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Original Research Article

First Insight Into Genetic Diversity of Alpine ibex (*Capra ibex*) in Slovenia

Key words

Capra ibex;
mitochondrial DNA;
MHC *DRB* exon 2;
reintroduction;
management

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Abstract: In Europe, the Alpine ibex (*Capra ibex*) was on the brink of extinction in the 19th century. Therefore, different conservation measures were implemented, and several reintroductions were made in the Alpine arc, starting from the only surviving population in Gran Paradiso, Italy. An extreme historical bottleneck and additional reintroductions have strongly shaped the genetic make-up of recent populations, resulting in significant genetic drift and profound inbreeding across the species range. To support science-based conservation actions, molecular methods have been increasingly used. However, such analyses did not include populations in Slovenia.We analysed neutral loci (partial fragment of mitochondrial cytochrome b, mtDNA) and the adaptive major histocompatibility complex (MHC *DRB* exon 2) of the Alpine ibex from both Slovenian populations (Julian and Kamnik-Savinja Alps) to understand how past reintroductions and recent management have affected the genetic diversity of the species. Results showed that both populations are genetically severely depleted, carrying only one mtDNA haplotype and one functional allele for MHC *DRB* exon 2, Caib-DRB*01. This calls for further conservation actions, including the reintroduction of individuals with different genetic background. However, the Alpine ibex is currently considered a non-native species in Slovenia, which makes conservation actions extremely difficult and threatens the long-term survival of the species. Therefore, scientists and population managers are urging policy/decision makers to change the status of the species to the native one and consequently to allow reintroductions. These appeals are supported by previous archaeological data on the existence of bones assigned to Alpine ibex in the Julian Alps, and evidence of severe genetic depletion in current ibex populations confirmed in this study.

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Introduction

Due to centuries-long intensive hunting, the Alpine ibex (*Capra ibex*) was on the brink of extinction in the 19th century (1). However, in the 20th century several reintroduction programmes were conducted in which captive-bred individuals from the only surviving population in Gran Paradiso, northern Italy, were translocated to various locations across the Alps (2, 3). As a result of these efforts, the Alpine ibex

has successfully recovered, and the number of individuals has increased from <100 to >53,000 within a century (4). The stepwise reintroduction strategies have been multidirectional and have included both primary translocation from captive breeding and secondary translocations from previously established populations (3, 5, 6, 7). Subsequent rounds of reintroductions and rapid increase in abundance have strongly shaped the genetic make-up of populations, and their isolation has also contributed to further genetic differentiation causing gradual genetic substructure in established (sub)populations (8, 9, 10).

Previous genomic studies revealed that the surviving population from Gran Paradiso has maintained a higher level of genetic variability compared to reintroduced populations, primarily due to the series of bottlenecks experienced by the reintroduced populations during translocations (9, 10). Moreover, new populations established by only a small number of individuals showed an increase in genetic drift and inbreeding, which leads to additional loss of genetic variation, reduces the efficacy of natural selection, and increases the expression of deleterious recessive mutations (8, 11). By analysing the genomic footprint and the consequences of sequential bottlenecks, Grossen et al. (10) found evidence for the concurrent purging of highly deleterious mutations and the accumulation of mildly deleterious ones. This suggests that recolonization bottlenecks induced both relaxed selection and purging, thus reshaping the landscape of deleterious mutation load. The accumulation of deleterious mutations was significantly lower in populations of >1,000 individuals in comparison with smaller populations (10). Maintaining an adequate effective population size of the Alpine ibex is hence of paramount importance for the species conservation (12, 13).

Current populations of Alpine ibex exhibit extremely low heterozygosity (8, 9, 10) and variability in major histocompatibility complex (MHC) genes (14). Introgression of the domestic goat *DRB* 2 allele (Caib-DRB*2) has been confirmed both in reintroduced populations in the Swiss Alps and in the source population of Gran Paradiso (15). In genetically depleted populations, introgression of the goat *DRB* allele likely reflects adaptation, as introgression increased the MHC *DRB* diversity. Based on genetic methods, Giacometti et al. (16) confirmed that wild Alpine ibex interbred with the domestic goats (*Capra hircus*) in a population in the southern Swiss Alps. The online survey recently performed by Moroni et al. (17) also showed that hybrids are present in most of the Alpine countries and that their occurrence is not a sporadic event, with some groups comprising 4–20 probable hybrids. The offspring of the hybrids are generally larger and heavier, have longer horns, some males do not have the characteristic horn folds, and their coat hair is darker (17). As one of the conservation actions in the Swiss Alps, all wild goats, including their hybrid offspring, were removed between 1998 and 2001 to protect the genetic purity of the Alpine ibex (16, 17).

