DivjaLabs Ltd.
Aljaževa ulica 35a, SI-1000 Ljubljana, Slovenia
info@divjalabs.com
www.divjalabs.com
a University of Ljubljana Spinout Company

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WWF

Assessing diet composition in Croatian wolf population: Insights from DNA metabarcoding analysis

Report – Contract no. 10/2023/HR00084

Project: Leveraging impact of Large Carnivores conservation efforts in Central and South-Eastern Europe (HR00084), implemented in Croatia

Client: WWF Adria (contract supervisor: Snježana Malić Limari)

Authors: Marta De Barba, Elena Pazhenkova, Barbara Boljte, Aleksandra Majić, Skrbinšek, Snježana Malić Limari, Tomaž Skrbinšek

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3 Nomenclature dictionary

To facilitate comprehension and interpretation of our study, we have included a comprehensive dictionary of animal species, genera, subfamilies and families mentioned in Latin language throughout this report. This list presents the used scientific nomenclature in Latin translated into English and Croatian languages for the convenience of our readers.

Latin (alphabetically)	**English**	**Croatian**
Bos (genus)	cattle	goveda (domaća)
Bovidae (family)	bovids	šupljerošci
Canidae (family)	canids	psi / kanidi
Capra (genus)	goats	koze (domaća)
Capreolus capreolus (species)	roe deer	obična srna
Cervidae (family)	the deer family	jeleni
Cervinae (subfamily)	Old World deer	jeleni Starog svijeta
Cervus elaphus (species)	red deer	obični jelen
Leporidae (family)	family of rabbits and hares	zečevi
Lepus (genus)	hare	obični zec
Ovis (genus)	sheep	ovce (domaća i muflon)
Suidae (family)	pigs	svinje
Sus scrofa (species)	European wild boar / domestic pig	divlja / domaća svinja
Vulpes vulpes (species)	red fox	lisica

4 Introduction

We carried out analysis for 60 putative wolf samples collected in Croatia within project "Provedba genetske analize populacije vuka u Hrvatskoj", 800/02-22/37JDN, which was financed by the Ministry of Economy and Sustainable Development (MESD). The samples were collected within the work of Animal signs Tracker Team led by the Institute for Environment and Nature MESD for Croatian wolf monitoring 2022 – 2023, and originally analysed using microsatellites to individually identify wolves (see Annex 1). Approval to use the samples for additional genetic analyses described in this report was obtained at a meeting with the representative of the Ministry on February 15th 2023 and later confirmed at Regional Large Carnivore Platform meeting in March 2023 in Skopje, North Macedonia. The

diet analysis provides added value to these samples and allows to add another dimension to the data that was obtained from the previous DNA analysis. The samples were selected from a larger set to have a good spatial coverage, and only the samples that provided a reasonably good genotype (meaning that DNA in them was of sufficient quantity and quality for genotyping) were selected. They were genotyped using genotyping by high-throughput sequencing.

To summarize, the scope of the project task according to the contract 10/2023/HR00084 commissioned to DivjaLabs by WWF Adria and presented in this report was:

- To analyse wolf diet by applying DNA metabarcoding analysis of the 60 selected faeces samples collected during 2022 for the purpose of Croatian national wolf monitoring.
- To perform spatial analysis of wolf diet in GIS with preparation of maps (distribution of samples, distribution of diet items detected).

DNA metabarcoding refers to the taxonomic identification of multiple taxa contained in complex mixtures, such as environmental samples or animal faeces, via a single experiment based on short genetic regions (Taberlet et al. 2012). The DNA sequences for these diagnostic regions obtained from field-collected samples are matched against reference sequences from available databases in order to assign the taxon. Major benefits of DNA metabarcoding, compared to traditional methods for taxonomic identification, are that it enables efficient and noninvasive taxa detection and greater taxonomic resolution. For these reasons, DNA metabarcoding is now widely applied in biodiversity monitoring and ecological studies, including diet analysis of wild predator species and derived applications, such as unravelling food webs, predator–prey interactions, herbivore ecosystem function and niche breadth. A parallel pilot study is currently underway, led by De Barba et al., as part of the LIFE WOLFALPS EU project. This study focuses on wolf samples sourced from the Alpine region of Slovenia. These two initiatives mark groundbreaking efforts, representing the first efforts in employing the DNA metabarcoding methodology to scrutinize the dietary patterns of the Dinaric-Balkan wolf population.

4.1 Wolf in Croatia

Croatian wolves are part of a larger Dinaric-Balkan wolf population which spreads from Slovenia to northern Greece, also including Croatia, Bosnia and Herzegovina, Serbia, Kosovo, Montenegro, Northern Macedonia, Albania and Bulgaria and is estimated to have around 5500 wolves (Boitani et al., 2022). In Croatia, the wolf is permanently present throughout the Dinaric Mountains, with Croatia population bordering to the wolf populations of Slovenia, Bosnia and Hercegovina and Montenegro to the south. Wolves in Croatia prey primarily on wild ungulates and sometimes also domestic animals which causes economic damage and social conflict (State Institute for Nature Protection, 2014).

From a limited pool of noninvasive wolf samples collected for the purposes of national wolf monitoring we selected samples that can be classified geographically into three distinctive groups that we describe as follows:

- Žumberak and Samobor Mountains is a hilly area and Nature park at the current edge of the wolf distribution in Croatia within the Continental biogeographical region. It is a mosaic-like landscape, result of human activities, with open grassland surfaces interweaving with forested areas, making it an ideal habitat for roe deer.
- Within the Alpine biogeographical region there is Gorski Kotar. Gorski Kotar is a mountainous forested area with relatively high densities of wild ungulates. Previous studies of wolf stomach contents report the main prey in this region being wild ungulates (red deer, roe deer and wild boar) which accounted for 84.21% of the diet (State Institute for Nature Protection, 2014).
- Southern Croatia, border of Alpine and Mediterranean biogeographical region, includes Lika area (Alpine) and Dalmatia area (Mediterranean). South of Croatia, especially Dalmatia is an area where availability of natural wolf prey is limited and wolves are reported to often incur damage to livestock (State Institute for Nature Protection, 2014).

5 Laboratory methods

DNA was extracted from collected samples with MagMax Multi Sample Kit (ABI, Thermo Fisher Scientific) implemented on a pipetting robot (Hamilton). Faecal DNA extractions were carried out in a room dedicated to processing low quality/quantity DNA samples. Three extraction negative controls (containing only extraction reagents and no faecal material) were included to monitor for contamination and were analysed following the same protocol used for the faecal samples.

Diet analysis was performed by amplifying a universal marker targeting a short and variable fragment of the mitochondrial DNA (Taberlet et al., 2018). Blocking oligonucleotides were included in the PCR to minimise amplification of wolf and human DNA. PCR amplifications contained 1x concentrated AmpliTaq Gold® Master Mix (ABI, Thermo Fischer Scientific), 0.2 µM each primer, 2µM blocking oligonuctleotides, 0.0032 mg bovine serum albumin (BSA, New England BioLabs, Inc.) and 2 µL DNA extract, in 20 µL reaction volume. Thermocycling conditions had an initial denaturation step of 10 minutes at 95°C, followed by 45 cycles of 30 seconds at 95°C, 30 seconds at 49°C, 60 seconds at 72°C, and a final elongation of 7 minutes at 72°C. Following De Barba et al. (2014), we used PCR negative controls (n=2) and aliquots (n=2) of a positive control sample in the experiment to monitor the performance of the amplification and the sequencing, and to guide the selection of filtering parameters in the sequence analysis process. The positive control was made by mixing known quantities of the DNA extract of 4 species. Four PCR replicates were performed for all samples. Primers used in each PCR were uniquely modified by the addition of molecular identifier tags on the 5' end, to allow the assignment of sequence reads to their source samples. Tags differed on both ends of a PCR product and were composed by eight nucleotides, containing at least five differences among them (Coissac, 2012). Empty PCR wells, corresponding to unused tag combinations, were included in the experiment to monitor potential tag-jumping events (Schnell et al., 2015).

