ANALYSIS OF DIETARY HABITS OF BROWN BEAR AND LYNX IN YENICE AND SARIKAMIŞ REGIONS, TURKEY

by

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ANALYSIS OF DIETARY HABITS OF BROWN BEAR AND LYNX IN YENICE AND SARIKAMIŞ REGIONS, TURKEY

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ABSTRACT

ANALYSIS OF DIETARY HABITS OF BROWN BEAR AND LYNX IN YENICE AND SARIKAMIŞ REGIONS, TURKEY

In this study, the dietary habits of brown bears (*Ursus arctos arctos*) living in Yenice and Sarıkamış regions and dietary habits of lynx (*Lynx lynx dinniki*) living in Sarıkamış were investigated by scat analysis, and using the DNA metabarcoding approach. Universal primers were used for plant, invertebrate and vertebrate detection. Diet compositions of bears in Yenice and Sarıkamış regions were analyzed and compared to determine the effect of garbage feeding, and diet compositions for the bear were consistent with literature. Shannon, Levin's, and Pianka indexes were used for comparison of different locations of the collected samples. The results did not confirm the hypothesis of low diet diversity and low niche width for garbage feeding. Vertebrate component of lynx diet was not identified, potentially due to biases introduced by the methods used. However, the lack of detection of vertebrates could be due to human pressure on habitats and decreased prey availability for lynx.

ÖZET

YENİCE VE SARIKAMIŞ (TÜRKİYE) BÖLGELERİNDEKİ BOZAYI VE VAŞAKLARIN DİYET ALIŞKANLIKLARININ ANALİZİ

Bu çalışmada, Yenice ve Sarıkamış'ta yaşayan bozayılar ile Sarıkamış'ta yaşayan vaşak türlerinin beslenme alışkanlıkları DNA metabarkodlama ile dışkı analizi yöntemi kullanılarak belirlendi. Diyet analizi için evrensel primerler ve bloklama primerleri kullanıldı. Çöplükten beslenmenin etkilerinin belirlenmesi için, Yenice ve Sarıkamış'ta yaşayan ayıların diyet kompozisyonları analiz edildi ve karşılaştırıldı. Ayıların diyet kompozisyonları literature ile uyumluydu. Toplanan örneklerin yerlerinin karşılaştırılması için Shannon, Levin's ve Pianka indeksleri kullanıldı. Sonuçlar çöpten beslenme için düşük diyet çeşitliliği ve düşük niş genişliği hipotezini doğrulamadı. Vaşak diyetinin omurgalılar bölümü kullanılan yöntemdeki potansiyel sorunlar sebebiyle tespit edilemedi. Fakat tespit edilemeyen bu omurgalı bölümü için olası diğer neden, vaşakların habitatlarının ve uygun av hayvanlarının üzerindeki insan baskısı olabilir.

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LIST OF SYMBOLS/ABBREVIATIONS

Symbol	Explanation	Unit
µl	Microliter	
°C	Degree Centigrade	
u	Unit	
H	Shannon Index	
B	Levin's Index	
α	Pianka's Index	
ng	Nanogram	

Abbreviation	Explanation
DNA	Deoxyribonucleic Acid
DNTP	Deoxyribonucleotide Triphosphate
G	Guanine
Kg	Kilogram
PCR	Polymerase Chain Reaction
Bp	Base-pair
dNTP	Deoxyribonucleotide Triphosphate
EMBL	European Molecular Biology Laboratory
NCBI	National Center for Biotechnology Information
ATE Buffer	Tris – EDTA (Ethylenediaminetetraacetic acid) buffer provided by Qiagen
A	Adenine
C	Cytosine
G	Guanine
T	Thymine
s	Seconds
TAE Buffer	Buffer made of acetic acid, Tris base and EDTA solution
P_i	Frequency of a food item in overall sample

1. INTRODUCTION

Habitat destruction/fragmentation is a major problem facing ecosystems throughout the world. Devastation of ecosystems negatively affects a diverse array of habitats and harms species located at different levels of the food pyramid. Studies on habitat destruction/fragmentation can separately focus on various effects on species (Mantyka-pringle *et al*., 2012; Ryall and Fahrig, 2006)

The large habitat requirements of large carnivores can increase the possibility of human – wildlife conflict in cases of habitat destruction or fragmentation (Ripple *et al*., 2014). One of the key large mammals globally, brown bears – (*Ursus arctos*) have vast home-ranges, which makes them more vulnerable to the effects of habitat destruction/fragmentation or human activity in general. Studies on destruction of habitats of bears are generally focused on habitat destruction/fragmentation by road constructions and industrial activities (forestry, mining etc.) (Mattson *et al*., 1986; McLellan and Shackleton, 1988). Destruction of habitats can diminish available food resources, limit nesting opportunities and also reduce territories available to roam. An increased level of habitat destruction/fragmentation and human activity can also cause changes in the feeding habits of bears (Mattson, 1989).

1.1. Brown Bear Diet

Brown bear is a generalist species which can feed on plants, vertebrates and invertebrates. Changes in dietary composition of brown bears mostly occur due to seasonal variations and individual habitat characteristics. Studies also record long term effects of environmental fluctuations and land-use changes on the diet composition of brown bears (Naves *et al.*, 2006). Several studies have composed a nearly full picture of dietary habits of brown bears. Despite having been conducted in different locations, seasons, and years; these studies outline a few main food sources for brown bears. These food sources include fruits, mast, insects, and if available, wild ungulates and livestock (Ciucci *et al*., 2014; Clevenger *et al*., 2016; Paralikidis *et al.*, 2010).

1.2. Determining Diets of Animals: Conventional versus Molecular Methods

Conventional methods of determining dietary habits range from direct observation of eating behavior to analyzing fecal samples for the hard parts remaining from prey species (bone, fur etc.). The direct observation method is not very effective for many species, since most organisms are small, elusive, and nocturnal or live underwater/underground. Analyzing feces or gut contents of species also has certain limitations. Feces or gut content analyses are labor-intensive, require a highly skilled researcher who can identify prey species from masticated and semi-digested fragments, and the method completely excludes studies of several fluid-feeder species (Sheppard *et al*., 2005). In addition to these conventional techniques, a variety of molecular techniques have been developed for the study of dietary habits. These include DNA–based methods, protein electrophoresis, immunoassays, fatty acid analysis, alkane fingerprints for plants and stable isotope analysis (Sheppard and Harwood, 2005; Symondson, 2002). However, all of these methods exhibit some form of limitation when it comes to studying the diets of most species.

1.3. DNA Barcoding and Diet Analyses

DNA based methods for studying dietary habits of species use DNA barcoding techniques to identify DNA fragments of prey species in feces or gut contents. DNA barcoding is a term used to describe the process of taxon identification using a standardized DNA region. For animals, this standard region proposed comprises a 658 base-pair (bp) fragment in the mitochondrial cytochrome c oxidase 1 gene (COI) (Hebert *et al.*, 2003; Meusnier *et al*., 2008). However for plants, the issue is not that clear, and multiple methods based on a single chloroplast region or combination of different regions have been proposed (Hebert *et al*., 2003; Valentini, *et al*., 2009). DNA barcoding can also be used in several areas like forensic science, biotechnology, and in the food industry. One problem with DNA barcoding for the purposes of dietary analyses is due to Sanger sequencing (Sanger *et al.*, 1977) not being appropriate for analyzing environmental samples including DNA fragments from different species, since it can only be used to sequence specimens individually, increasing cost and time to analyze environmental samples. However, with the advance of next-generation sequencing (NGS), it became possible to analyze samples with DNA from several species in parallel, reducing costs and time needed. When applied to the feces samples from the organism of interest, DNA barcoding methods coupled with NGS can identify prey species with a higher resolution than conventional methods (Casper *et al.*, 2007; Valentini *et al.*, 2009). Several types of

NGS technologies with diverse chemistries and base incorporation/selection tools are available to researchers with their own associated limitations, in terms of cost and time (Glenn, 2011).

1.4. Dietary Studies Using NGS

The method of parallel sequencing of environmental DNA can be used to identify prey DNA from feces or stomach contents of several species whose dietary habits cannot be studied effectively by conventional methods. Dietary habits of many different species [puffins (Bowser, *et al.*, 2013), tapirs (Hibert *et al*., 2013), seals (Deagle, *et al.*, 2009), bats (Bohmann *et al*., 2011), reptiles (Brown, *et al.*, 2012; Kartzinel and Pringle, 2015), penguins (Deagle *et al.*, 2010) and several fish species, among others)] have been studied with the DNA barcoding approach coupled with NGS. These studies mostly show that molecular analysis methods have a greater taxonomic resolution than conventional methods. Additionally, the fecal samples of some bird and fish species are not appropriate to study with conventional methods of visual identification and morphological examination (Bowser *et al*., 2013; Carreon-Martinez *et al.*, 2011). Molecular sequencing techniques prove to be more efficient and effective, especially with these organisms (Pompanon *et al*., 2012).