According to archaeological records, the Alpine ibex occurred in the area of present-day Slovenia during the last glaciation, when it inhabited most of Europe, including the lowland areas of France, Luxembourg, the Czech Republic, Slovakia, Romania, Hungary, and Slovenia (18). After the last glaciation with the succession of vegetation in the lowlands, the range of the species was restricted exclusively to the Alpine arc (4, 18). Although some bone remains indicated the presence of the Alpine ibex in Slovenia in the Late Pleistocene (19, 20) and Holocene (21, 22), the species is currently recognised as non-native in the country. This definition or perception is based on the fact that there is no reliable data on the historical occurrence of the Alpine ibex in the Slovene Alps (23, 24). In contrast to conservation measures/projects implemented in other countries of the Alpine arc (summarised in (14)), the recognised non-nativity of the species in Slovenia makes its conservation (almost) impossible and therefore poses a threat to its long-term existence. However, a recent analysis of ancient DNA confirmed that four bone remains from the Julian Alps dated to the $5^{th}/6^{th}$ century were indeed a part of the Alpine ibex skeleton, which scientifically support the appeal for reconsidering the formal status of the species, i.e., to classify and manage it as a native species (25).

The Alpine ibex is the least common wild ungulate in Slovenia, with a population size estimated at about 300 individuals (26, 27). The species has certainly been present in Slovenia since 1890, when Baron Born established a colony of 20 ibex in the Karavanke Mountains, northern Slovenia. During both World Wars, the colony experienced two severe declines; in spite of three reintroduction events in the 1950s and 1970s, this colony disappeared in the early 1990s (23). Currently, Alpine ibex is present in two Slovene areas: the Kamnik–Savinja Alps and in Julian Alps. In the Kamnik–Savinja Alps, 4 ibex from Switzerland were released in 1953, followed by an additional 8 (4 males, 4 females) from Switzerland (park Sankt Gallen) in 1961 and 1965, and 7 from Gran Paradiso in 1967. This population reached its peak with >80 individuals in 1991, but after two outbreaks of sarcoptic mange (in 1991 and 2011) the population size declined to 30–35 individuals in 2022 (28). In the Julian Alps, 24 individuals from Gran Paradiso were released in the Triglav National Park between 1963 (1964) and 1966; population reached its peak of 330 individuals in 1996, followed by a rapid decrease due to sarcoptic mange outbreaks, with the minimum around 100 individuals in 2003 (26, 29), and a population size of 150–160 individuals in 2022 (30, 31). In the most western part of the Slovenian Julian Alps, i.e., outside the Triglav National Park, eight ibex (two from Gran Paradiso and six from Switzerland) were also released in the 1970s (31), and since 2000, immigration of individuals from the Italian side of the Kanin mountain has been confirmed (32). In 2022, the population size across the Julian Alps was assessed at 250 individuals, with 50 of them being present on the Slovene side of the Kanin mountain (31).

The genetics of Alpine ibex in Europe and its connection with ecology and spatial distribution are relatively well-known thanks to several large-scale studies (8, 9, 10, 33). Numerous sets of microsatellite loci have been developed for the species, both for studying genetic diversity and levels of inbreeding (8, 34, 35, 36, 37), as well as the close link MHC complex (14, 15, 37). The microsatellite markers were also proved to be useful for confirming hybridization events with domestic goats (16). In the last decade, modern genomic analyses have also been performed on the Alpine ibex, including single nucleotide arrays and whole genome sequencing (10, 38, 39). Unfortunately, however, none of these analyses included Alpine ibex from Slovenia.

In our study, we used neutral loci (partial fragment of mitochondrial cytochrome b, mtDNA cytb) and the adaptive major histocompatibility complex (MHC *DRB* II exon 2) to analyse the genetic diversity of two Alpine ibex populations in Slovenia, i.e., from the Kamnik–Savinja Alps and the Triglav National Park (Julian Alps). Specifically, we explored whether past management is reflected in the genetic architecture and how the different reintroduction strategies influenced the genetic diversity of populations. We hypothesized that the two populations would show depleted genetic diversity compared to the source population from Gran Paradiso due to the founder effect and inappropriate conservation actions, i.e., as the Alpine ibex has the status of a non-native species, which has prevented additional reintroductions aimed at ensuring connectivity between populations as well as adding new individuals to existing populations.

Materials and methods

Study area and sampling

To assess the genetic diversity of the two Alpine ibex populations from Slovenia, we analysed DNA from 33 samples: 10 from the Kamnik–Savinja Alps and 23 from the Julian Alps, respectively. DNA was extracted either from bones collected from skulls or muscle tissue taken from carcasses found between 2002 and 2020 (Fig. 1, Table S1). Samples from both areas were collected by professional gamekeepers from the Triglav National Park and the Slovenia Forest Service. In addition, to compare the genetic diversity of the Slovenians with other wild and captive populations, we