PCR products were pooled together and purified using the MinElute PCR purification kit (QIAGEN GmbH) and sequenced as a single library prepared using a PCR-free procedure enabling a significant reduction in bias associated with library preparation and sequencing (Carøe & Bohmann, 2020). The sequencing was carried out on a Miniseq platform (2x150 bp) (Illumina Inc.).

6 Sequence analysis and filtering protocol

The sequence reads were first analysed using the OBITools package (Boyer et al., 2016). Forward and reverse reads corresponding to a single DNA molecule were assembled and primers and tags were identified. The amplified regions, excluding primers and tags, were kept for further analysis. Strictly identical sequences were clustered together, keeping the information about their distributions among samples. Sequences shorter than 50 bp were excluded. Each sequence within a PCR product was classified into the categories of 'head' (the most common sequence within a group of sequences differing by a single indel/substitution), 'internal' (sequences less frequent within the group of related sequences, i.e. corresponding to amplification/sequencing errors) or 'singleton' (a sequence with no other variant differing by a single indel/substitution). A sequence reference database for taxonomically identifying sequences detected in the samples was built by extracting the relevant DNA region from EMBL nucleotide library (downloaded on 22 February 2022) using *ecoPCR* program (Ficetola et al., 2010). Taxon assignation was achieved by finding highly similar sequences to the query sequence in the reference database and assigning a unique taxon to each sequence.

Taxonomically assigned sequences were then further filtered using R v. 4.2.1 (R Core Team, 2018). Following De Barba et al., (2014), we used the positive and negative controls included in the experiment to set filtering parameters (i.e. for identify erroneous/spurious sequences, sequences deriving from contamination and samples more likely to produce unreliable results) and to evaluate the performance of the experiment. Sequences not identified as "head" in ≥3 replicates, or "singleton" in 4 replicates for a sample were considered erroneous and therefore deleted. We discarded sequences with a per sample read frequency below a set threshold (0.05) based on the comparison of read counts of sequences of known taxa in the positive controls vs unexpected sequences. Sequences assigned to *Canis* (the predator), and with identity <80% over the entire query sequence length with any sequence in the reference database were removed. Sample replicates with read count less then 1000 were discarded based on the read count observed across the majority of the replicates for the negative controls. Only samples having at least 2 replicates after this step were retained. Within these samples, we kept sequences observed in >50% of sample replicates. Sample replicates were finally combined to obtain a consensus sequence profile for each sample, by taking the sum of the sequence read counts of sample replicates. Sequence read counts were then converted to frequencies for further analysis.

7 Results

Sequencing of the 60 scat samples processed for DNA metabarcoding generated a total of 9765461 paired-end sequences reads for which primers and tags were identified.

One extraction negative control sample contained a *Capreolus capreolus* sequence after the filtering. We scrutinized all other samples containing the same sequence and its amplification patterns and concluded that the sequence in the negative control likely originated from contamination from a wolf sample positioned just next to the control on the PCR plate and that other samples were not affected.

We discarded 6 faecal samples that yielded no sequence reads after sequence data analysis and filtering. Thus, we used 54 faeces for all further analyses of diet composition. Among these 54 samples, 4 contained fox (*Vulpes vulpes*). However, prior to applying data filtering, all these 4 samples had a disproportionate amount of fox reads compared to wolf reads, suggesting that they might be faeces deposited by fox rather than wolf. We therefore considered them as fox and report results of diet composition for 50 wolf and 4 fox samples.

From the 54 (50 wolf and 4 fox) samples retained after filtering, we detected 14 sequences corresponding to 10 different taxa (Table 1) belonging to 5 taxonomic families.

Table 1: Sequences identified in the samples analysed.

Sequence identifier	Taxon	Rank	Family	Best identity score	Total read count	% read count (RF)	N. of samples	Freq.of occurrence (FO)
18767:1096	Cervus elaphus	species	Cervidae	1	1825926	44.96%	21	38.9%
18697:1093	Sus scrofa	species	Suidae	1	828990	20.41%	10	18.5%
17798:1092	Vulpes vulpes	species	Canidae	1	493935	12.16%	4	7.4%
10904:1100	Capreolus capreolus	species	Cervidae	1	380975	9.38%	9	16.7%
20918:1098	Capreolus capreolus	species	Cervidae	1	272175	6.70%	8	14.8%
14249:1125	Caprinae	subfamily	Bovidae	1	100797	2.48%	4	7.4%
5436:1095	Capra	genus	Bovidae	1	42549	1.05%	1	1.9%
20749:1121	Bos	genus	Bovidae	1	20404	0.50%	3	5.6%
23647:1107	Ovis	genus	Bovidae	1	5766	0.14%	1	1.9%
8234:1477	Lepus	genus	Leporidae	1	3530	0.09%	1	1.9%
14259:1198	Capreolus capreolus	species	Cervidae	0.98	1989	0.05%	2	3.7%
25140:1341	Bos	genus	Bovidae	1	1804	0.04%	1	1.9%
21331:1117	Cervinae	subfamily	Cervidae	0.98	1505	0.04%	2	3.7%
24107:1261	Capreolus capreolus	species	Cervidae	0.98	521	0.01%	1	1.9%

The taxonomic resolution achieved was high, with 95% of sequences identified to species level. Ungulates of the Cervidae family comprised the majority of the sequence reads across all samples (63%) (Fig. 1), with *Cervus elaphus* (found in 21 samples, 39% FO) and *Capreolus capreolus* (found in 20 samples, 37% FO) being the most frequently detected species (Table 1, Fig. 2). *Sus scrofa* was the third most frequently detected species, found in 10 samples (18.5% FO; Table 1, Fig. 2).

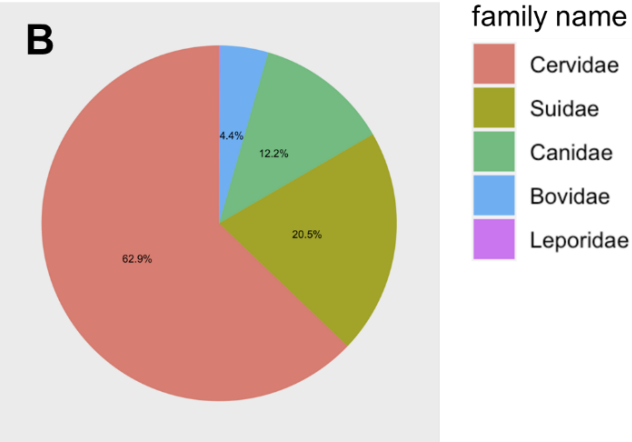

Figure 1: Proportion of sequences assigned to different families across the samples for which composition was determined.

