one of the major obstacles that prevents the realization of this alternative. For instance, Pedrotti & Gandolfi (2022) report that the total cost for the trapping of one red deer, considering the cost of materials and staff, is about 600-800 €. Therefore, although translocation may be considered a more socially accepted solution, in a perspective of reducing red deer densities, it is clear that this alternative is not often feasible.

Stakeholder groups perception

Increasing red deer hunting was considered the best alternative option by almost all stakeholder groups (Tables 4.5, 4.6, 4.7, 4.8, 4.9, 4.10): only the environmental associations (Table 4.6) and the park group (Table 4.10) considered also maintaining the status quo as an adequate solution for species management. Three groups (researchers, environmental associations, and farmers/breeders) (Tables 4.5, 4.6, 4.8 and Figure 4.3) out of six still considered the socio-cultural impact as the most important dimension to be taken into account within the alternative one (increasing red deer hunting quotas), confirming previous results obtained for the overall analysis (Table 4.4). The hunter group (Table 4.7) gave a slightly higher score to the environmental impact dimension (score = 1.07), but similar to the socio-cultural dimension (score = 1.04). The institutions group (Table 4.9) also gave a higher score to the environmental impact dimension (score = 0.96), although it did not show particularly evident score differences from the economic (score = 0.92) and socio-cultural dimension (score = 0.92). This result suggests that institutions, which represent the collectivity, put almost equal importance on all the dimensions considered.

Four groups (researchers, environmental associations, hunters and parks) (Tables 4.5, 4.6, 4.7, 4.10) out of six agreed in considering the translocation of red deer in other areas as the worst solution, in agreement with the overall result. The only two groups that differed were farmers/breeders and institutions (Tables 4.8 and 4.9). The institutions group considered the worst option “limiting sheep grazing in other areas” (Table 4.9, total score = 1.72). This result may reflect the importance for this group of preserving traditional mountain activities that are fundamental not only for the primary sector and tourism, but also for biodiversity conservation (Permanent Secretariat of the Alpine Convention, 2021). Indeed, in the last few years, the progressive abandonment of rural areas and the commercialization of agriculture caused a progressive decline of traditional agricultural and breeding practices, especially in mountain areas (MacDonald et al., 2000). For this reason, the EU’s Common Agricultural Policy (CAP) provides compensations and support to farmers in order to contribute to the sustainable development of rural areas (Italy CAP Strategic Plan 1.2, 2022).

On the other hand, farms/breeders (Table 4.8) considered maintaining the status quo as the worst solution (total score = 2.50) and, contrary to what expected, limitation of sheep grazing was considered as the second-best option (total score = 2.57). However, within this alternative, the economic impact dimension, which is the dimension that would most affect this group if this alternative option were applied (through the loss of the incentives provided by the EU’s Common Agricultural Policy, CAP, or the costs related to the rental of new areas for grazing), received the lowest score (score = 0.74).

Limitation of the study and future directions

One of the limitations of this study was the higher response rate from researchers, which constituted half of the sample size (24 out of 51) so that the overall final ranking may have been driven by the individual perception of this particular group. Therefore, a future direction of this study could be to increase the sample size in order to have an equal proportion of each stakeholder group and obtain a more homogenous evaluation of each dimension within each alternative option. Moreover, we did not consider the combination of two alternatives as an additional alternative option. An example could be the combination of increasing red deer hunting with limitation of sheep grazing in certain areas. A wider study is thus needed in order to include all the possible combinations of the considered alternative options.

This study showed that MCDA is a useful method for supporting the decision-making process by considering possible trade-offs among different perspectives and opinions related to stakeholder values, knowledge and perceptions.

Understanding the economic, socio-cultural, and environmental implications of chamois conservation in the Alps, is fundamental not only to mitigate the impact of disturbances for chamois, a symbol species for the Trentino region, but also to maintain an active and sustainable alpine economy and cultural traditions by considering the possible source of conflict of interest with local agricultural activities.

4.9 SUPPLEMENTARY MATHERIAL 4.1

MULTI-CRITERIA ANALYSIS PER LA GESTIONE DEL CAMOSCIO NELLE ALPI

Il camoscio alpino è una specie che vive in regioni montuose, principalmente in zone intervallate da pareti rocciose e scoscese (Corlatti et al, 2022a). Il suo habitat è dunque naturalmente frammentato e sempre più disturbato, oltre ad essere in progressiva riduzione a causa del cambiamento climatico. Negli ultimi anni, in alcune regioni del Trentino, le popolazioni di camoscio sono diminuite, mentre il cervo ha raggiunto densità sempre più elevate, aumentando il suo areale e la sua consistenza e facendo ipotizzare la presenza di un effetto di competizione tra le due specie (Corlatti et al., 2019). Inoltre, poiché nei mesi estivi le pecore vengono portate all’alpeggio (soprattutto a quote più elevate), si potrebbe verificare una sovrapposizione nell'uso delle risorse con i camosci e i cervi determinando quindi il potenziale per una competizione multispecifica: camoscio-cervo-pecora. Per descrivere la dieta e osservare possibili sovrapposizioni di nicchia trofica tra camoscio-cervo e camoscio-pecora abbiamo utilizzato la tecnica del DNA metabarcoding; i primi risultati suggeriscono un'elevata similarità di dieta tra le tre specie animali. Per ridurre la concorrenza delle risorse alimentari fra camoscio-cervo e camoscio-pecora, proponiamo 4 alternative per la gestione del cervo e della pecora. Utilizzeremo il metodo di Multi-Criteria Analysis (MCA): per ogni alternativa abbiamo individuato 3 tipologie di impatto (economico, socio-culturale e ambientale) e all’interno di ogni impatto abbiamo selezionato degli indicatori e sottoindicatori, comuni a tutte le alternative. Nel seguente questionario, Le chiediamo di assegnare un valore da 0 a 5 a ciascun indicatore individuato, i quali verranno utilizzati in seguito per la comparazione delle diverse alternative.

1. A quale categoria appartiene?
   - ☐ Ricercatore/ricercatrice
   - ☐ Associazione ambientalista
   - ☐ Cacciatore
   - ☐ Pastore/azienda agricola/allevatore
   - ☐ Istituzione/Ente Parco
   - ☐ Altro

ALTERNATIVA 1: Avviare un piano di controllo del cervo

Si propone l’avvio di piani di prelievo finalizzati alla riduzione delle consistenze.

Alternativa 1: **IMPATTO ECONOMICO**

Dimensione	Indicatori	Sotto-indicatori
IMPATTO ECONOMICO	Benefici	Privati
		Pubblici
	Costi	Privati
		Pubblici

IMPATTO ECONOMICO: Descrizione dei criteri e sotto-criteri

Nell'ambito dell'impatto economico generato dall'attuazione di questa alternativa, abbiamo individuato due indicatori e un totale di quattro sotto-indicatori.

