Community dynamics of saproxylic insect functional groups at tree-line

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"In nature, nothing exists alone." ~Rachel Carson

APPRECIATION

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CHAPTER 1

INTRODUCTION

1.1 Abstract: Saproxylic Coleoptera, Metabarcoding, and Tree-lines as Ecotones

While much attention has been given to the vulnerability of charismatic megafauna in the face of climate change and land use shifts (Petherick et al. 2021), it's becoming increasing apparent that earth's insects are already in global decline. 40% of world's insects are in danger of becoming extinct in the next few decades (Sánchez-Bayo and Wyckhuys 2019), leading to what some experts are calling an insect apocalypse (Wagner et al. 2021). Saproxylic Coleoptera, or beetles that at some point in their life cycle depend on dead or dying wood (Speight 1989), are especially threatened. In a 2010 European Red List assessment, only a small percent of the total number of saproxylic Coleoptera occurring in Europe were assessed. Of the 436 species evaluated, 14% were found to be declining, and almost a third were reported as data deficient, i.e., not enough information was known to accurately assess the species' risk (Nieto and Alexander 2010).

Saproxylic Coleoptera play critical roles within ecosystems (Stokland et al. 2012), yet still little is known about dynamics within functional groups (Ulyshen and Sobotnik 2018) or their interactions with predators such as Passerine birds. The ability to accurately measure population breadth within time and space is critical to predicting outcomes and creating effective management tools for the conservation of saproxylic Coleoptera. Time- and cost-effective technology is needed to effectively monitor insect populations. Metabarcoding, a relatively new technology, is becoming a popular way to detect species captured within insect traps (Batovska et al. 2021).

In many geographic areas across the world, basic information such as presence or absence of saproxylic Coleoptera is still unknown (Nieto and Alexander 2010). Tree-line communities are one such ecosystem (Öztürk et al. 2015). Tree-line ecosystems are especially pertinent to climate change monitoring and prediction as tree-lines present a visible boundary affected by climate- and disturbance-induced processes (Devi et al. 2020). The endemic flora and fauna at high elevation ecotones are under disproportionate extinction risk as climate change progresses (Dirnböck et al. 2011). Further, examining dynamics at tree-line is important because tree-lines are

an ecotone in flux, and are generally advancing throughout the world due to climate and other local factors (Holtmeier and Broll 2007).

1.2 Saproxylic Coleoptera

1.2.1 Decline of insects and saproxylic Coleoptera

In 2017, the popular science world was given a shock when a study was published that found flying insects in German natural areas had declined 75% in just 27 years (Hallmann et al. 2017). The study prompted a debate in popular culture about an impeding ecological Armageddon. However, to most entomologists, the results of the study were unsurprising as declines in insect diversity and biomass are well documented in numerous studies conducted worldwide over several decades (Leather 2018; Sánchez-Bayo and Wyckhuys 2019). These declines were found to be occurring in species across a wide ecological spectrum, suggesting the loss in biodiversity and abundance is being driven not only by the decline of specialized taxa that have traits that cause them to be more vulnerable (Rocha-Ortega et al. 2020), but also by the loss of widespread species, irrespective of niche breadth (Gaston and Fuller 2007).

The causes of insect loss are multifaceted and complex. Road collisions (Baxter-Gilbert et al. 2015), urbanization (Dennis et al. 2017; Merckx et al. 2019), and climate change (Baranov et al. 2020) are major drivers. Intensification of agriculture including higher usage of herbicide and pesticides, especially Neonicotinoids, are also likely causes (Morrissey et al. 2015; Habel et al. 2019). A recent study pointed to urbanization as a major cause for the reduction of terrestrial insect biomass, while conversion to agriculture was found to be a major cause of the reduction of terrestrial insect diversity (Uhler et al. 2021).

Many species of saproxylic Coleoptera are included in this dramatic decline and are especially vulnerable to land-use shifts and climate change (Nieto and Alexander 2010). European forests became increasingly industrialized over the last two centuries (Spiecker et al. 1996), and the contiguous United States lost almost all of the original old growth forests (Birdsey et al. 1993). Much of the decline of saproxylic Coleoptera in temperate and boreal regions can be directly linked to the loss of sufficient amounts of dead wood (Paillet et al. 2010; Toivanen and Kotiaho 2010; Gossner et al. 2013; Seibold et al. 2015; Haeler et al. 2021). Within Europe, about a third of assessed saproxylic Coleoptera are considered threatened or near threatened (Nieto and Alexander 2010). The extent of the decline outside Europe and the United States, however, is relatively unknown as a considerable amount of research has been concentrated only within these two areas (Ulyshen and Sobotnik 2018).