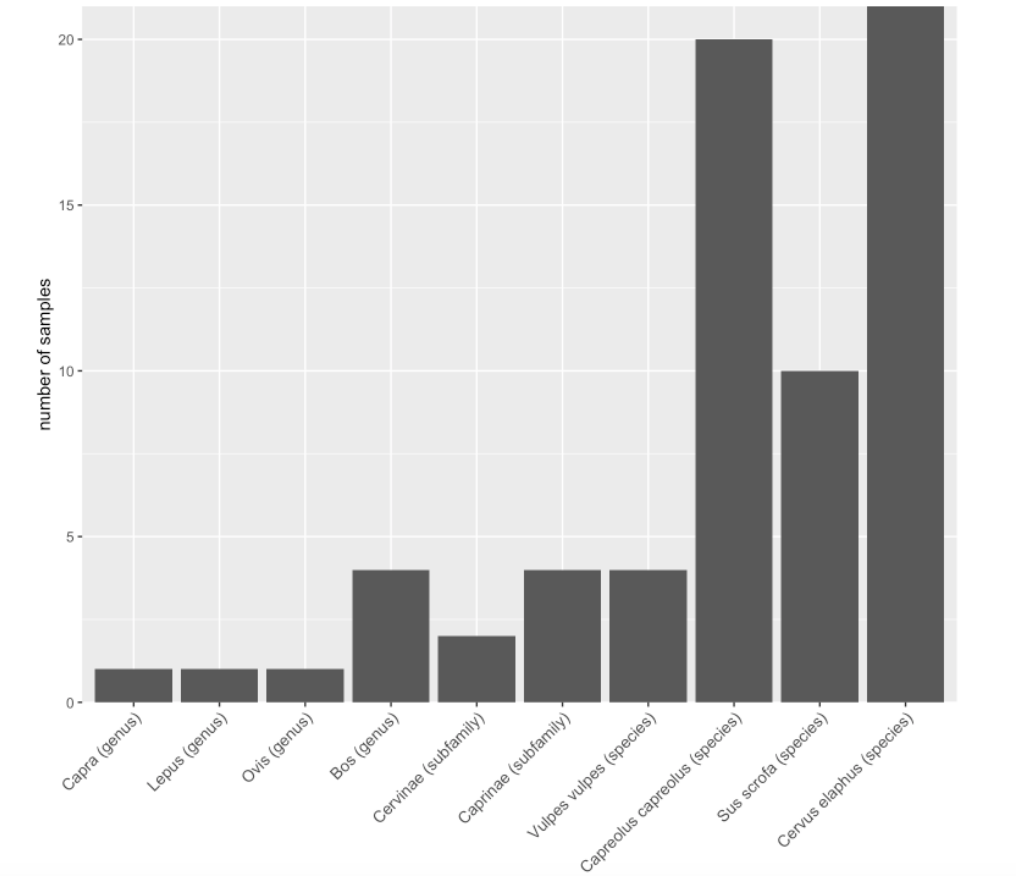

Figure 2: Number of samples in which a given taxon was detected.

Most wolf samples were comprised of a single prey item, except for 8 samples (16%), in which two taxa were detected (Fig. 3) with different relative abundance (Fig. 4). We detected a single prey item (*Cervus elaphus*) in only one of the 4 fox samples (Fig. 3 and 4).

Samples	Bos	Capra	Capreolus capreolus	Caprinae	Cervinae	Cervus elaphus	Lepus	Ovis	Sus scrofa	Vulpes vulpes
HRV003						■				
HRV004						■				
HRV005			■							
HRV007				■						
HRV008	■			■						
HRV00C			■							
HRV00E			■							
HRV00J			■							
HRV00K			■							
HRV011			■			■				
HRV012			■							
HRV014								■		
HRV015						■				
HRV016			■							
HRV017			■							
HRV018						■				■
HRV01C						■				
HRV01E						■				

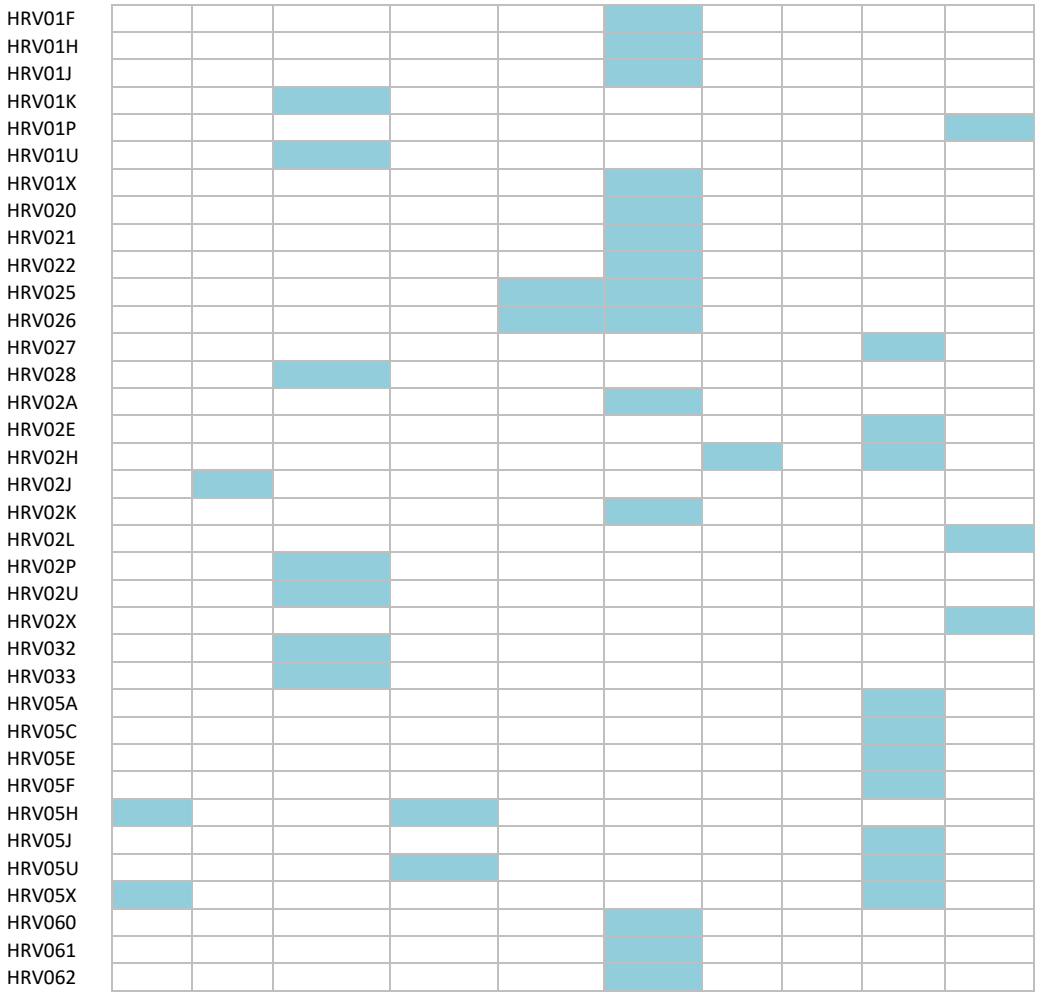

Figure 3: Taxa occurrence (presence/absence) in each sample for which composition was determined.