1.5. Objective of the Thesis

Aim of thesis is to analyze diet habits of brown bear in two separate regions (Yenice/Karabük and Sarıkamış/Kars/Ardahan) in Turkey and determine effects of human-wild life contact to the diet of brown bears. The case of Sarıkamış region is an example of habitat destruction/fragmentation and high level of bear-human interaction. Roaming of garbage dumps by bears in South Sarıkamış region is very well documented by Chynoweth *et al.* (2016), Cozzi *et al.* (2016), and also by national press. Figure 1.1 shows a bear group feeding on city garbage dump (Anadolu Agency, 2019). It is known that brown bears can use anthropogenic food sources like garbage dumps (Peirce and Daele, 2006). High levels of human activity near natural habitat of bears can severely affect feeding habits of these animals and cause them to survive by feeding on human waste and garbage. As opposed to South Sarıkamış, north of Sarıkamış and Yenice comprise more intact habitats, unaffected by human activity, thus providing a natural feeding source for bears in these regions. Our expectation is that, garbage feeding should cause a decrease in diet diversity and niche width, since garbage feeding bears do not need to search for food from natural environments with diverse food sources. Since Yenice and North Sarıkamış regions provide natural food sources, we also hypothesize that they should be more similar to each other, when compared to South Sarıkamış), according to diet diversity and niche width. The study also aims to establish a robust and simple

method to identify diet components of brown bears using fecal samples without the need of expertise and preliminary information about prey species. Even though the study's main focus was on brown bears, due to availability of lynx scat samples from Sarıkamış, these were analyzed as well, for dietary characterization.

Figure 1.1. Photo image of bear group feeding on Kars City Dump (Adopted from: Anadolu Agency).

2. LITERATURE REVIEW

2.1. DNA Metabarcoding

First study to include DNA barcoding and next generation sequencing for a scat sample analysis is that of Valentini *et al.* (2009). This study aimed to develop a universal diet analysis method for herbivores using P6 loop of chloroplast trnL (UAA) intron, and analyzed 12 scat samples of brown bears collected from Deosai National Park (Pakistan), using the 454 GS 20 sequencing system (Roche, Basel, Switzerland) following manufacturer's instructions.

Results showed that 31 % of different P6 loop sequences obtained from bear scat samples were identified to species level. Bear scat samples provided 557 different P6 loop sequences. Brown bear diet composed mainly of Poaceae and Polygonaceae, as well as, Cyperaceae and Apiaceae families of plants.

Overall results of study demonstrate a robust and reliable method to identify plants in scat samples for diet analysis. The *trnL* approach is easy to implement and has many advantages over conventional diet analysis methods. It is also suited for application to a wide range analysis of diet compositions to observe seasonal and geographical variations. Coupled with individual identification (Taberlet and Luikart, 1999), the method allows comparisons of diet of different individuals from different sex and age groups.

One of the two potential difficulties observed in this study are, sequencing errors due to large mix of DNA molecules to be sequenced. Many PCR products were pooled before sequencing step to reduce cost of sequencing. To separate unsorted sequences produced by a single sequencing run, each sample was tagged differently with 5'-CCNNNN-3' at the 5' end. This allowed identification of corresponding sequences in the sequencer output. Other potential problems included, the sequencing errors due to P6 loop itself resulting from degradation of DNA sample, nucleotide misincorporation and sequencing process errors.

De Barba *et al*. (2014) analyzes 91 brown bear samples collected in northern Italy for genetic monitoring of brown bear diet composition aiming to identify all components of diet; including plants, vertebrates and invertebrates. The study used a multiplexing method to reduce the number of PCRs with three different primer sets for each sample, targeting vertebrate (Vestheim and Jarman,

2008), invertebrate, and plant (Valentini *et al*., 2009) barcoding regions. The *trnL* approach is complemented with four sets of primers targeting Asteraceae, Cyperaceae, Poaceae and Rosaceae families (Baamrane *et al*., 2012). The study also attempted to standardize internal controls for filters for sequence analysis, thus removing erroneous sequences caused by DNA degradation, PCR and sequencing errors, chimeras, contamination and primer dimers (Glenn, 2011; Valentini *et al*., 2009; Qiu *et al*., 2001)

The results of De Barba *et al*. (2014) show that 59.3 % of samples have plant and invertebrate constituents. 16.5 % of samples only have plant species and 17.6 % have vertebrate, invertebrate and plants. The study found out that the plant component of the diet is more diversified with plant species belonging to a greater number of families.

Taxonomic resolution of chosen markers for the study shows that this approach is suitable for large scale studies for dietary analysis and for identifying changes over seasons, individuals, and different age and sex groups. Still, researchers advise complementary strategies for greater resolution, like using markers targeting important taxa or complementing reference database with sequences of specimens from the study area (De Barba *et al*., 2014).

2.2. Conventional Methods of Diet Determination

2.2.1. Visual and Morphological Analysis

Conventionally, diet composition of brown bears is identified with analysis of scat samples visually. Dahle *et al.* (1998) used a 7-30 power stereoscope and a 40-630 power microscope on washed scat samples to analyze diet components. The study aimed to compare diet composition of brown bears between Swedish and Norwegian locations. Results demonstrated that the presence of free-ranging sheep in Norway causes bears to switch food preferences; sheep seemed to be highly selected by bears. Berries, ungulates, ants and sheep were the main components of bear diets as determined in the study.

Grizzly bears (*Ursus arctos horribilis*) in Yellowstone National Park region have been heavily studied since the middle of the 20th century. One extensive study is Mattson (1991), which analyzed 3423 scat samples from a ten year period. Results of study underline opportunistic feeding behavior of bears. The study found that bear in the region switch their diet largely on a year to year basis. One example of various diet habit changes was the discovery of sweet cicely roots during 1986

without any evidence of consumption in the previous nine years. Study concludes Yellowstone bears sample a spectrum of potential foods continuously and prefer beneficial kinds. Diet variation among seasons and months also showed large-scale diet switching. The average diet of Yellowstone brown bears mainly consists of ungulates, graminoids and pine seeds during spring, summer, and fall, respectively. The study reports that the unavailability of fleshy fruits, and irregularity of pine seed production are the two main factors limiting the density of grizzly bears.

Rodríguez *et al.* (2007) documented brown bear food habits in Cantabrian Mountains-Spain spanning nearly a thirty-year period (1974 – 2003), taking effects of climate into account. 934 scat samples were analyzed. The study showed that berries, fleshy fruits and dry fruits constituted the bulk of brown bear's diet during the hyperphagic season (July – November). Rodríguez *et al*. (2007) reported that annual changes in diet composition occur, and results suggest that global and local factors may be partly responsible for this pattern.

A newer study using visual identification is by Ciucci *et al*. (2014). Using 2,359 scat samples from central Apennines, Italy, the study documents annual diet of Appenine brown bears and their seasonal dietary shifts. The study used a 7–30X stereoscope and a 400–600X microscope to identify food items at the lowest taxonomic level possible, and showed that fleshy fruits have the most energetic contribution to diet. Herbaceous vegetation and insects ranked second, and ungulates and livestock ranked third according to energetic contribution. The study also reports that hard mast (especially beechnuts) and fleshy fruits (especially buckthorn berries) are key foods during hyperphagy. Lastly, the study concludes that there was no evidence for nutritional stress, and therefore management goal should be protecting diversity and accessibility of key food items in the long term.

Paralikidis *et al*. (2010) is a comprehensive example of conventional scat analysis. The study analyzes 360 scat samples of brown bears from western Greece. Mammalian constituents of scat were identified by hair examination (Teerink, 2004). Plant parts were identified from the epidermal cells (Baumgartner and Martin, 1939; Dusi, 1949). Insects were identified using insect guides (Chinery, 2007). The results show that the diet habits of western Greece brown bears were similar to studies covering other European countries (Clevenger *et al*., 2016; Elgmork and Kaasa, 1992; Frackowiak and Gula, 1992). The study determined that green vegetation in spring, soft masts in summer and hard mast in autumn were major food sources for brown bears. Since ungulate density was low in the study area, wild ungulates made up a small part of the diet. The study concludes that

food availability and the ease of finding or capturing food are major factors affecting diet composition of the brown bears.

In a very recent study, Ogurtsov (2018) identified diet of brown bear population in Central Forest Nature Reserve (West-European Russia). Based on 474 scat samples, most commonly consumed food items and food groups were determined. The study found that the brown bears in the region were mostly vegetarian – frugivorous with apple as a main dietary component. Forbs, berries and shrubs were also important food items in the study.

All comprehensive studies report seasonal and annual changes of bear diet compositions according to availability of food items. Furthermore, opportunistic behavior of bears are supported by all studies showing the preference for highly nutritious foods.

2.2.2. Stable Isotope and Mercury Bioaccumulation Analysis

Stable isotope analysis is another method to estimate diet habits and assimilated diets. The method measures naturally occurring stable isotope concentrations of nitrogen, carbon and sulfur to detect changing ratios of stable isotopes as they move from plants to series of consumers (Robbins *et al*., 2004) . In addition to that, specific foods can be identified if they have unique isotope signatures. Felicetti *et al*. (2003) used this method to establish the importance of whitebark pine nuts in Yellowstone grizzly bear diet. The study found that whitebark pine nut is a critically important food for Yellowstone grizzly bears, in addition to meat. Felicetti *et al*. (2004) used mercury bioaccumulation in cutthroat trout (*Oncorhynchus clarki*) to detect dietary intake of grizzly bears using hair samples. The study discovered male grizzly bears consumed 92% of all trout ingested by grizzly bears.

One detailed study using stable isotope ratios to determine food habits of bears was undertaken by Hilderbrand *et al*. (1996). The study identifies diet habits of extinct cave bears, historical use of salmon by Pacific Northwest grizzly bears, and current seasonal diets of brown bears on Chigaof and Admiralty Islands. Carbon and nitrogen isotope ratios were determined from bone samples obtained from American Museum of Natural History for 10 cave bears. To determine historical use of salmon, bone and hair samples were obtained from the same museum. Results show that cave bears were not mostly herbivores as claimed in Bocherens and Mariotti (1994). Similarity of isotopic signatures between known omnivores (black and brown bears) and cave bears supports the finding. Historical diet analysis show that bears from central Montana and eastern Wyoming

obtained their assimilated carbon and hydrogen from terrestrially produced meat, but Alaskan coastal bears obtain nearly all their carbon and nitrogen from salmon.