I **benefici** sono divisi in 2 sotto-indicatori:

- I **benefici privati** si riferiscono ai benefici economici generati dall'applicazione di questa alternativa generati ad esempio dal valore economico dei capi abbattuti nelle filiere di trasformazione delle carni.
- I **benefici pubblici** si riferiscono ai benefici economici dei quali usufruirebbe direttamente o indirettamente la collettività a seguito dell'applicazione di questa alternativa, generati ad esempio da una riduzione delle collisioni stradali con la fauna selvatica e/o da un maggior ripopolamento dei camosci con effetti sull'attrazione turistica, ma anche di un maggior flusso turistico determinato dall'attività venatoria.

I **costi** sono divisi in 2 sotto-indicatori:

-I **costi privati** si riferiscono ai costi che i privati devono sostenere per poter applicare questa alternativa e poter dunque praticare l'attività venatoria, come ad esempio l'acquisto o il mantenimento dell'attrezzatura o dei cani da caccia.

-I **costi pubblici** si riferiscono ai costi necessari per l'applicazione di questa alternativa, come ad esempio i costi legati ai fondi da stanziare per l'avvio del piano di controllo e/o ai costi della burocrazia.

La **scala** per l'assegnazione del valore va da 0 a 5:

0 = benefici/costi prossimi allo 0

1 = benefici/costi molto bassi

2 = benefici/costi bassi
3 = benefici/costi medi
4 = benefici/costi alti
5 = benefici/costi molto alti

1. Secondo Lei, se venisse applicata questa alternativa, a quanto ammonterebbero i benefici privati? Valuti la sua risposta in una scala da 0 a 5.

	0	1	2	3	4	5	
Benefici prossimi allo 0	☐	☐	☐	☐	☐	☐	Benefici molto alti

2. Secondo Lei, se venisse applicata questa alternativa, a quanto ammonterebbero i benefici pubblici? Valuti la sua risposta in una scala da 0 a 5.

	0	1	2	3	4	5	
Benefici prossimi allo 0	☐	☐	☐	☐	☐	☐	Benefici molto alti

3. Secondo Lei, se venisse applicata questa alternativa, a quanto ammonterebbero i costi per i privati? Valuti la sua risposta in una scala da 0 a 5.

	0	1	2	3	4	5	
Costi prossimi allo 0	☐	☐	☐	☐	☐	☐	Costi molto alti

4. Secondo Lei, se venisse applicata questa alternativa, a quanto ammonterebbero i costi pubblici? Valuti la sua risposta in una scala da 0 a 5.

	0	1	2	3	4	5	
Costi prossimi allo 0	☐	☐	☐	☐	☐	☐	Costi molto alti

Alternativa 1: **IMPATTO SOCIO-CULTURALE**

Dimensione	Indicatori	Sotto-indicatori
IMPATTO SOCIO-CULTURALE	Accettazione sociale	Popolazioni locali Cacciatori Aziende agricole/allevatori Popolazioni non locali

IMPATTO SOCIO-CULTURALE: Descrizione dei criteri e dei sotto-criteri

Nell'ambito dell'impatto socio-culturale generato dall'attuazione di questa alternativa, abbiamo individuato un indicatore e quattro sotto-indicatori.

I quattro sotto-indicatori individuati sono descritti come segue:

- **Popolazioni locali**: accettazione di questa alternativa da parte di proprietari di orti e campi coltivati a uso privato in relazione agli eventuali minori danni subiti dal cervo.
- **Cacciatori**: accettazione di questa alternativa da parte dei cacciatori, a seguito ad esempio dell'aumento del numero di capi cacciabili.
- **Aziende agricole/allevatori**: accettazione di questa alternativa da parte di aziende agricole e/o allevatori a seguito, ad esempio, di una maggiore disponibilità della componente erbacea nei prati-pascoli dovuta alla riduzione degli impatti da brucamento.
- **Popolazione non locale**: accettazione di questa alternativa da parte di tutti quegli stakeholders (animalisti, cittadini) interessati per motivi etici e/o ideologici.

La scala per l'assegnazione del valore va da 0 a 5:

0 = livello di accettazione prossimo allo 0

1 = livello di accettazione molto basso

2 = livello di accettazione basso

3 = livello di accettazione medo

4 = livello di accettazione alto

5 = livello di accettazione molto alto

1. Secondo Lei, quale sarebbe il livello di accettazione di questa alternativa da parte delle popolazioni locali? Valuti la sua risposta in una scala da 0 a 5.

	0	1	2	3	4	5	
Livello di accettazione prossimo allo 0	☐	☐	☐	☐	☐	☐	Livello di accettazione molto alto

2. Secondo Lei, quale sarebbe il livello di accettazione di questa alternativa da parte dei cacciatori? Valuti la sua risposta in una scala da 0 a 5.

	0	1	2	3	4	5	
Livello di accettazione prossimo allo 0	☐	☐	☐	☐	☐	☐	Livello di accettazione molto alto

3. Secondo Lei, quale sarebbe il livello di accettazione di questa alternativa da parte delle aziende agricole/allevatori? Valuti la sua risposta in una scala da 0 a 5.

	0	1	2	3	4	5	
Livello di accettazione prossimo allo 0	☐	☐	☐	☐	☐	☐	Livello di accettazione molto alto

4. Secondo Lei, quale sarebbe il livello di accettazione di questa alternativa da parte delle popolazioni non locali? Valuti la sua risposta in una scala da 0 a 5.

	0	1	2	3	4	5	
Livello di accettazione prossimo allo 0	☐	☐	☐	☐	☐	☐	Livello di accettazione molto alto

Alternativa 1: **IMPATTO AMBIENTALE**

Dimensione	Indicatori	Sotto-indicatori
IMPATTO AMBIENTALE	Flora	Impatto su composizione e distribuzione delle foreste, della vegetazione erbacea e arbustiva e biodiversità
		Mantenimento dei pascoli
	Fauna	Impatto sulle altre specie autoctone
		Impatto sulla trasmissione di patologie
	Camoscio	Componente demografica e tassi di crescita

IMPATTO AMBIENTALE: Descrizione dei criteri e sotto-criteri

Nell'ambito dell'impatto ambientale generato dall'attuazione di questa alternativa, abbiamo individuato tre indicatori e un totale di cinque sotto-indicatori.