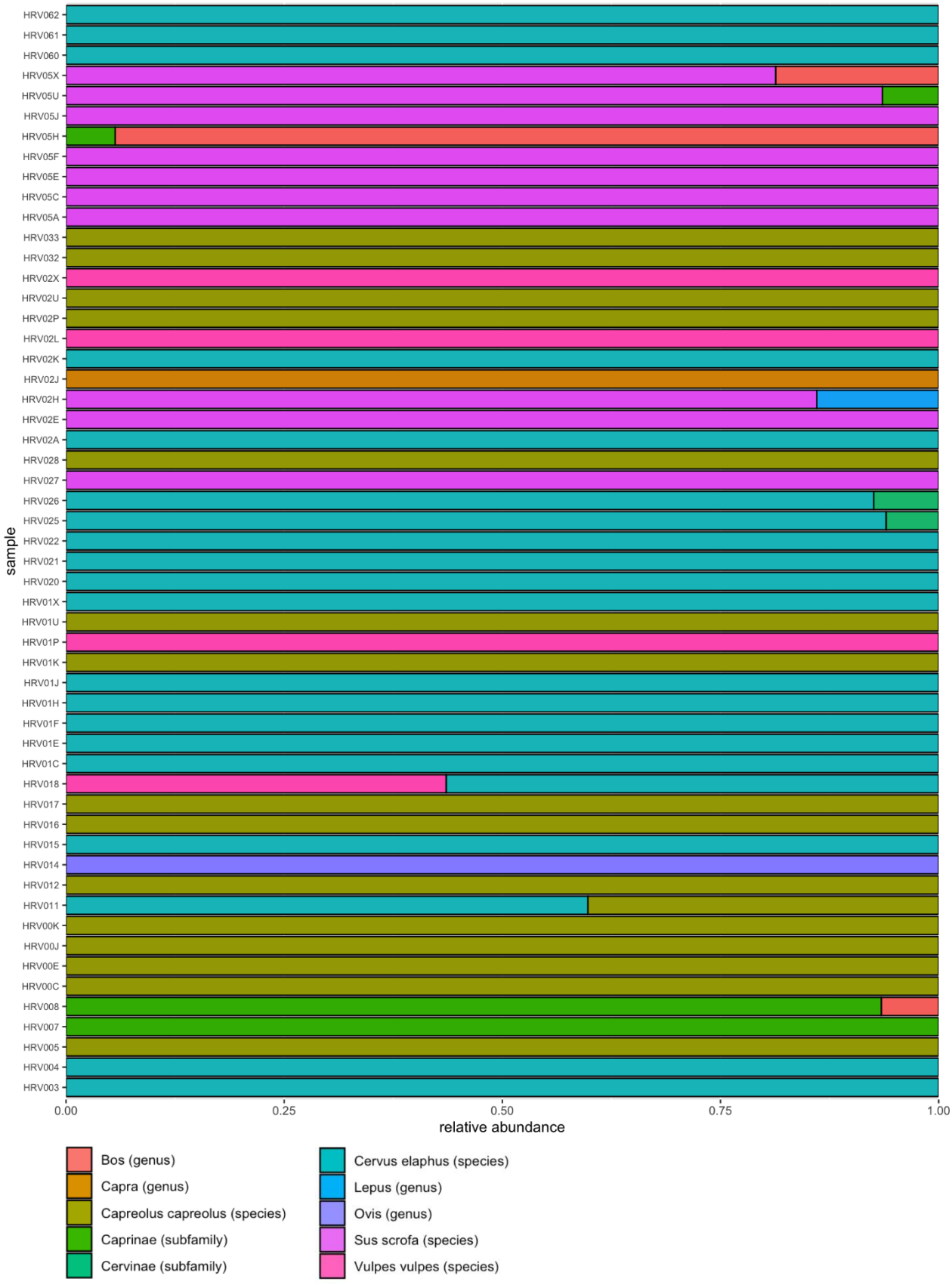

Figure 4: Relative abundance of taxa detected in the samples. Bars of different colours represent the proportion of reads for a given taxon in each sample.

When mapping wolf diet composition based on relative sequence abundance of taxa detected in each wolf sample, a certain degree of spatial clustering of prey items became apparent (Fig. 5).

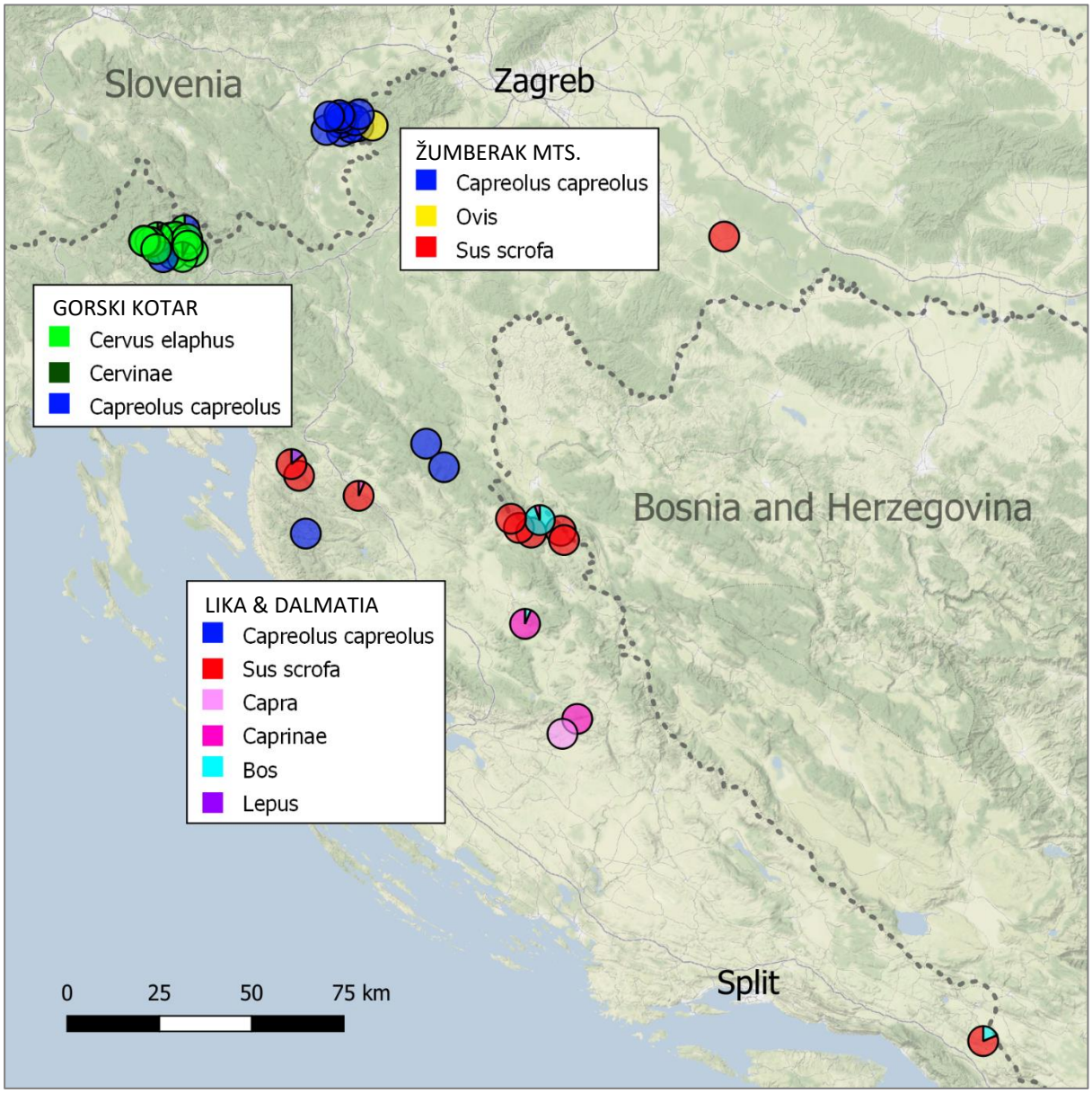

Figure 5: Map of wolf samples for which diet was determined showing spatial patterns in diet composition, entire study area. The map is constructed using sample locations and relative sequence abundance of taxa detected in each sample. Each pie chart represents a wolf sample; slices of the pie are proportional to the relative abundance of sequences for different taxa (prey items). Sample locations have been added a small amount of random noise to decrease overplotting.