1.2.2 Importance of saproxylic Coleoptera

The term "saproxylic Coleoptera" encompasses an ecological grouping whose members display an incredible amount of functional and taxonomic diversity and depend directly and/or indirectly on dying or dead wood for part of their life cycle. As an order, Coleoptera is incredibly under-described: the order is believed to account for around 24% (350,000-400,000) of all described flora and fauna species, yet current research estimates that 1.1 million Coleoptera species have yet to be described (Stork 2018). A sizable percentage of the order is saproxylic; studies in forests in Germany (Köhler 2000) and Maritime Provinces in Canada (Majka and Pollock 2006) found over 50% of the beetle species in those areas to be saproxylic. Due to their close association to the amount, diameter, and age of dead wood (and by association forest health), many saproxylic Coleoptera species or assemblages of species act as forest health indicators (Garcia-Lopez et al. 2016). Within the order, Cerambycidae (Karpiński et al. 2021) and Lucanidae (Lachat et al. 2012) have been identified as priority indicator groups. Some saproxylic Coleoptera, such as *Ips typographus* L. 1758, *Scolytus multistriatus* (Marsham 1802), and *Agrilus planipennis* Fairmaire, 1888, are considered pests outside and sometimes within their native range (Wermelinger 2004; Gandhi and Herms 2009; Jacobi et al. 2013).

1.2.3 Functional groups of saproxylic Coleoptera

The majority of saproxylic Coleoptera perform integral ecological functions, playing critical roles in the decomposition of wood and soil nutrient cycling (Hardersen and Zapponi 2018). Saproxylic functional feeding guilds include predators, parasites, fungivores, detritivores, myxomycophages (slime mold feeders), wood-consumers, and omnivores (Gimmel and Ferro 2018). Functional group interactions and correlations with environmental conditions have historically been used to describe community patterns of abundance and diversity (Vanderwel et al. 2006). For example, shaded rather than non-shaded logs harbor more wood-boring saproxylic Coleoptera and different assemblages of predator saproxylic Coleoptera (Johansson et al. 2007). Functional diversity of saproxylic Coleoptera was also found to be higher in cooler climates even when overall geographical gradients (latitude and elevation) were accounted (Hagge et al. 2019).

In addition, differences between taxonomic and functional diversity in saproxylic Coleoptera communities are often found as these communities respond differently to various drivers (Kozák et al. 2020). These differences indicate sound forest management practices should consider research dealing with both functional and taxonomic diversity. For instance, tree hollows were found to harbor higher functional diversity but less taxonomic diversity than other habitats (Micó et al. 2020). In Germany, species richness decreased and functional diversity increased with

increasing elevation (Thorn et al. 2018). In Canada, taxonomic diversity and abundance of saproxylic Coleoptera were unchanged across different amounts or type of coarse woody debris (Vanderwel et al. 2006). When the Canadian data was examined with functional groups in mind, fungivore abundance was found to be positively correlated with volume of course woody debris.

However, the diversity of saproxylic Coleoptera is vast, and basic life history traits and geographic range are unknown for many species (Ulyshen and Hanula 2009). A 2013 report found over a third of Nova Scotia's saproxylic Coleoptera may be at risk, although the robustness of this finding was limited due to insufficient collecting (Majka 2013). Even though the majority of saproxylic Coleoptera research has been performed in Europe, an extinction risk assessment could not be applied to a third of assessed European species because of insufficient biological and populational data (Nieto and Alexander 2010). More data are needed to measure both populational trends and examine species' biology. Baseline surveys play an integral role in identifying trends and are the starting point for many studies on biological and ecological aspects of insect life (Montgomery et al. 2020).

1.3 Passerines and Saproxylic Coleoptera at Tree-line

Predicting community patterns of arthropods as both prey and predator in a changing climate and landscape requires focus in various topographies and geographies. Research centered in certain ecotones, such as tree-lines, are particularly well suited for generating results with strong predictive power (Risser 1995; Kupfer and Cairns 1996). Tree-lines can be described as the geographical space in which the dominant stem of a tree no longer grows above 2-3m (Wieser and Tausz 2007; Körner 2012). Ecotones are transitional boundaries between two ecosystems or habitats (Lerner et al. 2021). The locational shifts in tree-lines and other ecotones have been used across the world to monitor effects of a warmer world under long term climate change (Beckage et al. 2008; Wieser et al. 2019; Jobe Iv and Gedan 2021).

It is important to note that tree-lines dynamics are also influenced by a number of local biotic and abiotic drivers, including species interactions (Liang et al. 2016), grazing (Hofgaard 1997), wind (Wieser et al. 2019), soil type and moisture (Holtmeier and Broll 2005; Jacob et al. 2015), and the decline of alpine farming (Gehrig-Fasel et al. 2007). Consideration of the amplitude and interaction of these effects is often a matter of scale, as course scale restraints can be disguised by local factors (Holtmeier and Broll 2017). Regardless, upward shifts of tree-lines has been documented in numerous mountain ranges (Grace et al. 2002) as measured by both tree densification and encroachment of the higher elevational boundary of tree growth (Feuillet et al. 2020).