La **flora** è divisa in 2 sotto-indicatori:

- **Impatto su composizione delle foreste, della vegetazione erbacea e arbustiva e sulla biodiversità vegetale**: impatto che l'applicazione di questa alternativa avrebbe a livello di rinnovazione forestale e sugli strati arbustivi del sottobosco, con conseguenze sulla diversità specifica e sulla stabilità ecologica delle foreste, nonché sulle relazioni di competizione tra le specie vegetali (in relazione a calpestìo e brucamento).
- **Mantenimento dei pascoli**: impatto che l'applicazione di questa alternativa avrebbe a livello di mantenimento di aree aperte di pascolo secondario con conseguenze sui tempi di ricolonizzazione di tali aree da parte delle essenze

arbustive e dunque sulla disponibilità trofica per altre specie (in relazione a calpestìo e brucamento).

La **fauna** è divisa in 2 sotto-indicatori:

- **Impatto sulle altre specie autoctone**: impatto che l'applicazione di questa alternativa genererebbe sulle altre specie autoctone presenti nel territorio, con conseguenze quindi sulla disponibilità di risorse e/o sull'eventuale riduzione del disturbo.
- **Impatto sulla trasmissione di patologie**: impatto che l'applicazione di questa alternativa avrebbe a livello di trasmissione di patologie nell'interfaccia selvatico-selvatico e selvatico-domestico. Elevate densità favoriscono infatti l'insorgere di malattie, con conseguenti maggiori tassi di contatto tra le specie e maggiori probabilità di trasmissione.

Il **camoscio** comprende un unico sotto-indicatore:

- **Componente demografica e tassi di crescita**: impatto che questa alternativa avrebbe sulla componente demografica e sul tasso di crescita del camoscio andandone ad influenzare quindi le densità di popolazione e i tassi di accrescimento annui.

La **scala** per l'assegnazione del valore va da 0 a 5:
0 = impatto prossimo allo 0
1 = impatto molto basso
2 = impatto basso
3 = impatto medio
4 = impatto alto
5 = impatto molto alto

1. Secondo Lei, quale sarebbe l'impatto di questa alternativa a livello di composizione delle foreste, della vegetazione erbacea e arbustiva e della biodiversità vegetale? Valuti la sua risposta in una scala da 0 a 5.

	0	1	2	3	4	5	
Impatto prossimo allo 0	☐	☐	☐	☐	☐	☐	Impatto molto alto

2. Secondo Lei, quale sarebbe l'impatto di questa alternativa a livello di mantenimento dei pascoli? Valuti la sua risposta in una scala da 0 a 5.

	0	1	2	3	4	5	
Impatto prossimo allo 0	☐	☐	☐	☐	☐	☐	Impatto molto alto

3. Secondo Lei, quale sarebbe l'impatto di questa alternativa sulle altre specie autoctone animali? Valuti la sua risposta in una scala da 0 a 5.

	0	1	2	3	4	5	
Impatto prossimo allo 0	☐	☐	☐	☐	☐	☐	Impatto molto alto

4. Secondo Lei, quale sarebbe l'impatto di questa alternativa a livello di trasmissione di patologie? Valuti la sua risposta in una scala da 0 a 5.

	0	1	2	3	4	5	
Impatto prossimo allo 0	☐	☐	☐	☐	☐	☐	Impatto molto alto

5. Secondo Lei, quale sarebbe l'impatto di questa alternativa sulla componente demografica e i tassi di crescita del camoscio? Valuti la sua risposta in una scala da 0 a 5.

	0	1	2	3	4	5	
Impatto prossimo allo 0	☐	☐	☐	☐	☐	☐	Impatto molto alto

ALTERNATIVA 2: Traslocare i cervi in altre zone

Si propone la traslocazione di cervi da alcune aree di alta vocazione per il camoscio in altre aree, eventualmente basandosi su studi genetici per la scelta del luogo adeguato in cui trasferire gli animali.

Alternativa 2: **IMPATTO ECONOMICO**

Dimensione	Indicatori	Sotto-indicatori
IMPATTO ECONOMICO	Benefici	Privati
		Pubblici
	Costi	Privati
		Pubblici

IMPATTO ECONOMICO: Descrizione dei criteri e sotto-criteri

Nell'ambito dell'impatto economico generato dall'attuazione di questa alternativa, abbiamo individuato due indicatori e un totale di quattro sotto-indicatori.

I **benefiici** sono divisi in 2 sotto-indicatori:

- I **benefici privati** si riferiscono ai benefici economici dei quali usufruirebbero i privati in riferimento all'eventuale riduzione dei danni che il cervo causa ai terreni coinvolti.
- I **benefici pubblici** si riferiscono ai benefici economici dei quali usufruirebbe direttamente o indirettamente la collettività, generati ad esempio da una riduzione delle collisioni stradali con la fauna selvatica e/o da un maggior ripopolamento dei camosci con effetti sull'attrazione turistica.

I **costi** sono divisi in 2 sotto-indicatori:

- I **costi privati** si riferiscono ai costi che i privati devono sostenere per praticare l'attività di traslocazione dei cervi, come ad esempio l'acquisto o il mantenimento dell'attrezzatura e/o del vestiario.

-I **costi pubblici** si riferiscono ai costi necessari per l'applicazione di tale misura, come ad esempio i costi legati alla realizzazione dei corral di cattura, costi di trasferimento degli animali, costi di assunzione del personale e costi della burocrazia.

La **scala** per l'assegnazione del valore va da 0 a 5:
0 = benefici/costi prossimi allo 0
1 = benefici/costi molto bassi
2 = benefici/costi bassi
3 = benefici/costi medi
4 = benefici/costi alti
5 = benefici/costi molto alti

1. Secondo Lei, se venisse applicata questa alternativa, a quanto ammonterebbero i benefici privati? Valuti la sua risposta in una scala da 0 a 5.

	0	1	2	3	4	5	
Benefici prossimi allo 0	☐	☐	☐	☐	☐	☐	Benefici molto alti

2. Secondo Lei, se venisse applicata questa alternativa, a quanto ammonterebbero i benefici pubblici? Valuti la sua risposta in una scala da 0 a 5.

	0	1	2	3	4	5	
Benefici prossimi allo 0	☐	☐	☐	☐	☐	☐	Benefici molto alti

3. Secondo Lei, se venisse applicata questa alternativa, a quanto ammonterebbero i costi per i privati? Valuti la sua risposta in una scala da 0 a 5.

	0	1	2	3	4	5	
Costi prossimi allo 0	☐	☐	☐	☐	☐	☐	Costi molto alti

4. Secondo Lei, se venisse applicata questa alternativa, a quanto ammonterebbero i costi pubblici? Valuti la sua risposta in una scala da 0 a 5.