Among the taxa identified, only *Bos*, found in three wolf samples, and Capra, found in one sample, are of anthropogenic origin in the region. Other taxa possibly associated with human activities, such as *Sus scrofa* and *Ovis*, have a domestic and a wild counterpart (domestic pig/wild boar and sheep/mouflon respectively) that cannot be distinguished with our method. Caprinae, found in 4 samples, include the genus *Ovis*, *Capra,* and *Rupicapra* (chamois). However, *Rupicapra* can usually be identified with our method. Knowledge about

the distribution of human activities and wild prey species in the study area can be used to disentangle these cases: for example, if the domestic species are not present in the sampling area, the taxa detected are most likely the wild counterparts. Knowledge about presence and distribution of potential prey species can also be used to refine taxonomic resolution. Specifically, sequences assigned to *Lepus* could be reasonably considered to originate from *Lepus europaeus*, if this is the only *Lepus* species present in the study area.

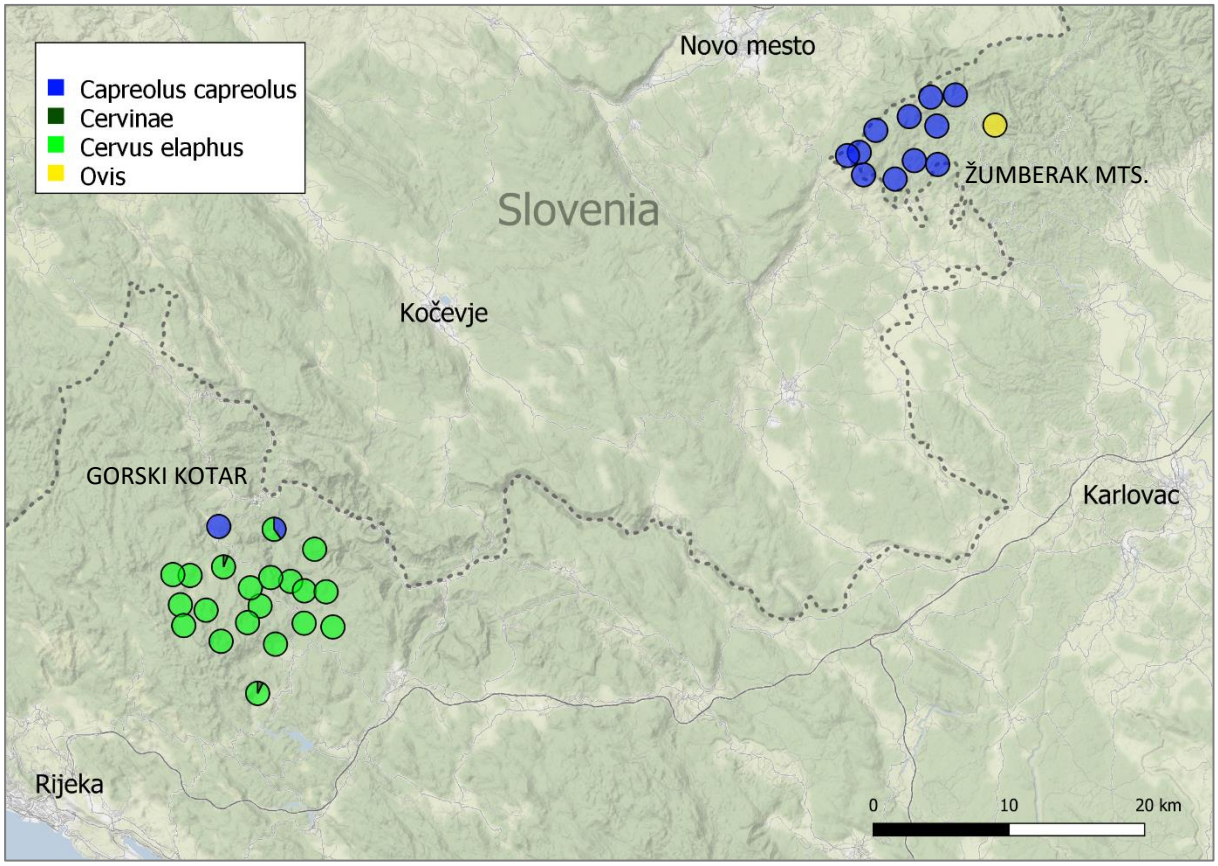

Figure 6: Map of wolf samples for which diet was determined showing spatial patterns in diet composition, northern part of the area (Gorski kotar and Žumberak Mountains).

Six sequences were identified above species level. This is generally due to 1. limited taxonomic resolution of the marker for certain taxa, i.e. different taxa have the same sequence for the marker analysed, so that the query sequence is identified at a higher taxonomic level that includes all these different taxa, or 2. incompleteness of the reference sequence database for certain taxa, i.e. the query sequence is not present in the reference database, so that it is assigned to the most similar sequence in the database, possibly of higher taxonomic level. In the case of Cervinae, found in two samples, the explanation is different. Here, the sequence is likely derived from the more abundant *Cervus elaphus* sequence in the samples due unfiltered PCR errors. Consequently, it was assigned to a higher taxonomic level, but should be considered as *Cervus elaphus*.