Few studies have examined saproxylic Coleoptera and their Passerine predators around and immediately below tree-line. Abundance and diversity of montane flora and fauna are mainly reported to decline or have a humped shaped distribution as elevation rises (Rahbek 2005; McCain 2009). At higher elevations, mean temperatures are lower and weather is more extreme (Navarro-Serrano et al. 2020). Although diversity and abundance of Coleoptera is species-specific (de Vries et al. 2021), in general insect community and functional group patterns are broadly and strongly driven by temperature (Bale et al. 2002; Reymond et al. 2013).

Population sizes of insectivorous birds and their prey are known to have a close positive correlation (Møller 2019; Møller 2020). Although some studies have examined bird community structure at tree-line (Lloyd et al. 2012, Ferrarini et al. 2017; Altamirano et al. 2020), to our knowledge no study has focused on the diet of insectivorous Passerines at tree-line. It is clear, however, that functional and community patterns of insectivorous Passerines at higher elevations are influenced by both biotic interactions and environmental filtering (Graham et al. 2009). In some areas, functional trait diversity and habitat specialization within montane Passerine communities has decreased, corresponding to a decrease in abundance of habitat specialist Passerine species at a populational level. These phenomena are linked to the upward advancement of the tree-line (Archaux 2007; García-Navas et al. 2020).

The Optimal Foraging Theory posits that overlap between closely related species is more likely in more favorable conditions, as reduced interspecies competition is probable when resources are plentiful (Rosenzweig 1991). While no study has examined dietary relationships between insectivorous Passerines and saproxylic Coleoptera specifically, other research has found that overlap within and among insectivorous Passerine diets is driven by prey quality and abundance. For example, Davies et al. (2022) found higher dietary overlap in reedbed warblers [*Acrocephalus scirpaceus* (Hermann, 1804)] in summer during peak emergence of Diptera. The diet of Lapland longspur [*Calcarius lapponicus* (L., 1758)] overlapped heavily with other shorebirds within its guild during the weeks of high Diptera emergence (Custer and Pitelka 1978). There are other examples, however, of high overlap in resource-limited times, as shown in the diet overlap among American redstarts (*Setophaga ruticilla* (L., 1758)] linked by availability of low-value prey (small ants) during resource-scarce time periods (Kent et al. 2022).

1.4 Collection Types and Identification Approaches

1.4.1 Conventional traps and Morphology

Traditionally, saproxylic Coleoptera surveys have used a variety of conventional devices, including malaise, baited or attraction, pitfall, flight intercept, and in-situ or

ex-situ emergence traps, and each trap is designed to capture a specific type of insect. Malaise traps are tent-like, and flying insects that contact the trap tend to crawl upward where the tent flaps direct them into a collection bottle. Baited or attraction traps capture insects using chemicals such as semiochemicals or decaying odors. Attraction traps can be defined as a simple hanging reservoir filled with the chemical in question. Lindgren funnel traps are a type of attraction trap consisting of a series of funnels attached loosely top to end with the lure and collection jar at the bottom of the funnel stack. Pitfall traps target crawling insects and are cups filled with liquid and placed in an indentation in the ground. Flight intercept traps (also known as window traps) consist of a clear plastic panel with a collection point below. Flying insects contact the clear panel and drop into the collection jar. Emergence traps (also known as eclector traps) consist of a container placed around wood for the duration of the study. Larvae or pupae inhabiting the interior of the wood are funneled into a collection jar and captured when they emerge as flying or walking adults. Emergence traps can be left in the field (in-situ) or potentially infested wood can be transported to enclosures within laboratories (ex-situ). Detailed descriptions and photos of insect traps can be found in following sources: (Hyvarinen et al. 2006; Bouget et al. 2008; Miller and Duerr 2008; Quinto et al. 2013; Allison and Redak 2017; Skvarla and Dowling 2017; Touroult and Witté 2020; Ruchin et al. 2021).

Insects captured in these trap types are historically identified to family, and family groups are sent to one or more group experts for identification. Identification using morphological characters is considered the primary source of species delimitation (Bybee et al. 2010), and the entire specimen is usually retained for posterity. Reconfirming the identification rarely involves damage to the specimen.

This process has both advantages and disadvantages: traditional traps often fail in severe weather, are easily disrupted by humans and animals, and can be difficult to locate resulting in decreased efficacy. However, traditional traps are often economically constructed and permitting for insect collection is usually more easily obtained than permitting for vertebrate collection. Collecting a wide and abundant range of insects in these trap types is relatively easy. The main drawback is sorting the resulting arthropod material. Family- and species-level morphological identification can be arduous due to the immense diversity of Coleoptera and mastery of some groups requires years of study (Mehle and Trdan 2012; Macfadyen et al. 2019). Insects make up 80% of all recorded life on Earth, and Coleoptera is the most speciose Insecta order (Erwin, 1996). For example, the rove beetle family (Staphylinidae) alone comprise the largest family of organisms in the world with almost 60,000 described species; in the tropics, approximately 75% of the species in this family are undescribed (Frank and Thomas 1999; Solodovnikov et al. 2013). For most Coleoptera species, larvae and female forms are unknown or under-described (Yeo et al. 2018). Some groups are difficult to delimit and display overlapping and

continuous morphological characteristics (Zapata and Jiménez 2012). Due to the vast diversity of Coleoptera, a network of group taxonomists is needed to identify trap catches to species-level, and the difficulty of cultivating species-level taxonomic identification expertise has created a critical knowledge gap (Petrović 2022). These holes in the fundamental knowledge base have far-reaching impacts such as hindering attempts to control insect pest outbreaks or manage pesticide resistance (Macfadyen et al. 2019).