Fortin *et al*., (2007) used stable isotope analysis of hair and feces to determine dietary overlap of black and brown bears in Kenai Peninsula/Alaska. Species, individuals and sex were identified by DNA analysis of samples. The study found that the presence of brown bears near salmon sources eliminate use of salmon by black bears. Therefore, black bears were mostly herbivorous and frugivorous. Salmon use of brown bears were similar to that reported in other studies at regions where salmon was abundant (Gende *et al.*, 2001; Mowat and Heard, 2006).

2.2.3. Near-Infrared Spectroscopy

Near-infrared spectroscopy (NIRS) uses the interactions among electromagnetic radiation at given wavelengths to detect special signatures from the analyte (Naes *et al.*, 1996). This method can be used to estimate diet quality and composition. Steyaert *et al*. (2012) applied this method to obtain faecal indices of dietary quality in brown bears in south-central Sweden. Nitrogen, crude fiber (CF), ether extracts (EE), acid detergent fiber (ADF), neutral detergent fiber (NDF), lignin and dry matter constituents of faecal samples and effects of exposure time to the constituents were measured. Elfström *et al*. (2014) combined NIRS and DNA metabarcoding methods to assess the quality and components of brown bear diet. The study revealed that Scandinavian brown bear diet did not differ between visits to human settlement areas and when they were in remote areas. Therefore it was concluded that bears do not approach settlements to find food in Scandinavia, contrary to the studies from North America (Hopkins *et al*., 2012).

Most studies of bear diet habits focus on identifying main components of diet to answer specific questions about behavioral changes due to season, food availability, sex and age groups, and obviously anthropogenic effects. As opportunistic omnivores, brown bear has a wide range of food sources which can change compositionally with a large number of effects. In addition, detecting whether plants or animals constitute the main energy source in a given time is critical for conservation efforts for brown bears. Whether it is stable isotope analysis of hair or DNA metabarcoding of faecal samples, new approaches try to establish robust, fast and reliable methods to enable identification of diet habits and food quality.

3. MATERIALS AND METHODS

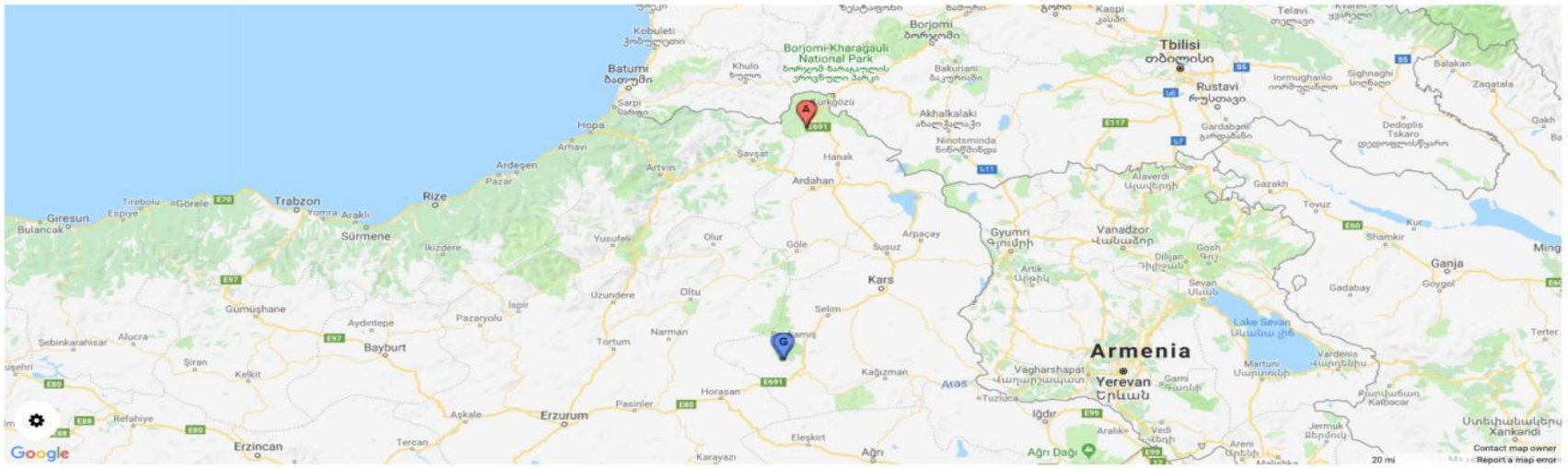
3.1. Field Sampling

Bear and lynx faecal samples were collected from Kars/Sarıkamış Region and Yenice Wildlife Enhancement Area between 2013 and 2015 (Figure 3.1). For diet analysis, a total of 28 faecal samples were chosen: five lynx samples were collected in 2013 from Sarıkamış, five lynx samples collected in 2014 from Sarıkamış, six bear samples collected in 2014 from North Sarıkamış, six bear samples collected in 2014 from South Sarıkamış, and six bear samples collected in 2015 from Yenice. Locations of bear and lynx samples used in this study can be seen in Figure 3.2, Figure 3.3 and Figure 3.4.

All samples were collected during June-September, a period of hyperphagia. All bear samples from north of Sarıkamış Region were collected in July 2014. Four bear samples from south of Sarıkamış Region were collected in June 2014, and two in July 2014. All bear samples from Yenice Region were collected in September 2015. Four lynx samples each were collected in August 2013 and July 2014, and one sample each in September 2013 and August 2014.

Figure 3.1. Yenice Wildlife Enhancement Area and Sarıkamış National Park.

Figure 3.2. Locations of Sarıkamış brown bear scat samples.



Sample Type North Sarıkamış Bear South Sarıkamış Bear

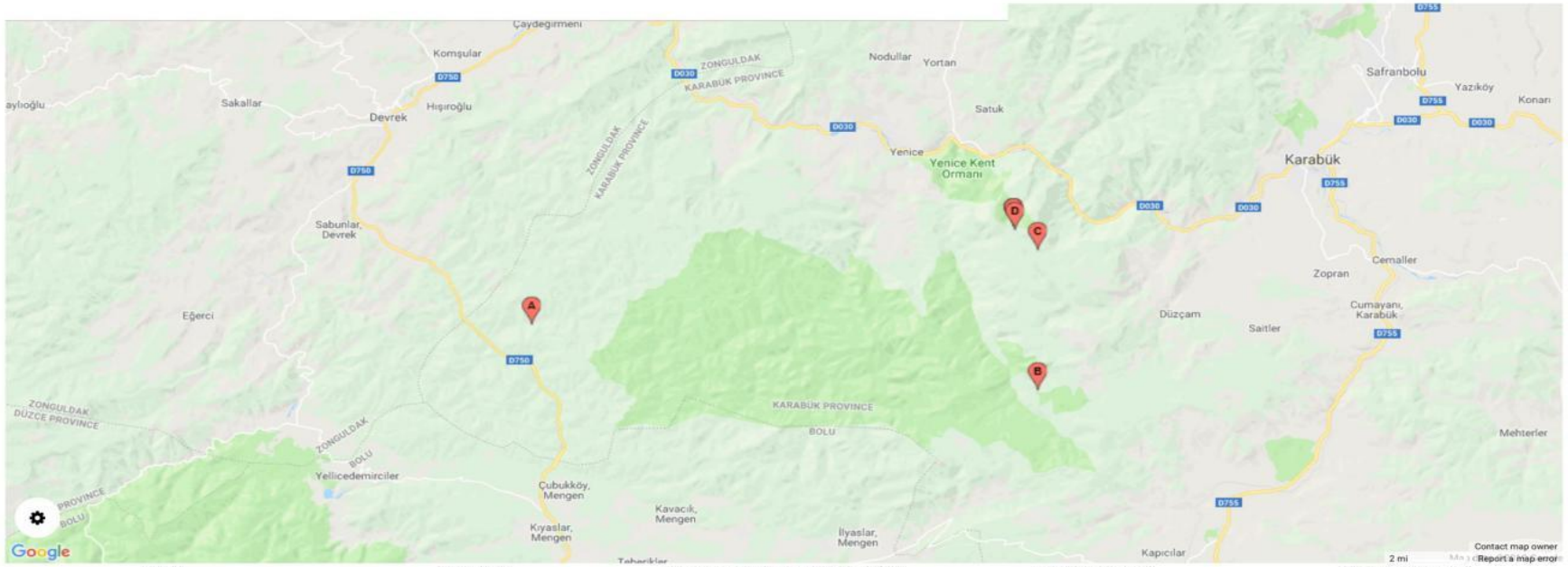
Marker	Sample Type	Sample Name&Number (r or 2=replicates)	GPS_point:Latitude	GPS_point:Longitude
A	North Sarıkamış Bear	3820	41.41213833	42.66516167
B	North Sarıkamış Bear	3831	41.41042333	42.66670667
C	North Sarıkamış Bear	3837	41.41205	42.66785833
D	North Sarıkamış Bear	3842	41.41260667	42.66767833
E	North Sarıkamış Bear	3843	41.41263833	42.667645
F	North Sarıkamış Bear	3844	41.41302	42.66736333
G	South Sarıkamış Bear	1113	40.23006333	42.53403167
H	South Sarıkamış Bear	1116	40.22912333	42.53545667
I	South Sarıkamış Bear	1130	40.22984	42.54274167
J	South Sarıkamış Bear	1133	40.23085167	42.542555
K	South Sarıkamış Bear	2777	40.22838333	42.51524333
L	South Sarıkamış Bear	2780	40.22793667	42.51870667

Figure 1.3. Locations of Yenice brown bear samples.