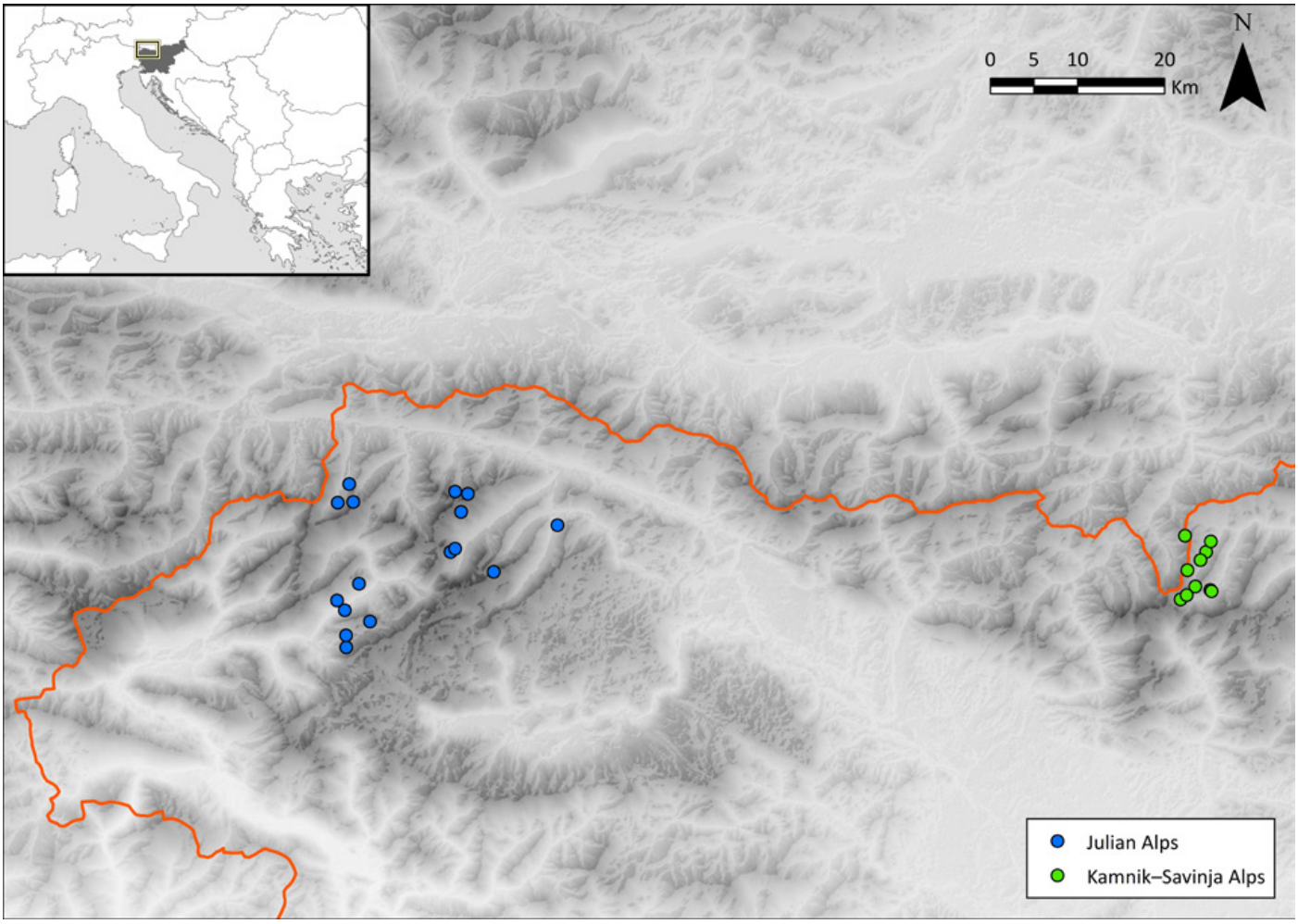

Figure 1: Sampling locations of Alpine ibex in Slovenia, period 2002–2020 (blue dots: Julian Alps (Triglav National Park); green dots: Kamnik–Savinja Alps); see Table S1 for details on the studied individuals and names of the localities

included in the analysis 17 blood samples of Alpine ibex from the Ljubljana Zoo (Slovenia; colony was established by male and female from Wildpark Feldkirch, Austria, and male and 3 females from Switzerland, unknown location), 5 tissue samples from Hohe Tauern (Austria), and 24 tissue or blood samples from Gran Paradiso (Italy).

DNA extraction and quality control

Bone samples from skulls were extracted with the High Pure Viral Nucleic Acid Kit (Roche, Switzerland), using the modified extraction method for highly degraded samples, developed and described by Rohland et al. (40). We used the E.Z.N.A.® Tissue DNA Kit (Omega Bio-Tek, USA) to isolate DNA from blood and tissue samples. The quality of extracts was assessed with the Qubit 3.0 using Qubit dsDNA BR Assay Kit (ThermoFisher Scientific, USA).

Amplification and statistical analysis of the mitochondrial cytochrome b region (cytb)

The partial mitochondrial cytochrome b gene (cytb; 623 bp) was amplified using the universal primer set L14724: 5'-CGAAGCTTGATATGAAAAACCATCGTTG-3' and H15347: 5'-GATGGGTTATTTGATCCTGTTTCGTG-3' (41, 42, 43). All polymerase chain reactions (PCR) were performed in a 20 µl reaction mix, using Platinum Direct PCR Universal Master Mix (ThermoFisher Scientific, USA) and amplified on a Thermal Cycler 2720 (Applied Biosystems, USA). After denaturation 3 min at 95°C, 35 PCR cycles with 30 s at 95°C, 45 s at 61°C and 60 s at 72°C were performed, followed by a final extension step of 10 min at 72°C. Sanger nucleotide sequencing was performed on the SeqStudio Genetic Analyzer (ThermoFisher Scientific, USA) using the BigDye Terminator v3.1 sequencing kit (Applied Biosystems, USA).