1.4.2 Metabarcoding

In the last decade, metabarcoding has become an increasingly popular technology for identification of bulk samples (Pompanon et al. 2012; Yu et al. 2012; Verkuil et al. 2022). The technology allows simultaneous taxonomic identification of multiple species from a single sample. DNA is extracted from environmental or bulk samples and conserved genes are amplified by a universal primer. The resulting amplicons of short, standard genes (barcodes) are sequenced using next generation sequencing (NGS). NGS generates millions of reads in parallel (Elbrecht et al. 2019; Liu et al. 2020; O'Rourke et al. 2020). Geneticists at the University of Guelph in Canada are credited for originating the idea of barcoding. The scientists proposed using a general sequence segment system for taxonomic identification across many animal taxa and identified the mitochondrial gene cytochrome c oxidase I (COI) region as a target profile (Hebert et al. 2003a; Hebert et al. 2003b). Less than a decade later, the technology has been used, for example, to identify the presence of potentially invasive birds in Brazilian rivers (Ritter et al. 2022), endangered freshwater mussels in Ohio (USA) (Marshall et al. 2022), and seasonal changes in rhizosphere bacterial communities in Spanish conifers (Lasa et al. 2022).

Costs associated with metabarcoding are decreasing and exclude the need for group-level morphological taxonomic expertise in groups well represented in DNA databases. Metabarcoding is fast and processing samples is becoming more streamlined (Flück et al. 2022). Quantitative analysis of metabarcoding results (i.e. absolute abundance counts of each taxa within the sample) was historically unattainable (Piper et al. 2019), although recent advances could give rise to quantitative results in the future (Di Muri et al. 2020). Methods for validating relative abundance results are becoming more common (Ershova et al. 2021; Laporte et al. 2022; Verkuil et al. 2022).

However, some disadvantages still exist. Metabarcoding and indeed all DNA-based identification methods are based on correct morphological identification of specimens submitted to DNA banks as references. Large gaps in the NCBI reference databases, especially in Arthropoda, hinder accurate results (Schoch et al. 2020), although these gaps are narrowing (Meiklejohn et al. 2019). False positives are not

uncommon, and can arise from inappropriate filtering thresholds, chimeric fragments, and/or laboratory or field contamination (Zinger et al. 2019; Sepulveda et al. 2020).

1.4.3 Metabarcoding and Passerines

Metabarcoding is also increasing in popularity as a tool for the identification of dietary components of birds (Rytkönen et al. 2019; Shutt et al. 2020; Silva et al. 2020; Shutt et al. 2021). European Passerines are one of the most studied bird groups in the world, yet metabarcoding is changing our understanding of their dietary diversity. Before the advent of metabarcoding, Passerine diets were mostly examined using nestlings, and these studies were less specific as well as invasive. Methods included video recording nests (Currie et al. 1996), morphologically identifying fecal sac contents (Kleintjes and Dahlsten 1992) or contents of undigested food from the throat using neck collars (ligatures) (Barba and Gil-Delgado 1990; Pagani-Núñez et al. 2011) or material flushed from the stomach (Senécal et al. 2021). Examining the diet of adult Passerines is more difficult. These techniques usually result in bird death, such as extracting gizzards to examine contents or euthanizing birds to analyze isotope-based niche metrics (Sehhatisabet et al. 2008; Maldonado et al. 2017).

Traditionally, these methods have found high overlap between or among European Passerines when dietary components are classified to a combination of class and family (Nour et al. 1998; Michalski et al. 2011; Grzędzicka 2018) (although see Atiénzar et al. (2013)). With metabarcoding, however, many prey items can be identified to species level, an incredible jump in quality of information. Recent studies of insectivore Passerine diets found surprisingly diverse diets. Most dietary components were rare (Rytkönen et al. 2019; Shutt et al. 2020; Shutt et al. 2021). In *Cyanestes caeruleas* (L. 1758), Shutt et al. (2020) reported 432 putative dietary items from just 793 fecal samples and conjectured that the high level of dietary diversity could be linked to dietary flexibility and prey availability.

1.5 Summation of Introduction

Dynamics between saproxylic Coleoptera and their Passerine predators are complex and in flux, and we expect climate change and land use shifts will continue to shape these communities. Responses to climate change can be measured at both a taxonomic and functional group level, as biotic and abiotic forces spark changes at different scales and measuring these developments will require a modern arsenal of tools. Finally, tree-line should be a focus of research as the changes occurring in this ecotone appear to be readily apparent and substantial.