	0	1	2	3	4	5	
Costi prossimi allo 0	☐	☐	☐	☐	☐	☐	Costi molto alti

ALTERNATIVA 2: **IMPATTO SOCIO-CULTURALE**

Dimensione	Indicatori	Sotto-indicatori
IMPATTO SOCIO-CULTURALE	Accettazione sociale	Popolazioni locali Cacciatori Aziende agricole/allevatori Popolazioni non locali

IMPATTO SOCIO-CULTURALE: Descrizione dei criteri e sotto-criteri

Nell'ambito dell'impatto socio-culturale generato dall'attuazione di questa alternativa, abbiamo individuato un indicatore e quattro sotto-indicatori.

I quattro sotto-indicatori individuati sono descritti come segue:

- **Popolazioni locali:** accettazione di questa alternativa da parte di proprietari di orti e campi coltivati a uso privato in relazione agli eventuali minori danni subiti dal cervo.
- **Cacciatori:** accettazione di questa alternativa da parte dei cacciatori, a seguito ad esempio della riduzione del numero di capi cacciabili.
- **Aziende agricole/allevatori:** accettazione di questa alternativa da parte di aziende agricole e/o allevatori a seguito, ad esempio, di una maggiore disponibilità della componente erbacea nei prati-pascoli dovuta alla riduzione degli impatti da brucamento.
- **Popolazione non locale:** accettazione di questa alternativa da parte di tutti quegli stakeholders (animalisti, cittadini) interessati per motivi etici e/o ideologici.

La **scala** per l'assegnazione del valore va da 0 a 5:

0 = livello di accettazione prossimo allo 0
1 = livello di accettazione molto basso
2 = livello di accettazione basso

3 = livello di accettazione medo
4 = livello di accettazione alto
5 = livello di accettazione molto alto

1. Secondo Lei, quale sarebbe il livello di accettazione di questa alternativa da parte delle popolazioni locali? Valuti la sua risposta in una scala da 0 a 5.

	0	1	2	3	4	5	
Livello di accettazione prossimo allo 0	☐	☐	☐	☐	☐	☐	Livello di accettazione molto alto

2. Secondo Lei, quale sarebbe il livello di accettazione di questa alternativa da parte dei cacciatori? Valuti la sua risposta in una scala da 0 a 5.

	0	1	2	3	4	5	
Livello di accettazione prossimo allo 0	☐	☐	☐	☐	☐	☐	Livello di accettazione molto alto

3. Secondo Lei, quale sarebbe il livello di accettazione di questa alternativa da parte delle aziende agricole/allevatori? Valuti la sua risposta in una scala da 0 a 5.

	0	1	2	3	4	5	
Livello di accettazione prossimo allo 0	☐	☐	☐	☐	☐	☐	Livello di accettazione molto alto

4. Secondo Lei, quale sarebbe il livello di accettazione di questa alternativa da parte delle popolazioni non locali? Valuti la sua risposta in una scala da 0 a 5.

	0	1	2	3	4	5	
Livello di accettazione prossimo allo 0	☐	☐	☐	☐	☐	☐	Livello di accettazione molto alto

ALTERNATIVA 2: **IMPATTO AMBIENTALE**

Dimensione	Indicatori	Sotto-indicatori
IMPATTO AMBIENTALE	Flora	Impatto su composizione e distribuzione delle foreste, della vegetazione erbacea e arbustiva e biodiversità
		Mantenimento dei pascoli
	Fauna	Impatto sulle altre specie autoctone
		Impatto sulla trasmissione di patologie
	Camoscio	Componente demografica e tassi di crescita

IMPATTO AMBIENTALE: Descrizione dei criteri e sotto-criteri

Nell'ambito dell'impatto ambientale generato dall'attuazione di questa alternativa, abbiamo individuato tre indicatori e un totale di cinque sotto-indicatori.

La **flora** è divisa in 2 sotto-indicatori:

- **Impatto su composizione delle foreste, della vegetazione erbacea e arbustiva e sulla biodiversità vegetale**: impatto che l'applicazione di questa alternativa avrebbe a livello di rinnovazione forestale e sugli strati arbustivi del sottobosco, con conseguenze sulla diversità specifica e sulla stabilità ecologica delle foreste, nonché sulle relazioni di competizione tra le specie vegetali (in relazione a calpestìo e brucamento).
- **Mantenimento dei pascoli**: impatto che l'applicazione di questa alternativa avrebbe a livello di mantenimento di aree aperte di pascolo secondario con

conseguenze sui tempi di ricolonizzazione di tali aree da parte delle essenze arbustive e dunque sulla disponibilità trofica per altre specie (in relazione a calpestìo e brucamento).

La **fauna** è divisa in 2 sotto-indicatori:
- **Impatto sulle altre specie autoctone**: impatto che l'applicazione di questa alternativa genererebbe sulle altre specie autoctone presenti nel territorio, con conseguenze quindi sulla disponibilità di risorse e/o sull'eventuale riduzione del disturbo.
- **Impatto sulla trasmissione di patologie**: impatto che l'applicazione di questa alternativa avrebbe a livello di trasmissione di patologie nell'interfaccia selvatico-selvatico e selvatico-domestico. Elevate densità favoriscono infatti l'insorgere di malattie, con conseguenti maggiori tassi di contatto tra le specie e maggiori probabilità di trasmissione.

Il **camoscio** comprende un unico sotto-indicatore:
- **Componente demografica e tassi di crescita**: impatto che questa alternativa avrebbe sulla componente demografica e sul tasso di crescita del camoscio andandone ad influenzare quindi le densità di popolazione e i tassi di accrescimento annui.

La **scala** per l'assegnazione del valore va da 0 a 5:
0 = impatto prossimo allo 0
1 = impatto molto basso
2 = impatto basso
3 = impatto medio
4 = impatto alto
5 = impatto molto alto

1. Secondo Lei, quale sarebbe l'impatto di questa alternativa a livello di composizione delle foreste, della vegetazione erbacea e arbustiva e della biodiversità vegetale? Valuti la sua risposta in una scala da 0 a 5.

	0	1	2	3	4	5	
Impatto prossimo allo 0	☐	☐	☐	☐	☐	☐	Impatto molto alto

2. Secondo Lei, quale sarebbe l'impatto di questa alternativa a livello di mantenimento dei pascoli? Valuti la sua risposta in una scala da 0 a 5.

	0	1	2	3	4	5	
Impatto prossimo allo 0	☐	☐	☐	☐	☐	☐	Impatto molto alto

3. Secondo Lei, quale sarebbe l'impatto di questa alternativa sulle altre specie autoctone animali? Valuti la sua risposta in una scala da 0 a 5.