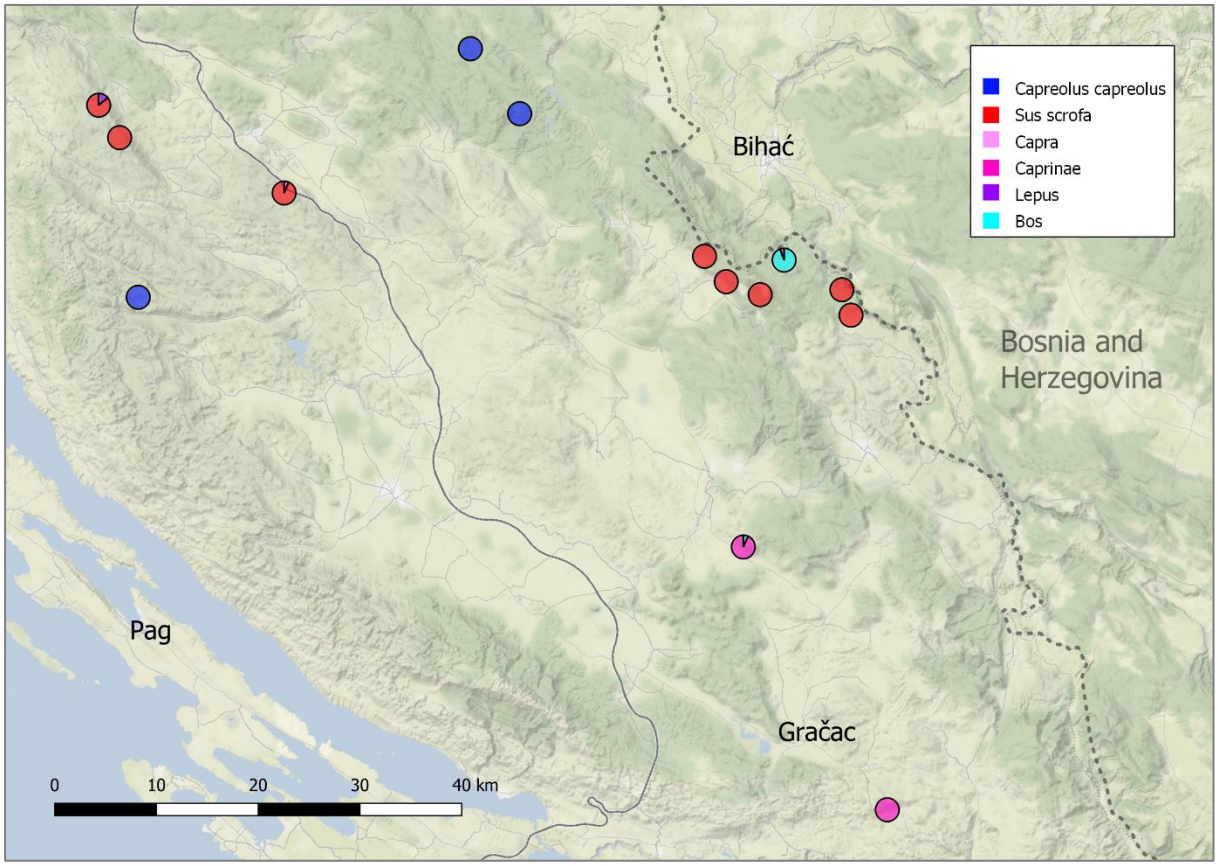

Figure 7: Map of wolf samples for which diet was determined showing spatial patterns in diet composition, central part of the area (region of Lika).

8 Discussion and recommendations

Although small, this study provides a first direct insight into wolf diet in Dinaric landscapes of Croatia. It is a pilot study, as well as a proof of concept of how noninvasive samples collected in genetic monitoring activities aimed at estimating population parameters can be used to gain insight into other important aspects of a species' life history and ecology. At the same time, a similar study was conducted within the LIFE WOLFALPS EU project in the northern part of the Dinaric – Balkan wolf population (Figure 8). In this study wolf diet from Slovenian Alps (n=70) was analysed and main prey species found were roe deer and red deer, found in 37 (53%) and 32 (46%) of the samples, respectively (De Barba et al., unpublished), which also directly reflects the availability of wild prey species in that area (Kavčič et al., 2011).

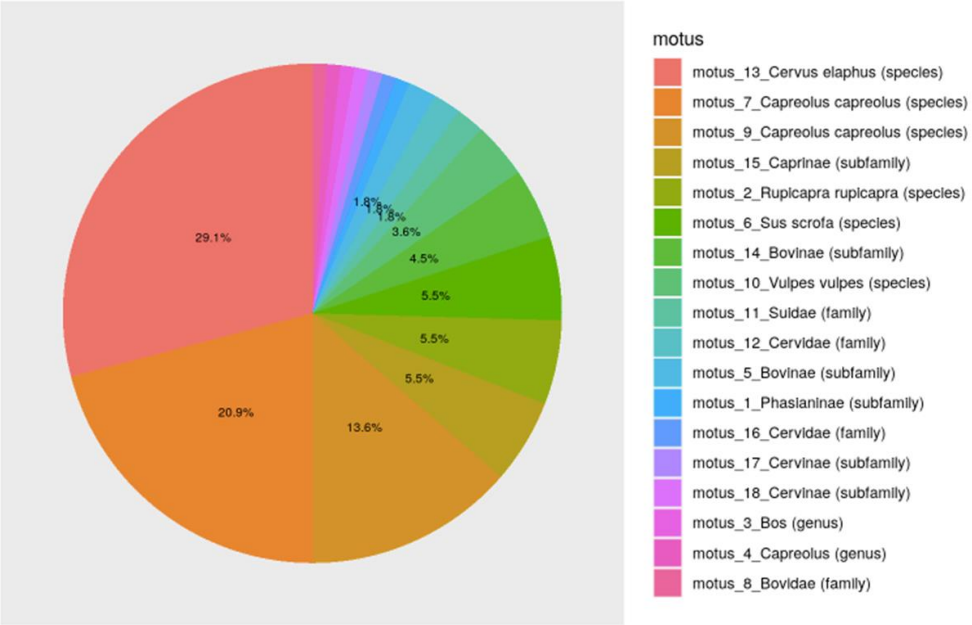

Figure 8: Results of the study from the Slovenian Alps – main species detected were roe deer and red deer (De Barba et al., unpublished).

The vast majority of the detected wolf prey was wild ungulates, with a quite clear spatial pattern. In Gorski Kotar the main prey was red deer. This mountainous area is covered by dense forests, with red deer as the predominant large herbivore, so it's not surprising that it features heavily in the wolf diet in the area. North from there, in the Žumberak – Samoborsko Gorje area, the landscape has a much more mosaic structure with a lot of forest edge, which is a typical landscape for roe deer. This is also directly reflected in the diet of the wolves. One of the detected preys there was *Ovis*; which could be either sheep or mouflon. We don't have the data about mouflon presence in the area, but it cannot be excluded as a possible prey. However, such animals would most likely be kept in an enclosure, while domestic sheep are much more abundant in the area, so this sample was most likely predation on domestic sheep.

Going towards south to Lika and Dalmatia, the landscape changes in character, and so does the wolf prey. The wild prey becomes much less abundant (Ocetnjak et al., 2020). While roe deer is still present in wolf diet, an important prey becomes the wild boar. We cannot distinguish with this method between wild boars and their domestic counterparts, but considering that damages to domestic pigs are rare, and wild boar is abundant, we can assume that the wolves in this area are mostly preying on the wild variety. Interestingly, wild boar is an abundant species also in the northern areas where we didn't detect it in wolf diet, and we can speculate that wolves preferentially select red deer and roe deer if they are sufficiently abundant but can utilize other prey if they are not. This dietary diversification has been reported in wolves before (Roffler et al., 2021). The authors have used DNA metabarcoding to study wolf diet in Alexander archipelago (SE Alaska, USA) and documented how wolves responded to biogeographical variation in availability of their primary prey by altering their foraging patterns. Wolves increased the number and diversity of species consumed and widened their dietary niche as the proportion of wild ungulates in their diet declined rather than switching to one or only few dietary items (Roffler et al., 2021).