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CHAPTER 2

FECAL MATTERS: IMPLEMENTING CLASSICAL COLEOPTERA SPECIES LISTS WITH METABARCODING DATA FROM PASSERINE BIRD FECES

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2.1 Abstract

Diversity inventories are critical to creating accurate species range maps and estimating population sizes, which in turn lead to better informed landscape and wildlife management decisions. Metabarcoding has facilitated large-scale environmental diversity surveys. However, the use of a metabarcoding approach with bird feces to survey arthropod diversity is still relatively undeveloped. The aim of this study was to see if and how a metabarcoding approach with bird feces could contribute to a saproxylic Coleoptera survey of traditional insect traps. We compared two methods of surveying saproxylic Coleoptera diversity (metabarcoding birds feces and deploying traditional traps) over two elevations in a mountain

system. The two methods caught different species and different levels of functional guild richness. The metabarcoding method successfully recorded both distinct and overlapping portions of diversity from traditional collections, and the approach was also effective in signaling the presence of both rare species and nine country records. Our results show that metabarcoding Passerine bird feces can be successful when used alongside traditional collection methods to capture a broad diversity of saproxylic Coleoptera. This method, however, has quantitative and qualitative limitations, including the inability to produce species abundance data as well as the generation of false positives and negatives due to biases within the metabarcoding pipeline. Implications for insect conservation: as many terrestrial ecosystems lose insect diversity, insect diversity surveys are essential to understand the scope of the loss. Despite metabarcoding approach shortcomings, the declining costs and shorter survey and processing time required for this approach compared to traditional survey methods indicate that it can be a valuable addition to the toolkit for saproxylic Coleoptera diversity surveys.

2.2 Introduction

Insect species make up 66% of all animal species found on earth (Zhang et al. 2011) and are conservatively estimated to provide more than 57 billion USD in economic value to the United States alone as both crop pollinators and vital building blocks for ecological functioning (Losey & Vaughan 2006). Recent studies have shown an alarming decline in terrestrial insect populations in the past half century (Dirzo et al. 2014; Sánchez-Bayo & Wyckhuys 2019; Møller 2020). For instance, one often cited study found a 75% reduction in flying insects in natural areas in Germany over the last 27 years (Hallmann et al. 2017). The stressors driving this decline of terrestrial insect populations are multifaceted and anthropomorphically derived and include pollution, the rise of industrial farming, climate change, and deforestation (Wagner et al. 2021).

Saproxylic Coleoptera [beetles that live or depend on dead or dying wood in some part of their lifecycle (Speight 1989)] are not immune to this trend. In Europe, roughly 11% of native saproxylic Coleoptera are considered threatened and a further 13% are considered near threatened (Nieto & Alexander 2010). The group is hyper-diverse with approximately 350,000-400,000 species worldwide (Storka et al. 2015). It is also functionally diverse and can be divided into multiple functional guilds within different life history stages including predatory, mycophagous, myxomycophagous (slime mold feeders), xylophagous, detritivorous, and parasitoid (Gimmel & Ferro 2018). This width and breadth of the taxonomic and functional variability of saproxylic Coleoptera have shown to be integral to nutrient cycling and food webs (Stokland et al. 2012; Gimmel & Ferro 2018). Many types of saproxylic Coleoptera play vital roles in the decomposition of dead wood through the digestion of polysaccharides and lignin with endosymbiotic fungi and/or bacteria (Micó et al. 2011; Strid et al. 2014; Hardersen & Zapponi 2017). Some saproxylic Coleoptera are restricted to a single host species, occupying distinct ecological niches (Milberg et al. 2014). In

addition to comprising essential ecosystem scaffolding, some families and species provoke economic damage, and climate change and increasingly even-aged stands are expected to compound this damage in the future (Pedlar et al. 2019; Sommerfeld et al. 2021).

Due to the ecological and economical importance of this group, surveys and inventories of saproxylic Coleoptera are employed throughout the world (see Saint-Germain et al. 2006; Karpiński et al. 2021) but the vast diversity of Coleoptera can hinder classification by morphological methods (Piper et al. 2019). Species level taxonomic identification expertise in many arthropod families often takes years to master (Macfadyen et al. 2019). The larval morphology of many species are completely unknown (Kamiński et al. 2019; Staniec et al. 2014). Furthermore, identifying fully cryptic species, or species that "morphology fails to delimit" (Liu et al. 2020) is only possible using molecular technology (DeSalle et al. 2005). For example, in the last decade *Brontispa longissima* (Gestro, 1885) (Coleoptera: Chrysomelidae) and two species of wireworms (Coleoptera: Elateridae) were split into cryptic species complexes due to distinct differences in genomes and geographic distributions despite only subtle differences in morphologic distinctions (Takano et al. 2011; Andrews et al. 2020, respectively).