	0	1	2	3	4	5	
Impatto prossimo allo 0	☐	☐	☐	☐	☐	☐	Impatto molto alto

4. Secondo Lei, quale sarebbe l'impatto di questa alternativa a livello di trasmissione di patologie? Valuti la sua risposta in una scala da 0 a 5.

	0	1	2	3	4	5	
Impatto prossimo allo 0	☐	☐	☐	☐	☐	☐	Impatto molto alto

5. Secondo Lei, quale sarebbe l'impatto di questa alternativa sulla componente demografica e i tassi di crescita del camoscio? Valuti la sua risposta in una scala da 0 a 5.

	0	1	2	3	4	5	
Impatto prossimo allo 0	☐	☐	☐	☐	☐	☐	Impatto molto alto

ALTERNATIVA 3: Limitare il pascolo delle pecore in determinate zone

Si propone la limitazione del pascolo delle pecore in determinate zone, ad esempio non aprire al pascolo delle pecore quelle zone in cui la densità del camoscio è più elevata, oppure zone in cui la disponibilità di risorse è inferiore.

Alternativa 3: **IMPATTO ECONOMICO**

Dimensione	Indicatori	Sotto-indicatori
IMPATTO ECONOMICO	Benefici	Privati
		Pubblici
	Costi	Privati
		Pubblici

IMPATTO ECONOMICO: Descrizione dei criteri e sotto-criteri

Nell'ambito dell'impatto economico generato dall'attuazione di questa alternativa, abbiamo individuato due indicatori e un totale di quattro sotto-indicatori.

I **benefici** sono divisi in 2 sotto-indicatori:

- I **benefici privati** si riferiscono ai benefici economici di cui beneficerebbero i privati a seguito dell'applicazione di questa alternativa generati ad esempio dall'affitto di nuovi pascoli e/o dall'acquisto di foraggio da parte degli allevatori.
- I **benefici pubblici** si riferiscono ai benefici economici dei quali usufruirebbe direttamente o indirettamente la collettività, generati ad esempio da un maggior ripopolamento dei camosci con effetti sull'attrazione turistica.

I **costi** sono divisi in 2 sotto-indicatori:

- I **costi privati** si riferiscono ai costi che i privati devono sostenere a seguito dell'applicazione di questa alternativa generati da mancati introiti per i premi PAC e/o dall'acquisto di foraggio andato perso e/o dall'affitto di nuovi terreni.
- I **costi pubblici** si riferiscono ai costi necessari per l'applicazione di tale misura, come ad esempio i costi della burocrazia e del monitoraggio permanente per il rispetto di questa alternativa.

La **scala** per l'assegnazione del valore va da 0 a 5:
0 = benefici/costi prossimi allo 0
1 = benefici/costi molto bassi
2 = benefici/costi bassi
3 = benefici/costi medi
4 = benefici/costi alti
6 = benefici/costi molto alti

1. Secondo Lei, se venisse applicata questa alternativa, a quanto ammonterebbero i benefici privati? Valuti la sua risposta in una scala da 0 a 5.

	0	1	2	3	4	5	
Benefici prossimi allo 0	☐	☐	☐	☐	☐	☐	Benefici molto alti

2. Secondo Lei, se venisse applicata questa alternativa, a quanto ammonterebbero i benefici pubblici? Valuti la sua risposta in una scala da 0 a 5.

	0	1	2	3	4	5	
Benefici prossimi allo 0	☐	☐	☐	☐	☐	☐	Benefici molto alti

3. Secondo Lei, se venisse applicata questa alternativa, a quanto ammonterebbero i costi per i privati? Valuti la sua risposta in una scala da 0 a 5.

	0	1	2	3	4	5	
Costi prossimi allo 0	☐	☐	☐	☐	☐	☐	Costi molto alti

4. Secondo Lei, se venisse applicata questa alternativa, a quanto ammonterebbero i costi pubblici? Valuti la sua risposta in una scala da 0 a 5.

	0	1	2	3	4	5	
Costi prossimi allo 0	☐	☐	☐	☐	☐	☐	Costi molto alti

ALTERNATIVA 3: **IMPATTO SOCIO-CULTURALE**

Dimensione	Indicatori	Sotto-indicatori
IMPATTO SOCIO-CULTURALE	Accettazione sociale	Popolazioni locali
		Cacciatori
		Aziende agricole/allevatori
		Popolazioni non locali

IMPATTO SOCIO-CULTURALE: Descrizione dei criteri e sotto-criteri

Nell'ambito dell'impatto socio-culturale generato dall'attuazione di questa alternativa, abbiamo individuato un indicatore e quattro sotto-indicatori.

I quattro sotto-indicatori individuati sono descritti come segue:

- **Popolazioni locali:** accettazione di questa alternativa da parte di proprietari di orti e campi coltivati a uso privato in relazione ai danni subiti dal pascolamento delle pecore.
- **Cacciatori:** accettazione di questa alternativa da parte dei cacciatori, a seguito ad esempio di una maggior disponibilità di pascolo per la fauna selvatica.
- **Aziende agricole/allevatori:** accettazione di questa alternativa da parte di aziende agricole e/o allevatori a seguito, ad esempio, di una minor disponibilità di pascolo con conseguente revisione dei piani d'alpeggio.
- **Popolazione non locale:** accettazione di questa alternativa da parte di tutti quegli stakeholders (animalisti, cittadini) interessati per motivi etici e/o ideologici.

La **scala** per l'assegnazione del valore va da 0 a 5:

0 = livello di accettazione prossimo allo 0
1 = livello di accettazione molto basso
2 = livello di accettazione basso
3 = livello di accettazione medo
4 = livello di accettazione alto
5 = livello di accettazione molto alto

1. Secondo Lei, quale sarebbe il livello di accettazione di questa alternativa da parte delle popolazioni locali? Valuti la sua risposta in una scala da 0 a 5.

	0	1	2	3	4	5	
Livello di accettazione prossimo allo 0	☐	☐	☐	☐	☐	☐	Livello di accettazione molto alto

2. Secondo Lei, quale sarebbe il livello di accettazione di questa alternativa da parte dei cacciatori? Valuti la sua risposta in una scala da 0 a 5.

	0	1	2	3	4	5	
Livello di accettazione prossimo allo 0	☐	☐	☐	☐	☐	☐	Livello di accettazione molto alto

3. Secondo Lei, quale sarebbe il livello di accettazione di questa alternativa da parte delle aziende agricole/allevatori? Valuti la sua risposta in una scala da 0 a 5.

	0	1	2	3	4	5	
Livello di accettazione prossimo allo 0	☐	☐	☐	☐	☐	☐	Livello di accettazione molto alto

4. Secondo Lei, quale sarebbe il livello di accettazione di questa alternativa da parte delle popolazioni non locali? Valuti la sua risposta in una scala da 0 a 5.