The CodonCode Aligner 4.27 (CodonCode Corporation, USA) was used to align the forward and reverse sequences. The resulting consensus sequences were aligned in MEGA 11 (44). The regions analysed in this study were combined with three previously published data downloaded from GenBank. Genetic diversity was estimated with haplotype diversity (Hd) and nucleotide diversity (π). All parameters were assessed with the programme DnaSP v.6.12 (45). The relationship among haplotypes was evaluated by constructing a median-joining haplotype network (46), using the PopART (47).

We included into the median-joining haplotype network three published nucleotide sequences of the Alpine ibex cytb from GenBank (nb. EU368877, AF034735, FJ207526), 23 sequences of samples from the Julian Alps and 10 from the Kamnik–Savinja Alps (Slovenia), 5 from Hohe Tauern (Austria), 24 from Gran Paradiso (Italy), and 17 from captive animals in Ljubljana Zoo (Table S1).

Amplification and statistical analysis of the major histocompatibility complex (MHC)

The Platinum Direct PCR Universal Master Mix (ThermoFisher Scientific, USA) was used to amplify the second exon of the MHC class II DRB gene using primers HL030: '5 -ATCCTCTCTCTGCAGCACATTTCC-3' and HL032: '5 -TCGCCGCTGCACAGTGAAACTCTC-3' (48). We performed PCR amplification in triplicate in 20-µl reaction mixtures (for details, see (49)).

The amplicons from the triplicates were pooled and purified with magnetic particles Agencourt® AmPure® (Agencourt Bioscience Corporation, A Beckman Coulter Company, USA), following the manufacturer's instructions. Concentrations of pooled and purified amplicons were quantified by Qubit 3.0 fluorometry using Qubit dsDNA BR (Broad range) Assay Kit reagents (ThermoFisher Scientific, USA). Samples were normalized to 3 ng and combined into a final library, which was again purified with Agencourt® AmPure® magnetic particles. For the separation, sizing and quantification of dsDNA final library of amplicons we used Agilent DNA High Sensitivity Kit on a 2100 Bioanalyzer (Agilent, Santa Clara, USA), according to the manufacturer's recommendations. We normalised the library to 100 pM, which was then multiplicated and bound with Ion Sphere particles (ISPs) using the Ion 520 & 530 Kit-OT2 reagent kit (ThermoFischer Scientific, USA) according to the protocol for sequencing 400 bp long fragments on Ion Torrent One Touch 2 (OT2) and sequenced following the ThermoFisher Scientific platform instructions on Ion Torrent S5, using the Ion 530 chip (ThermoFisher Scientific, USA).

For allele calling, we used the pipeline of the Amplicon Sequence Assignment (AmpliSAS) tool developed for high-throughput genotyping of duplicated polymorphic genes, such as MHC (50). The script was installed locally to analyse all the reads. Filtering of the raw data was performed with AmpliCLEAN by removing reads with a Phred quality score <20 and filtering all reads <250 bp and >300 bp. AmpliSAS clusters true variants with their potential artefacts based on platform-specific error rates. We used AmpliSAS's default parameters for Ion Torrent sequencing technology: a substitution error rate of 0.5 % and an indel error rate of 1 %. An accurate length was required to identify the dominant sequence within a cluster. We did not expect more than two *DRB* variants per individual, so we kept the "minimum dominant frequency" clustering threshold at 25 %, based on previously published works on Alpine ibex (14, 15, 37, 51). We discarded variants with a frequency <1 % within an amplicon. True variants of the *DRB* exon 2 fragments were aligned and translated into protein sequences to check for evidence of pseudogenes, such as the presence of premature stop codons or indels. A maximum of 200,000 reads per amplicon were used for allele calling. Since the web version of the AmpliSAS tool utilises only the first 5000 sample reads, the genotyping process was repeated with the same

parameters using the AmpliSAS script installed locally to analyse all reads.

The unique sequences were aligned, edited, and confirmed to be Alpine ibex MHC *DRB* exon 2 alleles by comparing them with alleles downloaded from GenBank (Table S1; GenBank nb. AY70631 from Albris, Switzerland) using MEGA 11 (44).

Results and discussion

Mitochondrial genetic diversity

We successfully sequenced mtDNA cytb from 78 out of the 79 samples (i.e., all except one from the Julian Alps). In the samples from Gran Paradiso, we detected haplotype H1 (already deposited in GenBank; Table S1) in 18 samples; three samples had haplotype H2, two samples had haplotype H3, and one had haplotype H4. The new haplotypes H2, H3 and H4 were deposited in GenBank under accession numbers OQ745823–OQ745825. Populations from the Julian Alps and the Kamnik–Savinja Alps had only the H1 haplotype. Haplotype diversity for all analysed samples was Hd = 0.431. The Alpine ibex populations revealed extremely low nucleotide diversity (π = 0.0008), possibly attributed to the historical bottleneck.