Surveys and inventories of saproxylic Coleoptera are most often carried out using traditional insect traps, such as malaise, flight intercept, and baited or attraction traps. These established sampling devises can suffer from shortcomings, including weather-related failures, human or animal disruption and/or sabotage, and difficulty of locating installed traps.

Therefore, to detect or monitor insects and other species of interest in a rapidly changing world, scientists are increasingly employing a metabarcoding approach to examine environmental diversity (Liu et al. 2020). Metabarcoding allows the simultaneous identification of taxa within bulk samples by the parallel sequencing of a portion of a gene (barcode) (Shokralla et al. 2012). Generally, DNA is extracted from a sample and a segment of a gene is amplified through PCR using a set of primers chosen with the organism or taxonomic group of interest in mind. The resulting amplicons are dual-tagged to facilitate re-assignment into the original samples and are pooled to form sequencing libraries. Finally, the amplicon sequence reads are assigned to taxonomic classifications using various bioinformatic methods (Piper et al. 2019; Liu et al. 2020). Over the last decade, this technology has advanced from a laboratory protocol (Yu et al. 2012) and enabled studies documenting arthropod diversity in bulk samples from a variety of habitats such as fresh water (Hajibabaei et al. 2019), caves (West et al. 2020), soil (Porter et al. 2019), and in bulk samples collected from traditional traps such as malaise traps (Hardulak et al. 2020).

To our knowledge, ours is the first study that morphologically identified insects captured from traditional insect traps and compared this potential prey list to dietary components identified by metabarcoding adult birds' feces. Herein, we test a metabarcoding-based method to document saproxylic Coleoptera diversity in Passerine feces collected from

birds captured and released from mist nets. Other studies have surveyed arthropod diversity in feces of birds caught in agricultural settings (Crisol-Martínez et al. 2016; Jedlicka et al. 2017), and in feces of nestlings (Rytkönen et al. 2019), and in feces in adults taken from nest boxes (Shutt et al. 2020; Shutt et al. 2021). Ribeiro (2019) examined dietary niches of an African Passerine by both metabarcoding the birds' feces and potential prey caught in pitfall traps.

Our aim was to ascertain whether metabarcoding bird feces could compliment traditional insect trap surveys in order to better describe the insect community in a given area. Therefore, we compared two methods (a species list of Coleoptera consumed by the birds characterized by metabarcoding and a species list of Coleoptera captured in traditional traps characterized by morphological identification) deployed at the same locations in two elevations to see if metabarcoding bird feces could be used as a viable addition to saproxylic Coleoptera surveys.

2.3 Methods

2.3.1 Study area and sample collection

Our study was conducted in Vall d'Ordino, a valley located within three km of Vall de Sorteny Natural Park in the parish of Ordino, Andorra. Five ~0.1km² plots were selected in black pine (*Pinus mugo* Turr) forest between 1719 and 2222masl. In each plot, one or two mist nets and seven traditional insect traps (three attraction, three flight intercept, and one white malaise trap) were deployed.

Percent open space around each trap or mist net (1000m radius) location was calculated using QGIS3.4 and the MCSA 2012 landcover map downloaded from the Institute of Andorran Studies (Centre de Biodiversitat de l'Institut d'Estudis Andorrans 2012). Plots were characterized as "high" or "low" elevation depending on placement above or below the median elevation of all plots (i.e., 2064masl).

Attraction traps consisted of a 1L plastic bottles with a single hole cut in the side, hung upright 30cm from a live tree trunk. Each bottle was filled with ~250g of a bulk bait mixture of 7L sangria (Don Simon), 2L peach juice (Spar), and one kilo each of salt and sugar (Viñolas et al. 2009). Flight intercept traps consisted of two clear plastic panes perpendicularly crossed below a 14cm diameter white hard disk attached to a white funnel 13cm in diameter. Each white malaise trap measured 120 x 100 x 150cm (Entosphinx S.R.O). A collection bottle containing 70% propylene glycol (VWR Chemicals) and a few drops of dish detergent was attached to each flight intercept and malaise trap. Malaise traps capture higher percentages of Hymenoptera and Diptera (Karlsson et al. 2020) but successfully capture Coleoptera as well (Skvarla & Dowling 2017). All traditional traps were spaced at least 30m apart. Traps were installed May 23-28, 2017, as mid-May is when snow traditionally recedes from the Andorran

tree-line, and their contents were removed and baits refilled every 13-15 days until September 30-31, 2017. All specimens captured in the traditional traps were kept in 70% ethanol until processed.

Mist nets were deployed in the same plots as the traditional traps between May 15, 2018, and September 30, 2018, and consisted of 3 x 6m and 3 x 9m long black polyester mesh nets with mesh size of 16mm² strung perpendicular to the ground between 2.5m high poles. Three nets were installed in all sites except one; in this site due to geography only two nets were needed in order to capture the maximum number of birds. Nets were installed for ~two hours every two weeks depending on weather (i.e., no rain or extreme wind) and on a rotating schedule in order to capture the most birds possible at a given site. Feces from Passerine birds captured in the mist nets were collected with a single use toothpick and stored in molecular grade alcohol on ice until longer term refrigeration was available. A total of 132 fecal samples were collected. All 14 bird species collected are common and eat a wide variety of insects, and a list of bird species collected can be found in Appendix 2.1.