	0	1	2	3	4	5	
Livello di accettazione prossimo allo 0	☐	☐	☐	☐	☐	☐	Livello di accettazione molto alto

ALTERNATIVA 3**: IMPATTO AMBIENTALE**

Dimensione	Indicatori	Sotto-indicatori
IMPATTO AMBIENTALE	Flora	Impatto su composizione e distribuzione delle foreste, della vegetazione erbacea e arbustiva e biodiversità
		Mantenimento dei pascoli
	Fauna	Impatto sulle altre specie autoctone
		Impatto sulla trasmissione di patologie
	Camoscio	Componente demografica e tassi di crescita

IMPATTO AMBIENTALE: Descrizione dei criteri e sotto-criteri

Nell'ambito dell'impatto ambientale generato dall'attuazione di questa alternativa, abbiamo individuato tre indicatori e un totale di cinque sotto-indicatori.

La **flora** è divisa in 2 sotto-indicatori:

- **Impatto su composizione delle foreste, della vegetazione erbacea e arbustiva e sulla biodiversità vegetale**: impatto che l'applicazione di questa alternativa avrebbe a livello di rinnovazione forestale e sugli strati arbustivi del sottobosco, con conseguenze sulla diversità specifica e sulla stabilità ecologica delle foreste, nonché sulle relazioni di competizione tra le specie vegetali (considerando ad esempio l'eventuale minore calpestìo e morsicatura).
- **Mantenimento dei pascoli**: impatto che l'applicazione di questa alternativa avrebbe a livello di mantenimento di aree aperte di pascolo secondario. Attraverso la selezione alimentare delle essenze, il calpestio e il rilascio di deiezioni, il pascolo può apportare cambiamenti notevoli nella struttura della vegetazione e nella composizione e varietà delle specie vegetali.

La **fauna** è divisa in 2 sotto-indicatori:

- **Impatto sulle altre specie autoctone**: impatto che l'applicazione di questa alternativa genererebbe sulle altre specie autoctone presenti nel territorio, con conseguenze quindi sulla disponibilità di risorse e/o sull'eventuale riduzione del disturbo.
- **Impatto sulla trasmissione di patologie**: impatto che l'applicazione di questa alternativa avrebbe a livello di trasmissione di patologie nell'interfaccia selvatico-selvatico e selvatico-domestico. Elevate densità favoriscono infatti l'insorgere di malattie, con conseguenti maggiori tassi di contatto tra le specie e maggiori probabilità di trasmissione.

Il **camoscio** comprende un unico sotto-indicatore:

- **Componente demografica e tassi di crescita**: impatto che questa alternativa avrebbe sulla componente demografica e sul tasso di crescita del camoscio andandone ad influenzare quindi le densità di popolazione e i tassi di accrescimento annui.

La **scala** per l'assegnazione del valore va da 0 a 5:
0 = impatto prossimo allo 0
1 = impatto molto basso
2 = impatto basso
3 = impatto medio
4 = impatto alto
5 = impatto molto alto

1. Secondo Lei, quale sarebbe l'impatto di questa alternativa a livello di composizione delle foreste, della vegetazione erbacea e arbustiva e della biodiversità vegetale? Valuti la sua risposta in una scala da 0 a 5.

	0	1	2	3	4	5	
Impatto prossimo allo 0	☐	☐	☐	☐	☐	☐	Impatto molto alto

2. Secondo Lei, quale sarebbe l'impatto di questa alternativa a livello di mantenimento dei pascoli? Valuti la sua risposta in una scala da 0 a 5.

	0	1	2	3	4	5	
Impatto prossimo allo 0	☐	☐	☐	☐	☐	☐	Impatto molto alto

3. Secondo Lei, quale sarebbe l'impatto di questa alternativa sulle altre specie autoctone animali? Valuti la sua risposta in una scala da 0 a 5.

	0	1	2	3	4	5	
Impatto prossimo allo 0	☐	☐	☐	☐	☐	☐	Impatto molto alto

4. Secondo Lei, quale sarebbe l'impatto di questa alternativa a livello di trasmissione di patologie? Valuti la sua risposta in una scala da 0 a 5.

	0	1	2	3	4	5	
Impatto prossimo allo 0	☐	☐	☐	☐	☐	☐	Impatto molto alto

5. Secondo Lei, quale sarebbe l'impatto di questa alternativa sulla componente demografica e i tassi di crescita del camoscio? Valuti la sua risposta in una scala da 0 a 5.

	0	1	2	3	4	5	
Impatto prossimo allo 0	☐	☐	☐	☐	☐	☐	Impatto molto alto

ALTERNATIVA 4: Mantenere lo status-quo

Questa alternativa prevede di mantenere l'attuale sistema di gestione delle popolazioni domestiche e selvatiche.

ALTERNATIVA 4**: IMPATTO ECONOMICO**

Dimensione	Indicatori	Sotto-indicatori
IMPATTO ECONOMICO	Benefici	Privati
		Pubblici
	Costi	Privati
		Pubblici

ALTERNATIVA 4: Descrizione dei criteri e sotto-criteri

Nell'ambito dell'impatto economico generato dall'attuazione di questa alternativa, abbiamo individuato due indicatori e un totale di quattro sotto-indicatori.

I **benefiici** sono divisi in 2 sotto-indicatori:

- I **benefici privati** si riferiscono ai benefici economici di cui beneficerebbero i privati a seguito dell'applicazione di questa misura, mantenendo dunque l'attuale stato di gestione delle specie.
- I **benefici pubblici** si riferiscono ai benefici economici dei quali usufruirebbe direttamente o indirettamente la collettività, mantenendo dunque l'attuale stato di gestione delle specie.

I **costi** sono divisi in 2 sotto-indicatori:

-I **costi privati** si riferiscono ai costi che i privati devono sostenere a seguito dell'applicazione di questa alternativa, mantenendo dunque l'attuale stato di gestione delle specie.

-I **costi pubblici** si riferiscono ai costi necessari per l'applicazione di tale misura, mantenendo dunque l'attuale stato di gestione delle specie.

La **scala** per l'assegnazione del valore va da 0 a 5:

0 = benefici/costi prossimi allo 0

1 = benefici/costi molto bassi

2 = benefici/costi bassi

3 = benefici/costi medi

4 = benefici/costi alti

5 = benefici/costi molto alti

1. Secondo Lei, se venisse applicata questa alternativa, a quanto ammonterebbero i benefici privati? Valuti la sua risposta in una scala da 0 a 5.