Sample Type Lynx 2013 Lynx 2014

Marker	Sample Type	Sample Name&Number (r or 2=replicates)	GPS_point:Latitude	GPS_point:Longitude
A	Lynx 2013	354	40.39095667	42.50679833
B	Lynx 2013	358	40.39012333	42.512525
C	Lynx 2013	405	40.28914833	42.61165833
D	Lynx 2013	702	40.36166	42.58219167
E	Lynx 2013	525r	40.72229667	42.44036
F	Lynx 2014	3553	40.18992667	42.51200333
G	Lynx 2014	3554	40.18976333	42.512205
H	Lynx 2014	3746	40.43666167	42.52261833
I	Lynx 2014	3750	40.43878833	42.51983167
J	Lynx 2014	4049	40.76110333	42.423775

Figure 3.4. Locations of Sarıkamış lynx scat samples.



Marker	Sample Type	Sample Name&Number (r or 2=replicates)	GPS_point:Latitude	GPS_point:Longitude
A	Yenice Bear	88	41.094291	32.057847
B	Yenice Bear	91	41.054023	32.423881
C	Yenice Bear	92	41.139537	32.424134
D	Yenice Bear	96	41.151818	32.406925
E	Yenice Bear	97	41.153974	32.405726
F	Yenice Bear	98	41.153983	32.405738

Samples collected in Sarıkamış were stored in ziplock bags at - 4 ℃. Yenice samples were stored in falcons filled with 70 % ethanol at - 4 ℃.

3.2. Methods

General protocol of diet analysis with next-generation sequencing involves three main steps: Extraction of DNA from samples, amplification of DNA with primers chosen to target and sequence diet DNA, and analysis of the data.

3.2.1. DNA Extraction

DNA from all samples were extracted with QIAamp DNA Stool Mini Kit from QIAGEN (Catalog no. 51504, Hilden, Germany). Extraction protocol was applied with modifications below:

- Metal beads were used during the first vortex and centrifugation steps. At the first step of extraction, homogenization of faecal samples is a critical step for the extraction of DNA. Faecal samples were stored at - 4 ℃, thus they become frozen, and bear faecal samples can contain seeds and chaff, which can be hard to homogenize. To overcome these issues, scalpels and centrifugation with metal beads were used. Beads were later collected from sample pellets and sterilized with bleach and autoclave sessions.
- Samples were incubated at 70 ℃ overnight to achieve complete lysis.
- 100 µl of ATE buffer was used for elution of DNA (instead of 200 µl), to increase final DNA concentration.

In addition to those steps, extraction bench was wiped with bleach solution and metal extraction tools like scalpels were sterilised with alcohol and fire from a bunsen burner for each sample, to avoid contamination.

3.2.2. Two-Step PCR Method

After amplification, sequencing adapters are added to the products of the target region PCR for sequencing purposes. Since adding of sequencing adapters via general kits can be costly, we applied a two-step PCR protocol proposed in 16S Metagenomic Sequencing Library Preparation (© 2019 Illumina, Inc) for MiSeq sequencing system, by designing unique primers for the study. First PCR amplifies the target regions and adds overhang adapter sequences to the target amplicons. Illumina

adapter and index sequences are added to the first PCR products at the second PCR step, by targeting the overhangs added at the first step (Figure 3.5). Table 3.1 shows amplification and blocking primers used in this two-step PCR approach.

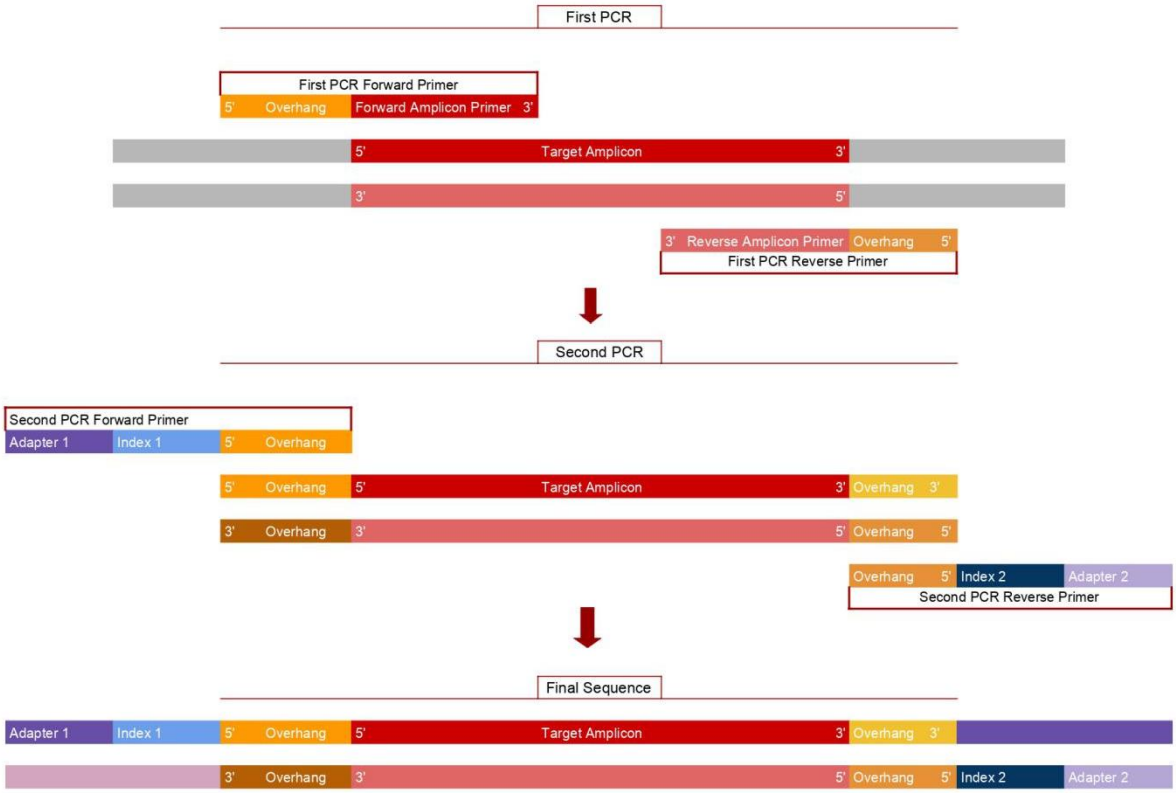


Figure 3.5. Amplification of target region and addition of sequencing adapters with the Two-Step PCR Method.

DNA markers for diet analysis were selected according to De Barba *et al*. (2014). In addition, blocking primers (Vestheim and Jarman, 2008) were used to prevent amplification of host (bear, lynx) DNA, and human and wolf DNA. Figure 3.6 displays how blocking primers prevent amplification for specific amplicons. Blocking primers for wolf and lynx were designed for the study.

Table 3.1. PCR primers used in the Two-Step PCR method.

	Diet Analysis Protocol - Primers Used			
Primer Name	**Type**	**Primer Sequence**	**Used in**	**Purpose**
Vertebrate	PCR Primer	TCGTCGGC	First PCR	Amplif

Forward Primer		AGCGTCAGATGTGTATAAGAGACAGTTTAGATACCCCACTATGC		ying vertebrate V5 loop of the mitochondrial 12S gene
Vertebrate Reverse Primer	PCR Primer	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTAGAACAGGCTCCTCTAG	First PCR	Amplifying vertebrate V5 loop of the mitochondrial 12S gene
Invertebrate Forward Primer	PCR Primer	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCAACATCGAGGTCRYAA	First PCR	Amplifying a short fragment of the mitochondrial 16S gene for invertebrates
Invertebrate Reverse Primer	PCR Primer	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGARTTACYNTAGGGATAACAG	First PCR	Amplifying a short fragment of the mitochondrial 16S gene for invertebrates

Plant Forward Primer	PCR Primer	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGGGCAATCCTGAGCCA	First PCR	Amplifying P6 loop of the chloroplast trnL (UAA) intron in angiosperms and gymnosperms
Plant Reverse Primer	PCR Primer	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCCATTGAGTCTCTGCACCTATC	First PCR	Amplifying P6 loop of the chloroplast trnL (UAA) intron in angiosperms and gymnosperms
Mammalian Blocking Primer	Blocking Primer	CCTAGGGATAACAGCGCAATCCTATT-(C3 SPACER)	First PCR	Blocking the amplification of mammalian DNA at invertebrate PCR
Human Blocking Primer	Blocking Primer	CTATGCTTAGCCCTAAACCTCAACAGTTAAATCAACAAAACTGCT-(C3 SPACER)	First PCR	Blocking the amplification of human DNA

Bear Blocking Primer	Blocking Primer	CCACTATG CTTAGCCTTAA ACATAAATAA TTTATTAAAC-(C3 SPACER)	First PCR	Blocking the amplification of brown bear DNA
Wolf Blocking Primer	Blocking Primer	CCACTATG CTTAGCCCTAA ACATAGATAA TTTTACAAC-(C3 SPACER)	First PCR	Blocking the amplification of wolf DNA
Lynx Blocking Primer	Blocking Primer	CCACTATG CTTAGCCCTAA ACCTAGATAG TTAACCTAAA-(C3 SPACER)	First PCR	Blocking the amplification of lynx DNA
Index PCR Barcode Primer	PCR Primer	AATGAATA CGGCGACCAC CGAGATCTAC ACNNNNNNNN TCGTCGGCAG CGTC	Second PCR	Amplifying first PCR product and adding index/sequence adapters
Index PCR Index Primer	PCR Primer	CAAGCAGA AGAACGGCAT ACGAGATNNN NNNNNGTCTC GTGGGCTCGG	Second PCR	Amplifying first PCR product and adding index/sequence adapters

3.2.3. First PCR

Three universal diet analysis markers were used to detect vertebrate, invertebrate and plant parts within the faecal samples. For the vertebrate component of brown bear diet, V5 loop of the mitochondrial 12S gene (Riaz *et al*., 2011) was targeted with primers from De Barba *et al*. (2014). To identify invertebrates, primers targeting a short fragment of mitochondrial 16S gene of molluscs, arthropods and vertebrates were used (De Barba *et al*. 2014). For plant species, a primer targeting P6 loop of the chloroplast trnL (UAA) intron in angiosperms and gymnosperms (Taberlet *et al*. 2007) was used.