2.3.2 Species identification and sequencing

Through consulting with group specialists (listed in Table 2.1), we morphologically identified all Coleoptera specimens from traditional traps to the species level, with the exception of Scydmaeninae. Morphotypes of Staphylinidae were sent to a Staphylinidae expert for species identification. Species were then assigned to functional larval feeding guilds based on the literature currently available regarding each species' lifecycle as well as the FRISBEE database (Bouget et al. 2008).

DNA from the feces samples (each sample weighed ~3mg) were isolated using QIAamp DNA Stool Mini Kits (QIAGEN) following the manufacture's protocol with adjustments as suggested by Davies (2022). These adjustments include lowering 25µl proteinaseK to 20µl in step four and 600µl supernatant to 400µl in step five. Four negative controls (i.e., no sample) were conducted alongside the extraction procedure. DNA concentration was quantified using a Qubit 3.0 Fluorometer (Thermo Fisher Scientific Inc.) and samples were sent to the Georgia Genomics and Bioinformatics Core (University of Georgia, Athens GA, USA) for library preparation and sequencing on the Illumina MiSeq platform (Illumina) using v3 chemistry with 600 cycles of 2x250bp paired-end read lengths. The primer pair ANML (Jusino et al. 2017) was used to amplify a 180bp segment of the mitochondrial gene, cytochrome oxidase C subunit 1 (COI).

Samples were sequenced together with five mock communities of arthropods created with specimens caught in traditional traps (mock community composition in Appendix 2.2), as mock communities allow verification of taxonomic coverage and sequencing bias (Braukmann et al. 2019). Each insect chosen for the mock community was identified morphologically to family, and Coleoptera specimens chosen for mock community were

morphologically identified to species before subsequent verification by sequencing. All specimens were dipped in a 1% concentration of detergent (Thermo Scientific Tween-80), placed in a sonicating water bath for 60 seconds, then moved to sterile distilled water. The head, wings and legs of individual specimens were collected, placed in a buffer solution, and macerated with a sterile pestle. DNA was extracted and quantified using the same protocol as for feces. Specimens were taxonomically identified by Sanger sequencing from the amplicons produced with conventional PCR using the LCO1490 and HC02198 primers (Folmer et al. 1994). The PCR was assembled as follows: for a final volume of 25ul, each reaction contained a final concentration of 0.2mM dNTP, 2.5mM MgCl2, 0.4uM of each primer, 1X buffer, Taq polymerase (Promega) and 1ul of template DNA. Reactions were run on a Mastercyler Gradient thermocycler (Eppendorf) following the conditions specified in Folmer et al. (1994) and amplicons were visualized in 1% agarose gel. Successfully amplified samples were sent for Sanger sequencing at Eton Bioscience Inc. (Raleigh, NC, USA) and results were queried using the NCBI BLAST algorithm tool (National Center for Biotechnology Information) and BOLD (Barcode of Life Data System) to identify the specimen to the species level. Species identity was assigned if the sequence with the highest percent identity had a value of 96% query cover or above (Jedlicka et al. 2013).

2.3.3 Bioinformatic analysis

The script of the bioinformatic pipeline used in this study can be found in Appendix 2.3. In brief, the paired-end amplicon fastq files generated from the Illumina MiSeq sequencer were demultiplexed and primers were removed using the open-source bioinformatics pipeline QIIME 2 2020.6 (Bolyen et al. 2019). Lower quality nucleotides on the amplicons were trimmed and truncated, and the amplicons de-noised with DADA2 (via q2-dada2) through a series of filtering, merging of paired reads, and de-replication (Callahan et al. 2016). The end product of the DADA2 pipeline is an ASV (amplicon sequence variant) table, which offers a higher specificity than traditional OTU-level flows (Prodan et al. 2020). An ASV value represents the number of times an error-free unique amplicon sequence variant is recovered from sequencing. The DADA2 pipeline uses error models to divide amplicon reads into partitions, bypassing clustering methods that use fixed dissimilarity methods (Callahan et al. 2016). Potential contaminants in the ASV table were identified by the package Decontam (Davis et al. 2018). Taxonomy was assigned using a classy-sklearn naïve Bayes via q2-feature-classifier approach referencing the "tidybug" database, a training set of arthropod records curated by O'Rourke et al. (2020). Complementing the taxonomy classification based upon the curated database, assignations were checked on a case-by-case basis (see Irion et al. 2020, Smith et al. 2020, Milazzo et al. 2021, and Ratcliffe et al. 2021) and the following protocol was carried out: 1.) After taxonomy assignation was complete, assignations were individually checked for locational range. If an ASV was assigned to a species not found in Europe, it was removed. 2.) If an ASV was assigned to a rare species or a species that is not currently found in the Pyrenees but is found in Europe, it was flagged. If multiple sequences were assigned to a single flagged species identity, the sequences were

aligned to check for sequencing error and the sequences below an 80% sequencing error were removed, leaving a consensus sequence. 3.) All flagged assignments were then queried using the NCBI BLAST algorithm tool, and assignments that did not score at or above 98% identity were removed (Brandt et al. 2021; Ritter et al. 2022) 4.) Multiple hits showing the same max score on the NCBI BLAST tool were removed from analysis.