	0	1	2	3	4	5	
Benefici prossimi allo 0	☐	☐	☐	☐	☐	☐	Benefici molto alti

2. Secondo Lei, se venisse applicata questa alternativa, a quanto ammonterebbero i benefici pubblici? Valuti la sua risposta in una scala da 0 a 5.

	0	1	2	3	4	5	
Benefici prossimi allo 0	☐	☐	☐	☐	☐	☐	Benefici molto alti

3. Secondo Lei, se venisse applicata questa alternativa, a quanto ammonterebbero i costi per i privati? Valuti la sua risposta in una scala da 0 a 5.

	0	1	2	3	4	5	
Costi prossimi allo 0	☐	☐	☐	☐	☐	☐	Costi molto alti

4. Secondo Lei, se venisse applicata questa alternativa, a quanto ammonterebbero i costi pubblici? Valuti la sua risposta in una scala da 0 a 5.

	0	1	2	3	4	5	
Costi prossimi allo 0	☐	☐	☐	☐	☐	☐	Costi molto alti

ALTERNATIVA 4: IMPATTO SOCIO-CULTURALE

Dimensione	Indicatori	Sotto-indicatori
IMPATTO SOCIO-CULTURALE	Accettazione sociale	Popolazioni locali Cacciatori Aziende agricole/allevatori Popolazioni non locali

IMPATTO SOCIO-CULTURALE: Descrizione dei criteri e sotto-criteri

Nell'ambito dell'impatto socio-culturale generato dall'attuazione di questa alternativa, abbiamo individuato un indicatore e quattro sotto-indicatori.

I quattro sotto-indicatori individuati sono descritti come segue:

- **Popolazioni locali:** accettazione di questa alternativa da parte delle popolazioni locali, facendo riferimento all'attuale status di gestione delle specie.
- **Cacciatori:** accettazione di questa alternativa da parte dei cacciatori, facendo riferimento all'attuale status di gestione delle specie.
- **Aziende agricole/allevatori:** accettazione di questa alternativa da parte di aziende agricole e/o allevatori facendo riferimento all'attuale status di gestione delle specie.
- **Popolazione non locale:** accettazione di questa alternativa da parte di tutti quegli stakeholders (animalisti, cittadini) interessati per motivi etici e/o ideologici, facendo riferimento all'attuale status di gestione delle specie.

La **scala** per l'assegnazione del valore va da 0 a 5:

0 = livello di accettazione prossimo allo 0

1 = livello di accettazione molto basso

2 = livello di accettazione basso

3 = livello di accettazione medo

4 = livello di accettazione alto

5 = livello di accettazione molto alto

1. Secondo Lei, quale sarebbe il livello di accettazione di questa alternativa da parte delle popolazioni locali? Valuti la sua risposta in una scala da 0 a 5.

	0	1	2	3	4	5	
Livello di accettazione prossimo allo 0	☐	☐	☐	☐	☐	☐	Livello di accettazione molto alto

2. Secondo Lei, quale sarebbe il livello di accettazione di questa alternativa da parte dei cacciatori? Valuti la sua risposta in una scala da 0 a 5.

	0	1	2	3	4	5	
Livello di accettazione prossimo allo 0	☐	☐	☐	☐	☐	☐	Livello di accettazione molto alto

3. Secondo Lei, quale sarebbe il livello di accettazione di questa alternativa da parte delle aziende agricole/allevatori? Valuti la sua risposta in una scala da 0 a 5.

	0	1	2	3	4	5	
Livello di accettazione prossimo allo 0	☐	☐	☐	☐	☐	☐	Livello di accettazione molto alto

4. Secondo Lei, quale sarebbe il livello di accettazione di questa alternativa da parte delle popolazioni non locali? Valuti la sua risposta in una scala da 0 a 5.

	0	1	2	3	4	5	
Livello di accettazione prossimo allo 0	☐	☐	☐	☐	☐	☐	Livello di accettazione molto alto

ALTERNATIVA 4: IMPATTO AMBIENTALE

Dimensione	Indicatori	Sotto-indicatori
IMPATTO AMBIENTALE	Flora	Impatto su composizione e distribuzione delle foreste, della vegetazione erbacea e arbustiva e biodiversità
		Mantenimento dei pascoli
	Fauna	Impatto sulle altre specie autoctone
		Impatto sulla trasmissione di patologie
	Camoscio	Componente demografica e tassi di crescita

IMPATTO AMBIENTALE: Descrizione dei criteri e sotto-criteri

Nell'ambito dell'impatto ambientale generato dall'attuazione di questa alternativa, abbiamo individuato tre indicatori e un totale di cinque sotto-indicatori.

La **flora** è divisa in 2 sotto-indicatori:

- **Impatto su composizione delle foreste, della vegetazione erbacea e arbustiva e sulla biodiversità vegetale**: impatto che l'applicazione di questa alternativa avrebbe a livello di rinnovazione forestale e sugli strati arbustivi del sottobosco, con conseguenze sulla diversità specifica e sulla stabilità ecologica delle foreste, nonché sulle relazioni di competizione tra le specie vegetali (considerando il mantenimento dell'attuale status quo di gestone delle specie).
- **Mantenimento dei pascoli**: impatto che l'applicazione di questa alternativa avrebbe a livello di mantenimento di aree aperte di pascolo secondario (considerando il mantenimento dell'attuale status quo di gestone delle specie).

La **fauna** è divisa in 2 sotto-indicatori:

- **Impatto sulle altre specie autoctone**: impatto che l'applicazione di questa alternativa genererebbe sulle altre specie autoctone presenti nel territorio (considerando il mantenimento dell'attuale status quo di gestone delle specie).
- **Impatto sulla trasmissione di patologie**: impatto che l'applicazione di questa alternativa avrebbe a livello di trasmissione di patologie nell'interfaccia selvatico-selvatico e selvatico-domestico (considerando il mantenimento dell'attuale status quo di gestone delle specie).

Il **camoscio** comprende un unico sotto-indicatore:

- **Componente demografica e tassi di crescita**: impatto che questa alternativa avrebbe sulla componente demografica e sul tasso di crescita del camoscio andandone ad influenzare quindi le densità di popolazione e i tassi di accrescimento annui (considerando il mantenimento dell'attuale status quo di gestone delle specie).

La **scala** per l'assegnazione del valore va da 0 a 5:
0 = impatto prossimo allo 0
1 = impatto molto basso
2 = impatto basso
3 = impatto medio
4 = impatto alto
5 = impatto molto alto

1. Secondo Lei, quale sarebbe l'impatto di questa alternativa a livello di composizione delle foreste, della vegetazione erbacea e arbustiva e della biodiversità vegetale? Valuti la sua risposta in una scala da 0 a 5.