In the south we can also find more domestic animals, namely cattle and goats, in the wolf diet. But altogether, in this limited sampling, the domestic livestock was a relatively rarely found in the analysed wolf scats. Although livestock breeding is a common practice, particularly in Lika and Dalmatia, there are relatively small number of samples with evidence of domestic livestock (sheep, goat, cattle). However, it is not possible to determine if these food items come from predation or scavenging since dumping offal remaining from slaughter of domestic animals is common practice, and sometimes the entire animals that have died from different causes are dumped in a place accessible to wolves. In some samples we detected a high number of reads from wild or putative wild (*Sus scrofa*) prey and a much lower number of reads from domestic livestock (*Capra*, *Bos*). The detection of a second item with lower relative abundance in a wolf scat can have multiple explanations; for example, it may originate from traces of a previous meal or from wolves supplementing their diet through scavenging on garbage dumps. Collection of additional diet data, as well as complementary field information about the ecological context could provide further insight.

A study based on analysis of stomach contents of dead wolves (n= 42) in Croatia found similar conclusions (Ocetnjak et al., 2020). The authors examined spatial variation and prey selection relative to availability of wild and domestic animals. The density ratio of domestic to wild ungulates increased gradually from north-west (5.8), through central (11.6) to south-east (134) Croatia, and the wolf diet followed this pattern with the ratio of domestic animals increasing from 0.7 in the north-west to 5.3 in the south-east. The relative share of wild ungulates in wolf prey was higher in all regions of Croatia, thus demonstrating wolves' selectivity for wild ungulates, rather than for abundant, but guarded livestock (Ocetnjak et al., 2020).

Another important issue determining the amount of preying on livestock was social organization, where wolves in stable packs were found to utilize predominantly wild prey while dispersing wolves more often used livestock as a food source (Imbert et al., 2016).

This is important since poaching or lethal control can lead to higher livestock depredations and have negative impact on wolf – human coexistence.

In accordance with the conclusions in the previous studies of wolf diet (e.g. Roffler et al., 2021, Octenjak et al., 2020, Imbert et al., 2016), our results also strongly suggest that understanding ecological interactions and dietary preferences is important for the development of effective conservation strategies and mitigation of human-wildlife conflicts. The two stakeholder groups most affected by the presence of wolves are livestock farmers and hunters as wolves come into conflict with them when they prey on game species or domestic animals. By understanding the specific diet composition of wolves, stakeholders can gain insights into which prey species are targeted and the extent to which livestock predation may occur. This knowledge allows for targeted communication efforts that address specific concerns of the stakeholders. Transparent and accurate information about wolf diet helps in building trust and fostering cooperation between stakeholders and conservationists. By sharing scientific findings on wolf diet composition, stakeholders can better understand the ecological role of wolves and the natural variability in their diet. This knowledge can help dispel misconceptions or exaggerated fears and foster a more informed and constructive dialogue among stakeholders, focusing on implementation of measures that minimize conflicts while ensuring long-term conservation of wolves. Those would include improvement of livestock husbandry practices (damage prevention and promotion of predator-friendly livestock husbandry) or targeted hunting regulations, to minimize conflicts. For game managers, understanding the wolf ecology can be crucial for maintaining sustainable hunting practices. By identifying the prey species targeted by wolves, hunting regulations can be tailored to ensure the conservation of both game species and wolves.

In conclusion, this DNA metabarcoding analysis provided valuable insights into the diet composition of the Croatian wolf population. By directly elucidating the composition of the prey species consumed, this study contributes to the wider understanding of the ecological role and conservation needs of wolves in the Dinaric region. Another interesting aspect is that the study "piggybacked" on samples collected for different purposes, giving additional value to the samples collected through the more "common" genetic monitoring. As genetic monitoring is becoming more and more commonplace, such synergies may hold considerable promise in the future since with thoughtful study designs, important ecological and conservation insights could be obtained without the need for additional field work and with reduced laboratory effort (DNA extraction from wolf samples is the same for both purposes). As for the case of wolves in the Dinaric landscapes of Croatia, further research with increased sample sizes, possibly longitudinally exploring geo-temporal and seasonal variations in diet and assessing impacts of human activities on prey availability and consumption, have the potential to considerably enhance our knowledge and inform conservation efforts in the future.

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10 Annex 1: List of analysed samples

Sample	Date collection	Population	Sample type	Animal Reference Code	X	Y	Sampling Note	Genetic Sex	Estimated Scat Age (days)	Scat thickness (cm)	Microlocation	Contents note
HRV001	21-Mar-22	Dinaric	Scat	HRV001	45.74358	15.35281	pada kiša -malo izpran izmet	M	1	3	cesta	blato z malo dlakami
HRV003	30-Mar-22	Dinaric	Scat	HRV028	45.454	14.673		F	1	3	cesta	blato s sivimi dlakami
HRV004	30-Mar-22	Dinaric	Scat	HRV022	45.462	14.667		F	1	2.8	cesta	blato s svetlimi dlakami
HRV005	22-Mar-22	Dinaric	Scat	HRV005	45.73841	15.284	leptiri na izmetu	F	2	3	cesta	svetle dlake, delčki listov
HRV007	13-Apr-22	Dinaric	Scat	HRV007	44.25388	15.96249	nedaleko je fotozam tuda redovito prolazi čopor vukova uz izmet je i markiranje.	F	5	2.5	cesta	svetle dlake z malo blata
HRV008	04-May-22	Dinaric	Scat	HRV008	44.45374	15.80205		F	1		planinarska staza	blato, skoraj brez dlak
HRV00C	17-Aug-22	Dinaric	Scat	HRV00K	45.72822	15.27626		F	1	3	cesta	blato, dlake
HRV00E	20-Apr-22	Dinaric	Scat	HRV00K	45.74312	15.35177	Proteklih dana dosta padala kiša - ispran izmet	F	5	3	cesta	blato, dlake
HRV00H	09-May-22	Dinaric	Scat	HRV00H	45.72715	15.2721		M	1	2.5	cesta	blato, dlake
HRV00J	21-Apr-22	Dinaric	Scat	HRV001	45.72596	15.26726		M	3	4	cesta	blato z malo dlakami

Sample	Date collection	Population	Sample type	Animal Reference Code	X	Y	Sampling Note	Genetic Sex	Estimated Scat Age (days)	Scat thickness (cm)	Microlocation	Contents note
HRV00K	23-May-22	Dinaric	Scat	HRV00K	45.72822	15.27775	intenzivan miris, puno muha, leptira, te insekti balegari koji su ga kotrjali, te je bio sav od pijeska.	F	2	3	cesta	blato, dlake
HRV00L	28-Mar-22	Dinaric	Scat	HRV00L	44.44681	15.5239		M	2	3	cesta	blato, dlake
HRV010	22-Mar-22	Dinaric	Scat	HRV010	43.83356	16.08689		U	4	4.5	cesta	blato, dlake
HRV011	02-Mar-22	Dinaric	Scat	HRV011	45.46747	14.70206	Pored ceste na plijenu (tele).	U	1	3	pored ceste	blato, dlake, kosti
HRV012	25-Mar-22	Dinaric	Scat	HRV001	45.7266	15.27097		M	2	4	cesta	blato, dlake
HRV014	31-Mar-22	Dinaric	Scat	HRV001	45.74874	15.36005	pada kiša, možda ispran uzorak	M	1	3	cesta	blato, dlake, kosti
HRV015	02-Mar-22	Dinaric	Scat	HRV015	45.461	14.667	Izmet na tragu čopora u snijegu (8 kom)	F	1	0	cesta	zelo malo blata
HRV016	24-Mar-22	Dinaric	Scat	HRV005	45.73194	15.35256		F	2	3	cesta	blato s kratkimi ščetinastimi dlakami
HRV017	25-Mar-22	Dinaric	Scat	HRV005	45.72581	15.26636	izmet izuzetno suh i tvrd, teško sam skupio uzorak	F	2	3	cesta	blato s kratkimi ščetinastimi dlakami
HRV018	07-Mar-22	Dinaric	Scat		45.49495	14.69437			3	2.5	hranilište	blato s kratkimi ščetinastimi dlakami
HRV01C	07-Mar-22	Dinaric	Scat	HRV02A	45.473	14.712		F	1	3	cesta	blato, dlake
HRV01E	02-Mar-22	Dinaric	Scat	HRV022	45.47	14.653	Trag čopora u snijegu (8 kom)	F	1	0	cesta	blato s kratkimi ščetinastimi dlakami