The median-joining network of mtDNA cytb haplotypes shows a star-shaped topology. The most common haplotype (H1) belongs to all studied populations. Alpine ibex from the Julian Alps and the Kamnik–Savinja Alps belong to the central, most common haplotype H1. Alpine ibex from Pointe de Calabre, France (sequences obtained from GenBank), Gran Paradiso (Italy), Hohe Tauern (Austria), and Ljubljana Zoo also share the same mitochondrial sequence. Haplotypes H2, H3 and H4, which belong only to the Gran Paradiso population, differ from the central haplotype only by one substitution (Fig. 2).

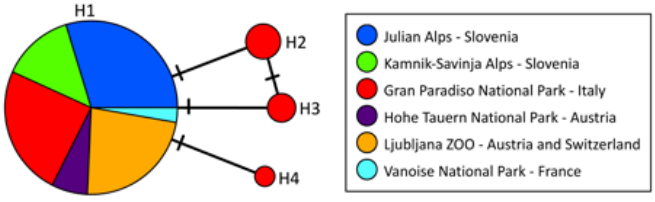

Figure 2: Median-joining network of mtDNA cytb haplotypes of the analysed Alpine ibex individuals. The size of the circles is proportional to the frequency of the haplotype, while the colours identify the area of the sample origin. The number of mutations separating the nodes is represented by lines crossing the branches of the grid.

MHC genetic diversity

We successfully analysed MHC *DRB* exon 2 from all 34 samples analysed, i.e., 24 from Gran Paradiso and 10 from the Julian Alps, Slovenia. We found one functional allele for MHC *DRB* exon 2, previously described by Schaschl et al. (48). No evidence of multiple locus amplification was found, confirming previous reports for Alpine ibex (52, 53). The samples from Gran Paradiso and the analysed Slovene population (Triglav National Park) had the same functional allele for MHC *DRB* exon 2, Caib-DRB*01, and we did not observe the presence of the allele Caib-DRB*02 in the studied populations. This allele was found by Grossen et al. (15) in a genetically severely depleted population of the Alpine ibex in Switzerland. The authors concluded that the introgression of the Caib-DRB*02 allele from domestic goats into the Alpine ibex was most likely due to adaptation, as introgression increased the diversity of the *DRB* gene in the MHC complex. The Caib-DRB*02 allele is otherwise identical to the *DRB* allele of the domestic goat (the so-called 'goat-like' *DRB* allele).

Possible consequences of low genetic diversity of Alpine ibex in Slovenia

Both mitochondrial and MHC genetic variability of Alpine ibex in the two Slovenian populations (the Julian Alps and the Kamnik–Savinja Alps) are very low. We found only one (the same) mtDNA cyt b haplotype and one MHC *DRB* exon 2 allele in both populations. Our results confirmed the low genetic variability of the Alpine ibex populations previously reported in France, Switzerland, and Italy (8, 14, 35). The presence of only one mtDNA haplotype and one MHC allele is likely the result of the founder effect but also of sequential bottlenecks in the 20th century due to improper management and the lack of connectivity among populations (23). Biebach and Keller (54) found that in Alpine ibex, the initial bottleneck reduced allele numbers more than subsequent bottlenecks, as predicted by theory (55, 56, 57). The preferential loss of low-frequency alleles is consistent, and a substantial proportion of alleles must be lost. If only higher frequency alleles remain in a population after an introduction, fewer founder individuals are required in subsequent reintroductions to retain most of the alleles present in the initial population. Thus, additional bottlenecks can reduce genetic variation even in the absence of an additional loss of alleles.

(Re)introductions and management history are the main determinants of today's genetic structure of the Alpine ibex in Slovenia; more than a hundred years after the first (re) introduction programmes we recorded depleted genetic diversity, which could lead to a severe population decline in the future (25). For example, in populations with low genetic variability, there is a risk of low disease resistance. In this respect, it is important to note that both in the Triglav National Park (Julian Alps) and in the Kamnik–Savinja Alps, periodic population declines were observed in the past due to increased mortality from infection with the scabies mite (*Sarcoptes scabiei*) (26, 28, 29, 31). Moreover, in ibex, sarcoptic mange has negative effects on the reproductive performance of both males and females, as already reported in Iberian ibex (*Capra pyrenaica*) (58, 59, 60).

The management of the Alpine ibex in Slovenia is in complete contrast to other successful managements throughout the Alpine arc. So far, >170 introduction events have been carried out in the Alps, leading to a dramatic increase in the abundance and spatial distribution of the species (4). Alpine ibex numbers in Europe have increased from only about 100 surviving individuals in the 19th century to >53,000 individuals, with an estimated population size increase of >400 % between 1975 and 2016, and the spatial distribution increase of 342 % between 1960 and 2020 (summarised in (61)). In contrast to this, the currently recognised non-nativity of the species in Slovenia hampers conservation efforts as reintroductions and releases of new individuals are not allowed throughout the ibex habitat as it completely overlaps with the Natura 2000 Network. This poses a severe threat to the long-term conservation of the species in Slovenia (25), particularly because genetic diversity (both mitochondrial and in immunogenes) is very low as revealed by our study. Therefore, there is an urgent need to change the status of the species, and subsequently implement active conservation/management, including new reintroductions, the success of which should be constantly monitored by the use of genomics tools to study the footprint and changes/improvement of Alpine ibex genetic diversity after a new conservation/management approach.