	0	1	2	3	4	5	
Impatto prossimo allo 0	☐	☐	☐	☐	☐	☐	Impatto molto alto

2. Secondo Lei, quale sarebbe l'impatto di questa alternativa a livello di mantenimento dei pascoli? Valuti la sua risposta in una scala da 0 a 5.

	0	1	2	3	4	5	
Impatto prossimo allo 0	☐	☐	☐	☐	☐	☐	Impatto molto alto

3. Secondo Lei, quale sarebbe l'impatto di questa alternativa sulle altre specie autoctone animali? Valuti la sua risposta in una scala da 0 a 5.

	0	1	2	3	4	5	
Impatto prossimo allo 0	☐	☐	☐	☐	☐	☐	Impatto molto alto

4. Secondo Lei, quale sarebbe l'impatto di questa alternativa a livello di trasmissione di patologie? Valuti la sua risposta in una scala da 0 a 5.

	0	1	2	3	4	5	
Impatto prossimo allo 0	☐	☐	☐	☐	☐	☐	Impatto molto alto

5. Secondo Lei, quale sarebbe l'impatto di questa alternativa sulla componente demografica e i tassi di crescita del camoscio? Valuti la sua risposta in una scala da 0 a 5.

	0	1	2	3	4	5	
Impatto prossimo allo 0	☐	☐	☐	☐	☐	☐	Impatto molto alto

5. GENERAL CONCLUSIONS

Understanding how ecological and genetic processes interact with the environment and shape the evolutionary processes, is fundamental for predicting long-term population responses to changing environments and to develop more effective management strategies (Crandall et al., 2000; Giménez et al., 2018). Indeed, faced with climate and human-induced changes, there is an increasing need for policies that incorporate a deep understanding of a species' genetic status, evolutionary history, and ecological factors that affect its survival and adaptation. The challenge I set out to tackle in this thesis was not only to integrate various approaches to contribute to the conservation of this species, but also to place the decision-making process for its management within an economic and socio-cultural context. The results obtained in this thesis thus, demonstrate how a multidisciplinary approach can produce relevant data and information useful to guide decision-making processes towards sustainable management practices and possibly improve conservation outcomes.

In Chapter I, I explored the phylogenetic relationships between the *Rupicapra* species and subspecies and investigated the genetic variability in the chamois before and after a time period in the last century strongly affected by human activities such as overharvesting and translocations. The phylogenetic reconstruction using the complete mtDNA sequence and the concatenated Cyt b – D-Loop sequence confirmed the results reported in previous studies (Crestanello et al., 2009; Iacolina et al., 2021; Rodríguez et al., 2009) identifying three main clades (mtC, mtE, mtW), mainly geographically separated, with a similar pattern in modern and historical samples. As observed in other studies (Leugger et al., 2022; Soglia et al., 2010; Zemanová et al., 2015), geographic features can act as barriers for chamois dispersal, and our study identified the Ticino Rivers as a possible barrier to gene flow between populations located east and west of this river valley. This result is particularly important for conservation, suggesting that translocation of chamois for reintroductions or restocking should consider the genetic background of populations from other geographic areas before deciding which animals to use.

Overall, this study contributes to the ongoing research effort to understand the mechanisms affecting chamois genetic structure and diversity. In addition, by comparing historical and modern samples of *R. rupicapra* and *R. pyrenaica*, results from this study provide insights into how the genetic variability of these chamois populations has changed over time with important implications for conservation. In addition, future research could integrate these findings with whole-genome data, providing valuable insights into the phylogenetic relationships within the *Rupicapra* genus and mechanisms enabling chamois to adapt to an evolving environment, and ultimately leading to improved conservation efforts for the species (Corlatti et al., 2022a).

In Chapter II, I investigated the diets of chamois, domestic sheep, and red deer, and I found a higher degree of overlap between chamois and red deer than between chamois and domestic sheep. This result suggests that the interspecific competition between these wild species, especially in areas where red deer densities are increasing (Corlatti et al., 2019) could have a negative impact on small chamois populations. Findings from this study offer thus valuable insights for developing management strategies aiming at reducing red deer densities in those areas where the two species' trophic niche strongly overlap. Furthermore, our preliminary analysis of the microbiota, not included in this thesis, can lay the groundwork for future research to investigate the potential effects of dietary changes on the microbiota composition of chamois. Indeed, the gut microbiota composition of an animal can change depending on several factors such as diet, age, or habitat, affecting the individual's ability to adapt to changes in the environment and thus impacting on its health, survival, and fitness (Hauffe & Barelli, 2019). Combining diet studies with the study of gut microbiota yields a more holistic comprehension of the effects of these interactions and would have, thus, even greater implications for the conservation of the species.

Finally, Chapter III explored the trade-offs of four management strategies that could be implemented to reduce the potential interspecific competition between chamois, domestic sheep and red deer. Rather surprisingly, our findings showed that although bearing very different values and perceptions of the problem, most of the stakeholder groups agreed in considering increasing red deer hunting as the best alternative option. The findings of this study demonstrate the effectiveness of MCDA in facilitating the decision-making process by accounting for diverse stakeholder values, knowledge, and perspectives, and acknowledging potential trade-offs among them. I showed that the application of this method for species management, can lead to more sustainable outcomes that balance ecological, economic, and socio-cultural considerations. Moreover, our study can serve as a foundation for future research, providing a framework for decision-making that incorporates diverse stakeholder perspectives and criteria and ensuring that decisions are both effective and sustainable.

In conclusion, results from this research will contribute to chamois management and highlight the need for integrated approaches for the study of the interactions between socio-economic and ecological systems. The analysis conducted in this study highlighted the importance of avoiding translocations between genetically distinct populations, which is crucial for the preservation of genetic diversity and the long-term success of chamois management efforts. At the same time, it is fundamental to identify management strategies that are not only effective but also socially acceptable and financially sustainable, which can lead to more effective and sustainable management strategies.

Achieving conservation goals requires a holistic approach that recognizes the importance of biodiversity for the well-being of both the environment and human societies. As human beings, we rely on biodiversity for the benefits that it indirectly provides to humans, which are fundamental for our cultural and economic welfare, serving the purposes of research, education, recreation, and tourism and generating the so-called ‘ecosystem services’ (Singh & Sharma, 2017), which are fundamental for environmental sustainability and well-being. I strongly believe that despite the numerous challenges and the inherent difficulties, the achievement of conservation goals needs a collaborative effort and a multidisciplinary approach. Conserving biodiversity is a complex and multifaceted task that requires bridging gaps not only between different disciplines but also between divergent values, knowledge, and point of view. However, only by working together, pooling our resources, and sharing our expertise can we hope to contribute to biodiversity conservation and to sustainable and impactful long-term management strategies.

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