Since universal primers were used to detect diet components of faecal samples, amplification of host DNA can highly compromise detection of diet DNA. To prevent this, specially designed blocking primers were used, following Vestheim and Jarman (2008). Another type of blocking primer was used to prevent amplification of human and wolf sequences in case of a contamination (wolf blocking primers were used as wolf scat was also studied in the lab that the analyses for this thesis were undertaken in).

Figure 3.2. Binding region of blocking primers to eliminate unwanted PCR amplification.

PCR conditions were optimized based on De Barba *et al*. (2014). PCR conditions for first PCR step are:

- An initial denaturation step at 95 ℃
- 55 cycles of 30 s at 94 ℃ and 90 s at 55 ℃

Elongation step is removed from PCR profile to reduce the +A artefact (Brownstein *et al*. 1996)

Qiagen Multiplex PCR Kit (QIAGEN, cat. no. 206143, Hilden, Germany) is used in first step PCR. However, multiplexing samples for three types of primers could not be optimized to give

meaningful results in gel electrophoresis. For this reason, three separate PCRs (one each for vertebrate, invertebrate and plant detection) and one replicate for all samples was undertaken. In addition, negative controls are used to detect possible contamination in the PCRs. A blank sample is used through all workflow, to monitor the performance of the protocol from start to finish (See Table 2 below for details of the chemical composition of different PCR categories). In total, 168 PCR reactions [28 samples x 2 independent DNA extractions / sample (*i.e.* replicates) x 3 PCRs (one each for vertebrate, invertebrate and plant) / extraction) were made to characterize the dietary components of 28 scat samples. One negative control was also processed, for which the entire steps from DNA extraction to data analyses was employed.

Table 3.2. Quantities of chemicals used for the first PCR step.

	Vertebrate (µL)	Invertebrate (µL)	Plant (µL)
Qiagen Master Mix	12.5	12.5	12.5
Qiagen Q Solution	2.5	2.5	2.5
Vertebrate Forward Primer	0.20	-	-
Vertebrate Reverse Primer	0.20	-	-
Invertebrate Forward Primer	-	0.50	-
Invertebrate Reverse Primer	-	0.50	-
Plant Forward Primer	-	-	0.25
Plant Reverse Primer	-	-	0.25
Bear Blocking	0.40	0.40	-
Human Blocking	0.40	0.40	-
Wolf Blocking	0.40	0.40	-
Mammalian Blocking	-	1.00	-

Lynx Blocking (Instead of Bear Blocking Primer in lynx samples)	0.40	-	-
Water	6.4	4.80	7.50
DNA	2.00	2.00	2.00
Total	25.00	25.00	25.00

3.2.4. Gel Extraction

Since a two step modification of the amplification process of De Barba *et al*. (2014) was used, a gel extraction step was added to remove unwanted PCR by-products. An agarose gel is prepared with 340 ml of TAE buffer, 7 gr of agarose (peqGold Universal Agarose, Cat. no. 732-2789P, PEQLAB Biotechnologie GmbH, Erlangen, Germany) and 34 µL of dye (RedSafe™ Nucleic Acid Staining Solution, iNtRON Biotechnology, Burlington, USA). For 25 µL of PCR sample, 4 µL of loading dye is used. The gel was run at 100 volts for 2 hours. MinELute Gel Extraction Kit (QIAGEN, cat. no. 28604, Hilden, Germany) is used for gel extraction of samples following manufacturer's protocols.

3.2.5. Second PCR

The second PCR is prepared with 12.5 µL concentrated Qiagen Multiplex Master Mix, 8 µL pure water, 1 µL of PCR primers and 2,5 µL of DNA. The second PCR conditions in the 16S Metagenomic Sequencing Library Preparation Manual (© 2019 Illumina, Inc) were optimized to determine the most ideal annealing temperature.

PCR conditions were optimized as follows;

- Initial denaturation step at 95 ℃ for 3 minutes
- 20 cycles of 95 ℃ for 30 s, 60.5 ℃ for 30 s and 72 ℃ for 30 s.

- Final elongation step at 72 ℃ for 5 minutes.

3.2.6. Purification and Pooling

Second PCR products are purified with Ampure magnetic beads (AMPureXP for PCR Purification, cat. no. A63880, Beckman Coulter Life Sciences, Indianapolis, USA) according to the manufacturer's protocols. 34 of 169 PCR products which had concentrations lower than 11 ng/ul were removed, and remaining 135 purified products of vertebrate, invertebrate and plant PCR products of samples were first pooled based on their DNA concentrations into 28 pools, aiming for 250 ng sample DNA in each pool. After that, first pools were pooled again, aiming for 200 ng DNA from the first set of pools. The final sample was sent to University of Tennessee for paired-end 75 bp Illumina Miseq sequencing using V3 chemicals. The removal of 34 PCR products with low concentrations caused the complete loss of data from three samples coded as 525, 113r, and 2777r (r refers to replicate samples).

3.2.7. Data Analysis

Analyis of sequences identified from 53 samples (28 samples x 2 replicates minus three samples or replicates for which no data were produced, see above) was made by Obitools v. 1.2.13 (Boyer *et al*., 2014). Illuminapairedend command was used to align sequences, and Obiuniq command was used to group identical sequences and produce count numbers for each sequence. Obigrep command was used to filter sequences which have a count numbers lower than 10 and base lengths lower than 30. To clean variant sequences with small differences and low count numbers, obihead command was used; sequences with no variants with a count greater than 5% of their own count were kept. Three reference databases were build with obiconvert and ecopcr commands using the EMBL database, and plant, vertebrate and invertebrate primers discussed above. Taxonomic assignment was done with the ecotag command. However, there were some problems with the final taxonomic assignment: taxonomic assignment of vertebrate and plant groups was generally at the division level. In addition to that, taxonomic assignment of the invertebrate group was incorrect most of the time; sequences assigned to certain species were providing different results when analyzed with BLAST applications provided through EMBL and NCBI.

Therefore, taxonomic assignment of sequences was done manually, using Blast application provided by NCBI. Sequences having counts of 500 and higher were analyzed with application and species and genus results were recorded. Sequences resulting in hits to multiple genera were merged

at a higher taxonomic level, e.g. families, order and superorders and reported that way. If merged sequences were from the same families (but nonoverlapping genera), they were reported as *1, *2 etc.

To measure species diversity of dietary intakes, Shannon index (H) (Shannon, 1948) was calculated replacing species with food items according to this formula:

$$H = -\sum p_i \ln p_i \quad (3.1)$$

where p_i is the frequency of food items in overall sample (P_i = Total number of samples with a particular food item/Total number of dietary items in a particular group). Higher Shannon diversity index means a high number of different items and more even distribution of these items.

Width of the trophic niche was calculated with Levin's index (B) (Levins, 1968) following the formula:

$$B = 1/\sum p_i^2 \quad (3.2)$$

Levin's Index is maximum when diet items are equally distributed in numbers, which means width of niche is highest.

To compare different trophic niches between bear location groups and lynx year groups, Pianka's index of trophic niche overlap (α) (Fischer *et al.*, 2005) was used. The formula for the index is:

$$\alpha_{xy} = \sum p_{xy} / \sqrt{(\sum (p_{ix})^2 (p_{iy})^2} \quad (3.3)$$

When α = 0, two groups are completely seperate in terms of their niches, and when α = 1 they are completely identical.

4. RESULTS

Analysis of 53 samples including replicates produced 35 unique sequences, which can be assigned to different taxonomic levels. Of these 35 unique taxonomic levels, 23 are at the family level, one at the class level (Magnoliopsida), one at the superorder level (Asteranae), one at the order level (Passeriformes), four at the genus level, and five at the species level.

Table 4.1 shows diet items identified in all samples as a binary table (1=presence, 0=absence). It should be noted that the negative control sample, which has no DNA source material but was processed with the entire sequencing protocol also resulted in two sequences, labeled as Apiaceae2 and Apiaceae3. These two taxonomic groups were removed from further statistical analyses below.

Table 4.1. Numbers of assigned sequences for all samples and their replicates (shown with the suffix ‘r’ after the sample code).