Conclusion

The study on mitochondrial genetic diversity of the Alpine ibex populations in Slovenia revealed limited haplotype variation, with only one predominant haplotype (H1) present both in the Julian Alps and the Kamnik–Savinja Alps populations. The analysis of MHC genetic diversity in the same populations showed very limited variability, with only one functional allele (Caib-DRB*01) present. Our findings highlight the negative effects of management history on the genetic structure of the Alpine ibex in Slovenia. The depletion of genetic diversity would probably lead to additional population declines and reduced disease resistance. The non-nativity status of the species in Slovenia hampers conservation efforts, preventing reintroductions and new releases throughout the ibex habitat. To ensure the preservation of Alpine ibex in Slovenia, urgent action is required to change the species' status and implement active conservation and management strategies, including reintroductions. Genomic tools should be utilized to monitor the genetic diversity of the population and evaluate the success of conservation efforts over time. Such measures are essential to safeguard the future of the Alpine ibex in the region.

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Originality statement: The material submitted for publication has not been published except in abstract form, and it is not currently under consideration for publication elsewhere.

Ethical statement: All bone samples taken from the trophies used in the study were legally harvested during regular hunting activities prescribed by the state of Slovenia in annual wildlife management plans. No animals were sacrificed for the purpose of obtaining samples for this study.

Competing interest: The is no competing interest.

Author's contributions: Conceptualization: E.B., B.P.; sample providing: A.B., S.H., P.B., I.M.; sequences providing: N.P, S.H., I.M.; laboratory and statistical analyses: L.D., A.B., E.B.; writing – draft preparation: EB.; writing – review and editing: E.B., B.P., A.B., L.D., S.H., N.P., P.B. I.M.; funding: E.B. All authors have read and agreed to the published version of the manuscript.

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Data availability: The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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Table S1: Data on Alpine ibex samples included in the study

Lab/GenBank ID	Sample ID	Country	Location	Year of sampling	Lat	Long	Cyt b hpt	MHC allele
LME2748	ALPKOZ- 3	Slovenia	Julian Alps – Kriški podi – pod Kolenom	2019	46.402	13.801	H1	/
LME2749	ALPKOZ- 4	Slovenia	Julian Alps – Jalovec	2017	46.421	13.680	H1	Caib-DRB*01
LME2750	ALPKOZ- 5	Slovenia	Julian Alps – Kriški podi – Gorenja luknja	2014	46.405	13.808	H1	/
LME2751	ALPKOZ- 6	Slovenia	Julian Alps – Kriški podi – pod Šplevto	2001	46.402	13.801	H1	/
LME2752	ALPKOZ- 7	Slovenia	Julian Alps – Kriški podi – pod Kolenom	2019	46.402	13.801	H1	Caib-DRB*01
LME2753	ALPKOZ- 8	Slovenia	Julian Alps – Jalovec	2008	46.421	13.680	–	Caib-DRB*01
LME2754	ALPKOZ- 9	Slovenia	Julian Alps – Jalovec	2011	46.421	13.680	H1	Caib-DRB*01
LME2756	ALPKOZ- 11	Slovenia	Julian Alps – Plazi – pod Pejči	2015	46.315	13.698	H1	/
LME2757	ALPKOZ- 12	Slovenia	Julian Alps – Kriški podi – Stružnik	2018	46.402	13.801	H1	/
LME2758	ALPKOZ- 13	Slovenia	Julian Alps – Plazi – pri Skali	2012	46.343	13.696	H1	Caib-DRB*01
LME2759	ALPKOZ- 14	Slovenia	Julian Alps – Kriški podi	2020	46.343	13.696	H1	Caib-DRB*01
LME2760	ALPKOZ- 15	Slovenia	Julian Alps – Kriški podi – pod Debelo pečjo	2009	46.392	13.933	H1	Caib-DRB*01
LME2761	ALPKOZ- 16	Slovenia	Julian Alps – Plazi – na Laberju	2009	46.343	13.696	H1	/
LME2762	ALPKOZ- 17	Slovenia	Julian Alps – Plazi – pri Skali	2019	46.343	13.696	H1	/
LME2763	ALPKOZ- 18	Slovenia	Julian Alps – Plazi – pod Risjem	2019	46.315	13.698	H1	Caib-DRB*01
LME2764	ALPKOZ- 19	Slovenia	Julian Alps – Plazi – Pejča	2019	46.318	13.683	H1	Caib-DRB*01
LME2765	ALPKOZ- 20	Slovenia	Julian Alps – Kriški podi	2002	46.380	13.834	H1	/
LME2766	ALPKOZ- 21	Slovenia	Julian Alps – Kriški podi – Korito	2004	46.429	13.710	H1	Caib-DRB*01
*	TNPCapr aIbexSI	Slovenia	Julian Alps – Krn	/	46.265	13.660	H1	/
*	WILD1C apralbex SI	Slovenia	Julian Alps – Krn	/	46.265	13.660	H1	/
*	WILD2C apralbex SI	Slovenia	Julian Alps – Krn	/	46.265	13.660	H1	/
*	WILD3C apralbex SI	Slovenia	Julian Alps – Bavšica	/	46.369	13.628	H1	/
*	WILD4C apralbex SI	Slovenia	Julian Alps – Log pod Mangartom	/	46.411	13.594	H1	/
LME3986	Kozorog 1	Slovenia	Kamnik–Savinja Alps	2013	46.366	14.562	H1	/
LME3987	Kozorog 2	Slovenia	Kamnik–Savinja Alps	2008	46.366	14.562	H1	/
LME3988	Kozorog 3	Slovenia	Kamnik–Savinja Alps	2013	46.366	14.562	H1	/
LME3989	Kozorog 4	Slovenia	Kamnik–Savinja Alps	2011	46.366	14.562	H1	/