Sample	Date collection	Population	Sample type	Animal Reference Code	X	Y	Sampling Note	Genetic Sex	Estimated Scat Age (days)	Scat thickness (cm)	Microlocation	Contents note
HRV01F	02-Mar-22	Dinaric	Scat	HRV01F	45.46711	14.70253		F	1	3	cesta	blato, dlake
HRV01H	07-Mar-22	Dinaric	Scat	HRV01F	45.475	14.709		F	2	3.5	cesta	večinoma dlake, površina blatna
HRV01J	02-Mar-22	Dinaric	Scat	HRV01J	45.463	14.667	Trag čopora u snijegu (8 kom)	M	1	0	cesta	blato
HRV01K	31-Mar-22	Dinaric	Scat	HRV00K	45.74881	15.36069	pada kiša, možda ispran uzorak	F	1	3	cesta	glodalčje dlake z blatom
HRV01P	10-May-22	Dinaric	Scat	HRV01P	45.76227	15.36781	1m dalje u blatu otisak vučje šape	F	2	3	cesta	blato, dlake
HRV01U	28-Apr-22	Dinaric	Scat	HRV01U	44.75586	15.05404	Izmet je bio na cesti pregažen, pa nije izmjeren promjer.	M	5		cesta	blato, dlake
HRV01X	02-Mar-22	Dinaric	Scat	HRV028	45.466	14.716		F	1	3	cesta	blato, dlake
HRV020	03-Mar-22	Dinaric	Scat	HRV020	45.426	14.689		F	2	2.75	cesta	blato, dlake
HRV021	07-Mar-22	Dinaric	Scat	HRV021	45.462	14.732		M	1		cesta	blato, dlake
HRV022	02-Mar-22	Dinaric	Scat	HRV022	45.46727	14.70228		F	1	2.75	cesta	večinoma dlake, kost
HRV024	12-Jul-22	Dinaric	Scat	HRV024	45.423	17.674		M	1	3.5	cesta	dlake z malo blata
HRV025	13-Apr-22	Dinaric	Scat	HRV025	45.465	14.625		F	5	2.25	cesta	črno blato, dlake, koža
HRV026	21-Apr-22	Dinaric	Scat	HRV026	45.419	14.681		F	1	3	cesta	dlake
HRV027	02-May-22	Dinaric	Scat	HRV027	45.36014	16.56021	Padala kiša večer prije	U	5	2.5	ovanica	blato, dlake
HRV028	13-Apr-22	Dinaric	Scat	HRV028	45.471	14.644		F	2	2.75	cesta	blato, dlake, kosti
HRV02A	02-Mar-22	Dinaric	Scat	HRV02A	45.469	14.715		F	1	2.75	cesta	blato, dlake
HRV02C	18-Jul-22	Dinaric	Scat	HRV02C	44.89035	15.04075	visoka vručina, sunce	M	2	2.5	cesta	dlake z malo blata

Sample	Date collection	Population	Sample type	Animal Reference Code	X	Y	Sampling Note	Genetic Sex	Estimated Scat Age (days)	Scat thickness (cm)	Microlocation	Contents note
HRV02E	18-Jul-22	Dinaric	Scat	HRV02E	44.89397	15.04028		F	1.5	2	cesta	dlake
HRV02H	18-Jul-22	Dinaric	Scat	HRV02C	44.88825	15.04165		M	1	2	cesta	blato, dlake
HRV02J	23-Apr-22	Dinaric	Scat	HRV02J	44.21586	15.87522	Bilo je kiše. Uzorak sam vzeo jer je miris još intenzivan.	M	5	2.5	cesta	blato, dlake
HRV02K	25-Feb-22	Dinaric	Scat	HRV02K	45.47694	14.62052		F	5	3	sijsko križanje	blato, dlake
HRV02L	27-Apr-22	Dinaric	Scat	HRV02L	44.25695	15.94281	Iako je izmet star još daje intenzivan miris pa sam uzorkovao.	M	5	2.5	cesta	dlake z malo blata
HRV02P	08-Jul-22	Dinaric	Scat	HRV00K	45.72956	15.27884		F	3	3	cesta	blato, dlake
HRV02U	17-Aug-22	Dinaric	Scat	HRV005	45.72675	15.27125		F	1	3	cesta	blato, dlake
HRV02X	24-Mar-22	Dinaric	Scat	HRV02X	44.91478	15.53141		M	5	2	staza	blato, dlake
HRV032	07-Jun-22	Dinaric	Scat	HRV033	44.92952	15.52261		F	2	3	cesta	blato, dlake
HRV033	28-May-22	Dinaric	Scat	HRV033	44.88309	15.56065		F	2	3	cesta	blato, dlake
HRV05A	03-May-22	Dinaric	Scat	HRV05J	44.6974	15.976		F	1	3.5	cesta	blato, malo dlak
HRV05C	12-Apr-22	Dinaric	Scat	HRV05C	44.7356	15.8412		U	2		cesta	blato, malo dlak, smrdi
HRV05E	12-Apr-22	Dinaric	Scat	HRV05J	44.736	15.8397		F	2		cesta	blato, dlake
HRV05F	09-Apr-22	Dinaric	Scat	HRV05J	44.70592	15.9785		F	1	3	cesta	blato, malo dlak
HRV05H	08-Apr-22	Dinaric	Scat	HRV05H	44.7431	15.8205		F	3		cesta	blato, dlake
HRV05J	12-Apr-22	Dinaric	Scat	HRV05J	44.7355	15.842		F	2		cesta	blato, dlake, kosti
HRV05U	26-Jul-22	Dinaric	Scat	HRV05U	44.82528	15.27517		F	0	2.5	cesta	blato, malo dlak
HRV05X	18-Jul-22	Dinaric	Scat	HRV05X	43.36228	17.2427	Vrlo suh	M	2	2.7	cesta	blato, par dlak

Sample	Date collection	Population	Sample type	Animal Reference Code	X	Y	Sampling Note	Genetic Sex	Estimated Scat Age (days)	Scat thickness (cm)	Microlocation	Contents note
HRV060	28-Jul-22	Dinaric	Scat	HRV060	45.4544	14.6044	Tomaž: Koordinate nisu sasvim ok (bio je dvaput upisan lattitude), pa je lokacija dana po geografskom imenu .	M	2	3.5	cesta	blato, malo dlak
HRV061	08-Aug-22	Dinaric	Scat	HRV011	45.48129	14.6044		U	4	2.5	cesta	blato
HRV062	28-Jul-22	Dinaric	Scat	HRV011	45.45922	14.63486		U	5	3	cesta	blato, malo dlak