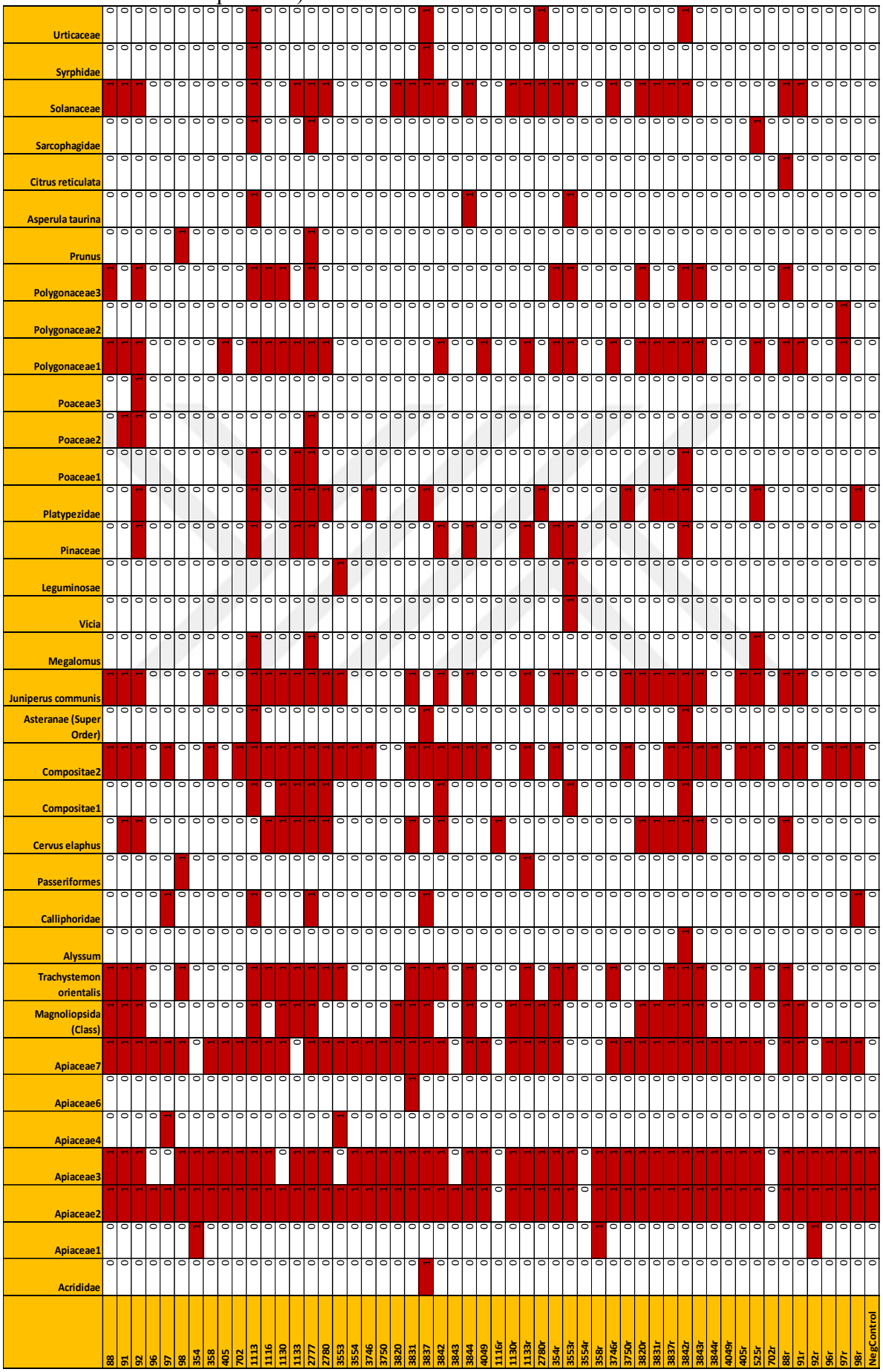


Figure 4.1 and Figure 4.2 show the numbers and percentages of diet items found in bear samples, grouped according to locations (North Sarıkamış, South Sarıkamış and Yenice), respectively. Figure 9 and Figure 10 shows the numbers and percentages of diet items found in lynx samples, grouped according to years (2013 and 2014, respectively). Numbers in Figure 4.1 and 4.3 display the number of samples in which a diet item is identified. Figure 4.2 and 4.4 gives information of percentage of identified food items according to location groups for bears and years for lynx.

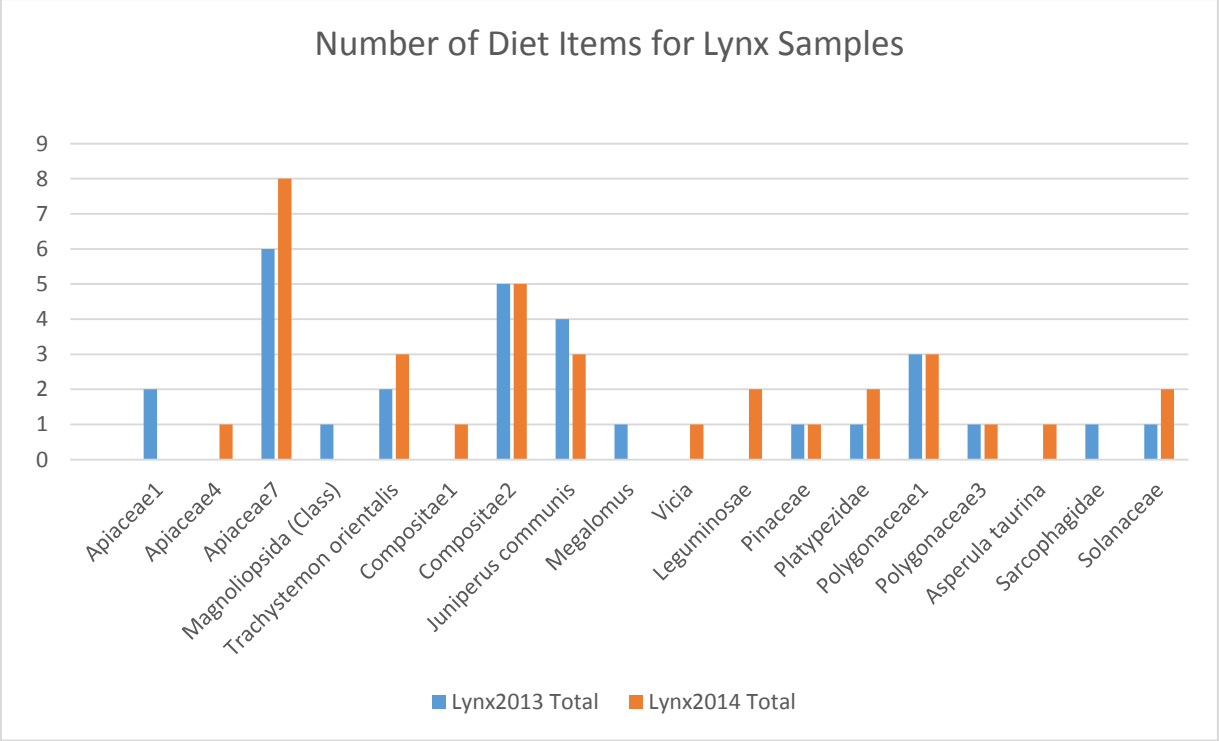


Figure 4.1. Number of diet items for lynx samples grouped according to years.

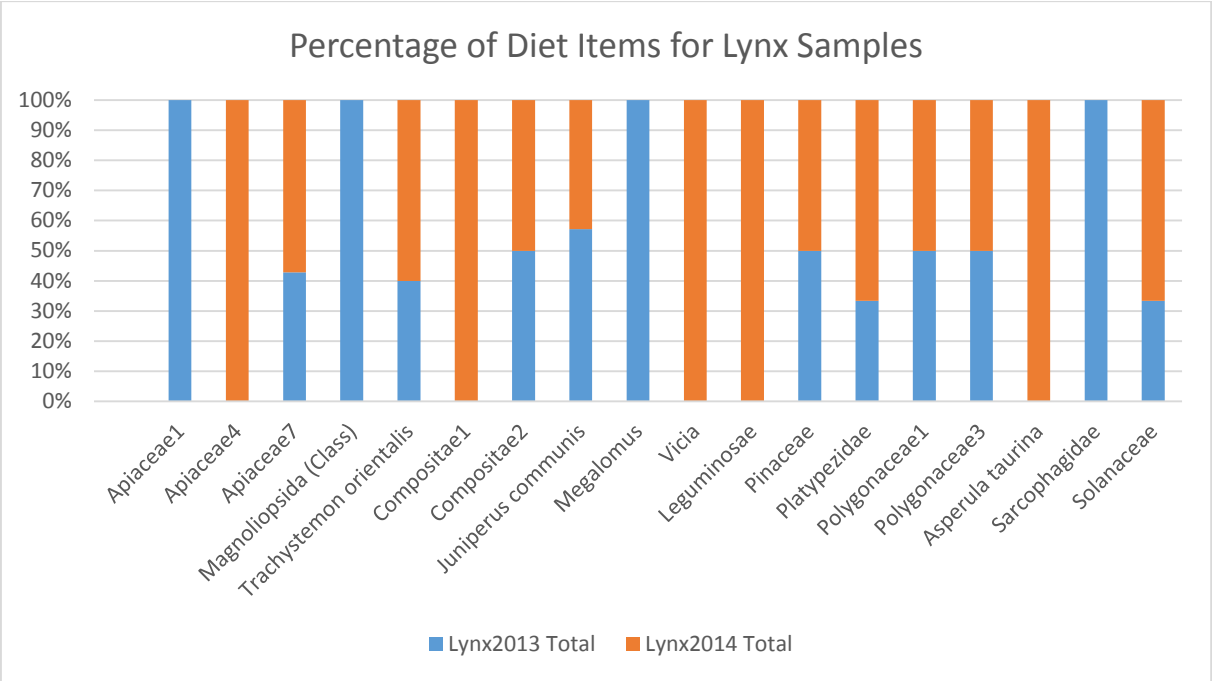


Figure 4.2. Percentage of diet items for lynx samples grouped according to years (2013 and 2014).