Table S1: Continued

Lab/GenBank ID	Sample ID	Country	Location	Year of sampling	Lat	Long	Cyt b hpt	MHC allele
LME3991	Kozorog 6	Slovenia	Kamnik–Savinja Alps	2008	46.366	14.562	H1	/
LME3992	Kozorog 7	Slovenia	Kamnik–Savinja Alps	2008	46.366	14.562	H1	/
LME3993	Kozorog 8	Slovenia	Kamnik–Savinja Alps	2019	46.366	14.562	H1	/
LME3996	Kozorog 11	Slovenia	Kamnik–Savinja Alps	2011	46.366	14.562	H1	/
LME3997	Kozorog 12	Slovenia	Kamnik–Savinja Alps	2010	46.366	14.562	H1	/
LME3995	Kozorog 10	Slovenia	Kamnik–Savinja Alps	2013	46.366	14.562	H1	/
LME3660	O01Q	Italy	Orco	2017	45.402	7.510	H2	Caib-DRB*01
LME3661	O01R	Italy	Orco	2018	45.402	7.510	H1	Caib-DRB*01
LME3662	O02Q	Italy	Orco	2017	45.402	7.510	H1	Caib-DRB*01
LME3663	O02R	Italy	Orco	2018	45.402	7.510	H1	Caib-DRB*01
LME3664	O03Q	Italy	Orco	2017	45.402	7.510	H3	Caib-DRB*01
LME3665	O03R	Italy	Orco	2018	45.402	7.510	H1	Caib-DRB*01
LME3666	V01N	Italy	Valsavarenche	/	45.582	7.218	H1	Caib-DRB*01
LME3667	V01P	Italy	Valsavarenche	2016	45.582	7.218	H1	Caib-DRB*01
LME3668	V02N	Italy	Valsavarenche	/	45.582	7.218	H1	Caib-DRB*01
LME3669	VO2P	Italy	Valsavarenche	2016	45.582	7.218	H3	Caib-DRB*01
LME3670	VO3P	Italy	Valsavarenche	2016	45.582	7.218	H2	Caib-DRB*01
LME3671	V04N	Italy	Valsavarenche	/	45.582	7.218	H1	Caib-DRB*01
LME3672	V04P	Italy	Valsavarenche	2016	45.582	7.218	H1	Caib-DRB*01
LME3673	V04Q	Italy	Valsavarenche	2017	45.582	7.218	H1	Caib-DRB*01
LME3674	V05N	Italy	Valsavarenche	/	45.582	7.218	H1	Caib-DRB*01
LME3675	VO5Q	Italy	Valsavarenche	2017	45.582	7.218	H4	Caib-DRB*01
LME3676	V06N	Italy	Valsavarenche	/	45.582	7.218	H2	Caib-DRB*01
LME3677	V06Q	Italy	Valsavarenche	2017	45.582	7.218	H1	Caib-DRB*01
LME3678	V07N	Italy	Valsavarenche	2017	45.582	7.218	H1	Caib-DRB*01
LME3679	V08N	Italy	Valsavarenche	/	45.582	7.218	H1	Caib-DRB*01

Table S1: Continued

Lab/GenBank ID	Sample ID	Country	Location	Year of sampling	Lat	Long	Cyt b hpt	MHC allele
LME3681	V08Q	Italy	Valsavarenche	2017	45.582	7.218	H1	Caib-DRB*01
LME3682	V09Q	Italy	Valsavarenche	2017	45.582	7.218	H1	Caib-DRB*01
LME3683	V11N	Italy	Valsavarenche	/	45.582	7.218	H1	Caib-DRB*01
LME3684	V20L	Italy	Valsavarenche	2018	45.582	7.218	H1	Caib-DRB*01
EU368877(62)	CiGP1	Italy	/	/	/	/	H1	/
*	AIB4574 CapraIb exAT	Austria	Döllach Hohe Tauern	2013	46.980	12.939	H1	/
*	AIB4832 CapraIb exAT	Austria	Hohe Tauern	2016	47.163	12.505	H1	/
*	AIB5332 CapraIb exAT	Austria	Heiligenblut Hohe Tauern	2017	47.014	12.808	H1	/
*	AIB5333 CapraIb exAT	Austria	Heiligenblut Hohe Tauern	2017	47.014	12.808	H1	/
*	AIB5336 CapraIb exAT	Austria	Heiligenblut Hohe Tauern	2017	47.014	12.808	H1	/
*	ZOO17C apralbex SI	Austria and Switzerland	ZOO Ljubljana	/	46.054	14.472	H1	/
*	ZOO18C apralbex SI	Austria and Switzerland	ZOO Ljubljana	/	46.054	14.472	H1	/
*	ZOO19C apralbex SI	Austria and Switzerland	ZOO Ljubljana	/	46.054	14.472	H1	/
*	ZOO20C apralbex SI	Austria and Switzerland	ZOO Ljubljana	/	46.054	14.472	H1	/
*	ZOO28C apralbex SI	Austria and Switzerland	ZOO Ljubljana	/	46.054	14.472	H1	/
*	ZOO29C apralbex SI	Austria and Switzerland	ZOO Ljubljana	/	46.054	14.472	H1	/
*	ZOO30C apralbex SI	Austria and Switzerland	ZOO Ljubljana	/	46.054	14.472	H1	/
*	ZOO31C apralbex SI	Austria and Switzerland	ZOO Ljubljana	/	46.054	14.472	H1	/
*	ZOO40C apralbex SI	Austria and Switzerland	ZOO Ljubljana	/	46.054	14.472	H1	/
*	ZOO42C apralbex SI	Austria and Switzerland	ZOO Ljubljana	/	46.054	14.472	H1	/
*	ZOO43C apralbex SI	Austria and Switzerland	ZOO Ljubljana	/	46.054	14.472	H1	/
*	ZOO47C apralbex SI	Austria and Switzerland	ZOO Ljubljana	/	46.054	14.472	H1	/