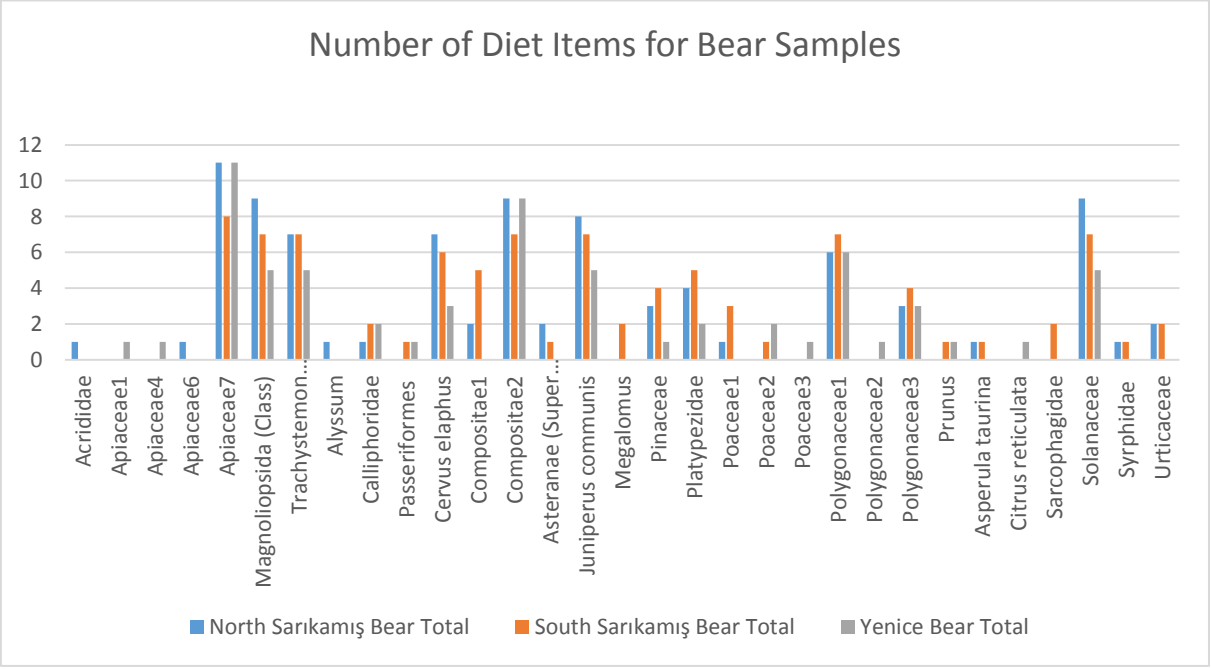


Figure 4.3. Number of diet items for bear samples grouped according to three main location categories (North Sarıkamış, South Sarıkamış, Yenice).

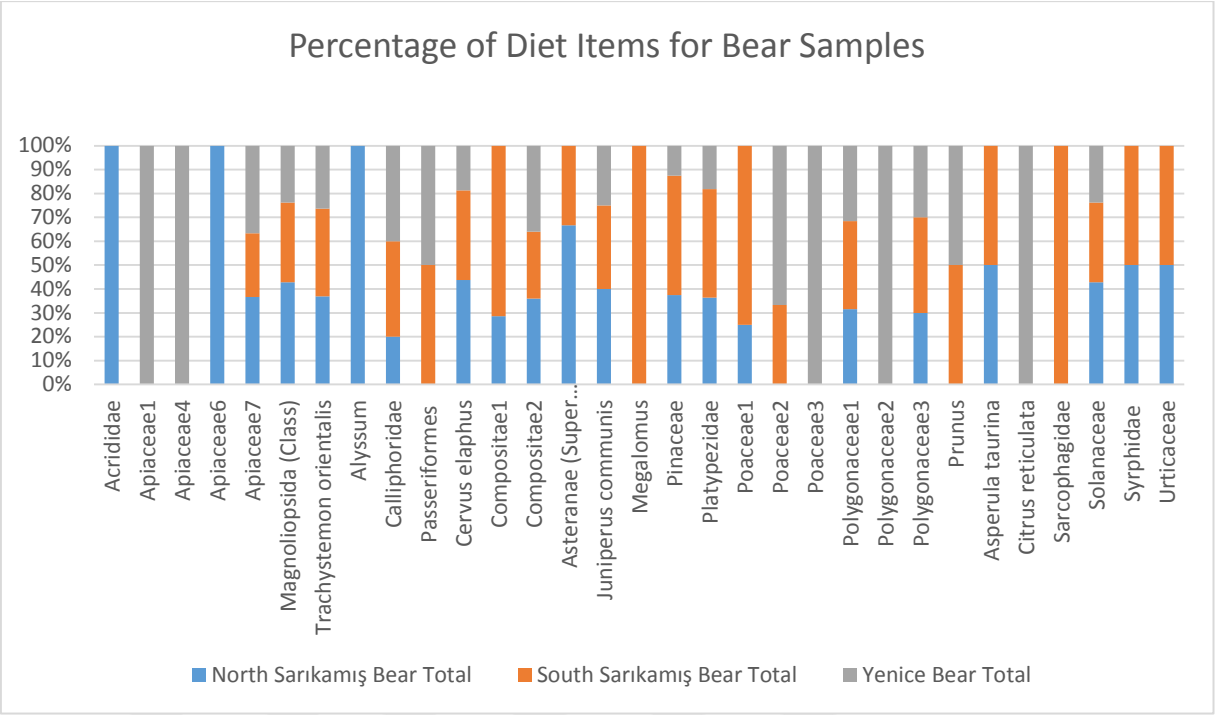


Figure 4.4. Percentage of diet items for bear samples grouped according to three main location categories (North Sarıkamış, South Sarıkamış, Yenice).

Figures 4.1, 4.2, 4.3 and 4.4 indicate that both lynx and bear have diets with a high diversity of plants. Largest component of both species diets is from Apiaceae7, plant family of nutritious plants like carrot, parsnip and celery. Second largest component of both species diets is from Compositae2, the family of lettuce, endive and artichoke. Bear diet is more diverse than the lynx diet, with 31 components and 18 components, respectively. Lynx diet contains no vertebrate component. Bear diet includes *Cervus elaphus* (red deer) and order passeriformes (perching birds) as vertebrate preys. In addition, both diet compositions include fly families; Calliphoridae (Carrion flies) Platypezidae (flat-footed flies) and Sarcophagidae (flesh flies), probably digested during scavenging. Lynx diet includes two items from the same family which are not in bear diet; one item from genus *Vicia* (vetches) of Leguminosae family and one item from Leguminosae family (family of peas, beans and legumes) different than the item from genus *Vicia*.

Figure 4.5 shows the percent contributions of location groups to total amount for the red deer, *Cervus elaphus.* Both Sarıkamış groups have a higher percentage of red deer in their diets when compared to the Yenice group.

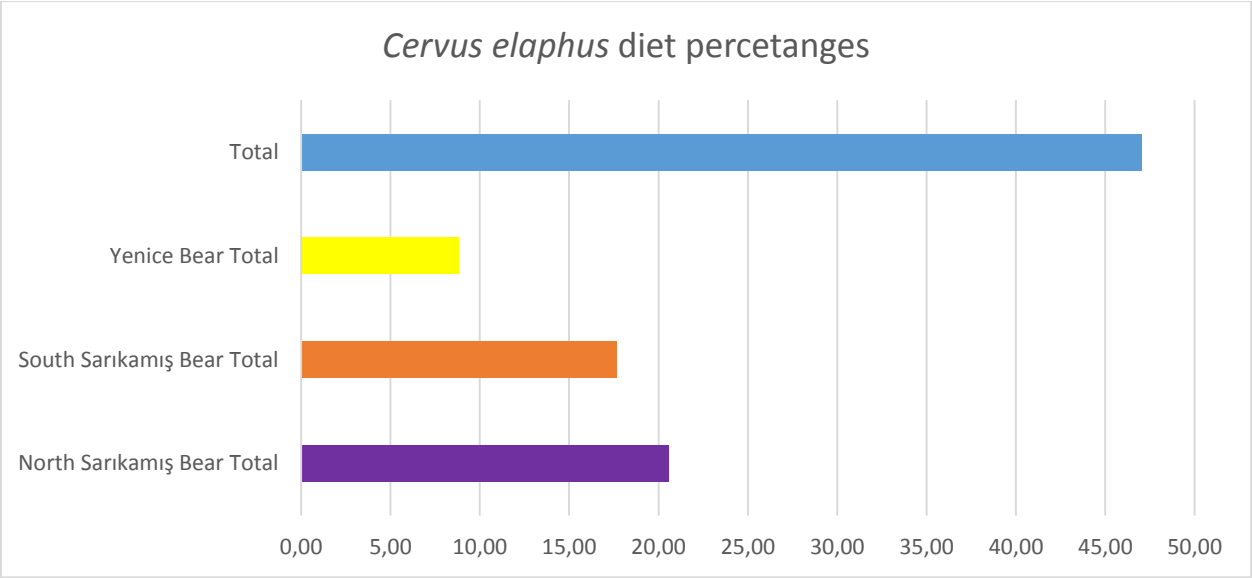


Figure 4.5. Percentage of *Cervus elaphus* at location groups.

For bears, Shannon diversity index values in Table 4.2 show that the group with the most diverse diet composition is South Sarıkamış (H'=0.83), other groups following it with minimal difference (North Sarıkamış, H'=0.78) and Yenice being the least diverse (H'=0.73). For niche width, Levin's index shows that South Sarıkamış has the greatest width (B'=0.48) according to diet composition for bears. For both lynx groups (2013 and 2014) diversity and width are similar to each other (H'= 0.80, B'= 0.43, H'=0.83 and B'=0.45, respectively).

Table 4.2. Shannon diversity and Levin's niche width indexes for all food groups. Values were standardized with reported methods for comparison.

	Standardized Shannon Diversity Index (H'=H/Hmax)	Standardized Levin's Niche Width Index (B'=B-1/N-1, N being total number of food items)
North Sarıkamış Bear Samples	0.78	0.37
South Sarıkamış Bear Samples	0.83	0.48
Yenice Bear Samples	0.76	0.33
Lynx2013 Total	0.80	0.43
Lynx2014 Total	0.83	0.45

Table 4.3 shows the similarity of niches according to diet composition. Values display higher similarity between the two Sarıkamış groups (α=0.95) and slightly lesser similarity between North Sarıkamış and Yenice (0.93), and lowest values between South Sarıkamış and Yenice groups (0.88), for bears. Niche overlap is high between both lynx groups (0.91).

Table 4.3. Pianka's Niche overlap index for comparison of different food groups. Three location groups for bear samples, two group of collection year for lynx samples exists.

	North Sarıkamış * South Sarıkamış	North Sarıkamış * Yenice	South Sarıkamış * Yenice	2014 Lynx * 2013 Lynx
Pianka's Niche Overlap Index	0.95	0.93	0.88	0.91

5. DISCUSSION

5.1. Diet Composition

Our study determined 35 unique taxonomic level with 23 families, one class, one superorder, one order, four genus and five species for diet analysis. 28 of these taxonomic levels were found by targeting chloroplast *trn*L (UAA) intron. Valentini *et al.* (2009) showed slightly higher coverage of plant diet than our study with 12 species, six genus, four tribes, three sub-families and one family as plant components of bear diet.

Results show that the majority of bear and lynx diet consist of plants. For bears, red deer (*Cervus elaphus*) is present in 47 % of samples, and one sample includes a food item from Passeriformes, an order of perching birds. The consumption of red deer is consistent with literature (Mattson, 1997; Mattson *et al*., 1991), where bears acquire most of their energy from feeding on large ungulates (Mattson *et al*., 1991), and red deer is one of the available ungulates both in Yenice and Sarıkamış regions. It is not possible to determine the source of ungulate meat as being due to scavenging or hunting, since bears use both methods to secure meat. One indication of scavenging can be the presence of fly families on samples. Mattson (1997) reports insects as a diet item for bears, however they are mostly ants and pupae which can be excavated. Flies does not represent a diet group for bears, so two possible explanations for the detection of fly DNA in our results include either the devouring flies with scavenged meat or ingesting flies (and their DNA) after they have swarmed the scat samples. Source of food item from perching birds can be from a bird nest or a carcass. In addition, one sample includes a food item from family Acrididae, a family of grasshoppers. Dahle *et al*. (1998) reports insects and birds as components of bear diet, however grasshopper has not been reported in the literature to the best of our knowledge; insects which are rich in proteins and accessible by bears (e.g. ants) have been previously discussed as insect dietary items for bears (Dahle *et al*., 1998; Elfström *et al*., 2014).

The absence of vertebrate from lynx diet is an unexpected result. Lynx diet studies report deer, wild boar, birds and small carnivores as main food items (Krofel *et al.,* 2011; Weber and Weissbrodt, 2012), and only negligible amounts of plant material. The reason for this result can be implicit bias introduced by the DNA metabarcoding method. Several steps of method present possibilities to produce erroneous sequences: PCR with universal primers have a tendency to amplify certain sequences in higher ratios and PCR can also introduce insertions, deletions and

substitutions (Coissac *et al.*, 2012; Taberlet *et al.* 2012). All these sources of error may be responsible for a bias which caused underrepresentation of vertebrate sequences. Our decision to identify sequences with only 500 and higher counts of reads, coupled with underrepresented vertebrate sequences may have caused the filtering out of the vertebrate sequences with low reads counts. Since lynx and bear samples were processed with the same method, it can be assumed that underrepresentation of vertebrates is also an issue in terms of bear diet composition. However, it should be noted that, these same protocols were able to detect one mammal species (*i.e. Cervus elaphus*) in half of the bear samples, and hence the lack or low level of vertebrates in the diets of lynx could also be a real biological phenomenon for the lynx diets in the region. Chynoweth *et al.* (2015) reports high threats of habitat loss and prey depletion for lynx at the Sarıkamış region, and these may be have resulted in the absence of vertebrate prey in lynx diet, and a switch to a predominantly plant-based diet.

Composition of plant food materials for bears is similar to ones reported by De Barba *et. al.* (2014), when comparisons are made with the main reported plant families (Asteraceae (Compositae), Rosaceae, Apiaceae, and Poaceae) in that study. In addition, Valentini *et al.* (2009) report Poaceae, Polygonaceae, Cyperaceae, and Apiaceae families as main components of the brown bear diet. These findings are also consistent with our study. Notable food items for bears in this study are *Trachystemon orientalis* (Abraham-Isaac-Jacob plant, Turkish: *Kaldırık otu*, at 55 % of bear samples), *Juniperus communis* (Common Juniper, Turkish: Ardıç, at 58% of bear samples) and *Polygonum cognatum* (Indian knotgrass, Turkish: *Madımak otu*, at 55% of bear samples).

Ambarlı (2016) studied diet of brown bears in Turkey with conventional scat analysis. The study reports diet components as mainly plants (87.5 %) with only 12.5 % meat and animal protein. Plant component of the reported bear diet mainly consists of family Rosaceae, consistent with our results. However, some of the other reported plant species from Ambarlı (2016) do not overlap with the findings of families of Vitaceae, Ericaceae, Rhamnaceae and Caprifoliaceae in this study.

5.2. Comparison of Diet Location Groups

Our main expectation was that feeding on garbage can decrease food diversity and niche width, since bears feeding on dumps should not need to relocate (Cozzi *et al.*, 2016) for searching for food, as they can acquire energy from very localized anthropogenic food sources, rather than utilizing a variety of natural food resources. In contrast to these expected results, Shannon and Levin's Indexes show a slightly higher diversity and niche width for South Sarıkamış group than the North

Sarıkamış and Yenice groups, which utilize more natural food sources. Hence neither of these index values supports our initial expectation. Shannon and Levin's Indexes does not indicate a major difference between South Sarıkamış, and North Sarıkamış and Yenice. A possible explanation for the higher (or lack of lower) diversity and niche width values of South Sarıkamış group can be access to an increased food spectrum due to feeding from both garbage dump and the nearby natural forest areas, as well.

Similarly, we expected that the diet composition in Yenice and North Sarıkamış regions would be more similar to each other than either is to South Sarıkamış, even though the former ones are much farther apart. The Pianka Index shows higher similarity between South and North Sarıkamış regions than between North Sarıkamış and Yenice. Also, the distribution of different food items shows no clear difference between bear groups; 10 food items (Apiaceae7, Magnoliopsida, *Trachystemon orientalis*, *Cervus elaphus*, Compositae2, *Juniperus communis*, Pinaceae, Polygonaceae1, Polygonaceae3 and Solanaceae) with highest frequencies (Appendix 1) are present in all three sample groups. In addition, eight food items (Acrididae, Apiaceae1, Apiaceae4, Apiaceae6, Alyssum, Poaceae3, Polygonaceae2, *Citrus reticulata*), which are present in only one group are the least frequent, with all of their count numbers being lower than or equal to two (Appendix 1). These results indicate that all feeding areas for bears provide a similar spectrum of available dietary items for food, and there is no localization specific specialization for certain food items.

6. CONCLUSIONS AND RECOMMENDATIONS

The dietary composition of brown bear and lynx has not been studied with metabarcoding analyses in Turkey previously. The identified food items (red deers and various plants) for bears are in line with previous research. However, our analysis failed to identify expected vertebrate dietary components for lynx. This could be a methodological issue, *i.e.* the DNA metabarcoding approach with steps of primer choice, PCR, and sequencing can add biases, which can potentially prevent the identification of vertebrate components of diet. However, this lack of detection of vertebrate component could also be representing a real situation for lynx in Sarıkamış region, with threats of habitat loss, decreasing prey availability and dangerous human interactions like vehicle collisions. The fact that vertebrate dietary components were recovered for bears, for which identical methodological steps were used suggests that the situation might indeed be a biological reality. These results suggest the necessity for further studies to identify lynx diet and effects of human–wildlife interactions.

The expectation of lower diversity and niche width for garbage diet of bears is not supported with our results. In addition, our expected higher niche similarity between Yenice and North Sarıkamış is not confirmed. However, our findings for dietary components of bear are consistent with the literature and many major plant components of bear diet are identified. Further diet studies can focus on temporal changes of bear diet habits in the region and can provide deeper insight by featuring individual determination of bears, larger samples sizes for diet analysis and also accounting for the migratory movements of bears.

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APPENDIX A: FREQUENCIES AND COUNTS OF ALL FOOD ITEMS FOR LOCATION AND YEAR GROUPS