Table S1: Continued

Lab/GenBank ID	Sample ID	Country	Location	Year of sampling	Lat	Long	Cyt b hpt	MHC allele
*	ZOO48C apralbex SI	Austria and Switzerland	ZOO Ljubljana	/	46.054	14.472	H1	/
*	ZOO49C apralbex SI	Austria and Switzerland	ZOO Ljubljana	/	46.054	14.472	H1	/
*	ZOO52C apralbex SI	Austria and Switzerland	ZOO Ljubljana	/	46.054	14.472	H1	/
*	ZOO56C apralbex SI	Austria and Switzerland	ZOO Ljubljana	/	46.054	14.472	H1	/
*	ZOO58C apralbex SI	Austria and Switzerland	ZOO Ljubljana	/	46.054	14.472	H1	/
AY70631(48)	/	Switzerland	Albris, Kanton Graubuenden	2005	46.658	9.608	/	Caib-DRB*01
FJ207526(63)	Cyto 2002-037, MNHN	France	/	2002	/	/	H1	/
AF034735(64)	Bone 1938-1296	France	Pointe de Calabre, Savoie	1900s	45.473	7.077	H1	/

Notes: Lat – latitude; Long – longitude; Cyt b hpt – cytochrome b haplotype; MHC allele – MHC DRB exon 2 allele. Caib-DRB*01 was detected in *Capra hircus* and deposit in GenBank with accession number AY706312.

Prvi vpogled v genetsko raznolikost alpskega kozoroga (*Capra ibex*) v Sloveniji

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Izvleček: V Evropi je bil alpski kozorog (*Capra ibex*) v 19. stoletju na robu izumrtja. Po tem času so se izvajali različni ukrepi za njegovo ohranjanje. V alpskem loku je bilo izvedenih več ponovnih naselitev, najprej z edino ohranjeno populacijo v narodnem parku Gran Paradiso v Italiji. Ozko grlo v preteklosti in dodatne ponovne naselitve so močno vplivale na genetski sklad populacije, tudi zaradi prisotnega genetskega zdrsa in parjenja v ožjem sorodstvu.V podporo znanstveno utemeljenim ukrepom ohranjanja so se za vse zasnovane populacije z izjemo Slovenije uporabljale tudi molekularne analize. Da bi razumeli, kako je ponovno naseljevanje in upravljanje vplivalo na genetsko variabilnost populacij v Sloveniji, smo analizirali nevtralni lokus (delni fragment mitohondrijskega citokroma b, mtDNA) in adaptivni poglavitni histokompatibilnostni kompleks (MHC DRB ekson 2) alpskega kozoroga iz dveh populacij (Julijske in Kamniško-Savinjske Alpe). Rezultati so pokazali, da sta obe populaciji genetsko zelo osiromašeni, saj nosita le en haplotip mtDNA in en funkcionalni alel za MHC DRB ekson 2, Caib-DRB*01. Zato so potrebni nadaljnji ukrepi za ohranjanje, vključno s ponovno naselitvijo živali iz populacij z večjo genetsko variabilnostjo. Vendar alpski kozorog v Sloveniji trenutno velja za tujerodno vrsto, kar zelo otežuje ukrepe za njegovo ohranitev in ogroža dolgoročno preživetje vrste.Znanstveniki in upravljavci populacij zato pozivajo politike/odločevalce, naj spremenijo status vrste v avtohtono in posledično omogočijo ponovno naselitev. Ti pozivi so podprti s predhodnimi arheološkimi podatki o obstoju kosti alpskega kozoroga v Julijskih Alpah in z dokazi o izraziti genetski osiromašenosti sedanjih populacij kozoroga, potrjenimi v tej študiji.

Ključne besede: *Capra ibex*; mitohondrijska DNA; MHC *DRB* exon 2; ponovna naselitev; upravljanje