2.3.4 Mock community

The species assignments given to the mock community ASVs by metabarcoding were compared with the species assignments of the mock community by sanger sequencing, and the ratio of bias according to taxonomic rank (i.e. the level of uncertainty) was calculated.

2.3.5 Statistical analyses

Mean species richness among plots in high and low elevation and the four collection types were calculated, and the number of Coleoptera species aggregated by family caught by traditional and feces collections were tallied. To compare species richness among collection types, the data was fit to a generalized linear mixed-effects model using the lme4 v.26 package in R (Bates *et al.* 2015). Open space within a 1km radius, elevation, and collection type were used as explanatory factors, and plot as random. After the richness model was fit, post-hoc Tukey tests were carried out to investigate error rates of the categorical factor of season and trap type. The Jaccard dissimilarity index was applied to the data using the "vegdist" function of the vegan package and "jaccard" as the method (Oksanen *et al.* 2020) to examine differences within and among collection types. The above analyses were performed on collection type results that consisted of all Coleoptera species found, and just saproxylic (*sensu* Speight, 1989) species. They were also performed on all the traditional traps combined versus feces collection.

Further examination of differences in species richness between the collection types was conducted by creating sample-size based rarefaction and extrapolation curves of Shannon diversity with 95% confidence intervals using the iNEXT package in R (Chao & Jost 2012; Chao *et al.* 2014). The iNEXT procedure uses presence/absence data to create a sample-based (in our case traditional and feces collection based) rarefaction curve for the species found in the four collection types, and then estimates the numbers of sample units or percentage of sample coverage present in the assemblage but not represented in the traditional and/or feces collections. Sample coverage can be defined as "the percentage of the total number of individuals in a community that belong to the species represented in the sample" (Chao & Jost 2012).

Finally, the species richness of each larval functional feeding guild per collection type was calculated. Differences of collection types within a guild were examined using zero-truncated poisson regression with models fit by the glmmTMB package (Brooks *et al.* 2017) in

R (R Core Team 2021), and P values were calculated using Tukey tests. Parasitic and myrmecophilous Coleoptera were considered predators for the purpose of this analysis. Rhizophagous and herbivorous Coleoptera were considered phytophages, and coprophagous, necrophagous, and fungivorous Coleoptera were considered detritivores. Differences in functional guild composition among the collection types were also plotted using the elbow method to define the optimal number of clusters, followed with a k-means cluster analysis (Maechler *et al.* 2021).

2.4 Results

A total of 8995 individual specimens was collected in the traditional traps, representing 36 Coleoptera families, 153 species, and 15 unresolved taxa (interpreted herein as separate species). In the feces samples, 8.95 million sequence reads were produced, identified as the DNA of 19 Coleoptera families, 51 species, and 11 unresolved taxa (interpreted herein as separate species) (Table 2.2). The ANML primer amplified Coleoptera taxa in 74.2% of the samples, and the ASV counts per feces sample ranged from 4 to 126095 (Appendix 2.4). The comparison of species assignments given to the mock community ASVs by metabarcoding sanger sequencing displayed a 16.67% uncertainty in the assignment to order, and an additional 33.3% uncertainty in the assignment to family.

2.4.1 All Coleoptera species

Collection type was a significant factor in the model, with malaise (mean of 6.35±7.59 species per sample) and flight intercept (mean of 6.35±4.65) containing the highest species richness, followed by feces (2.06±1.18) and attraction (1.52±0.754) (beta-estimates and P values in Table 2.3) (Fig. 2.1A). Tukey tests showed richness levels between malaise and flight intercept collection types and between attraction and feces collection types were not significantly different (Table 2.3). Elevation and percent of open space within a 1k radius (beta-estimates and P values in Table 2.3) was not significant.

2.4.2 Saproxylic Coleoptera

As with all Coleoptera captures, collection type (with only saproxylic Coleoptera was taken into consideration) was a significant factor (attraction, mean of 1.32±0.58 species per sample; feces collection, 1.37±0.58; flight intercept, 4.94±3.58; and malaise, 4.2±4.2) (estimates and P values in Table 2.3) (Fig. 2.1B). Similar to results of all Coleoptera, richness levels between malaise and flight intercept collection types and between attraction and feces collection types were not significantly different (Table 2.3). The percent of open land within a 1k radius and elevation also did not influence richness (beta-estimates and P values in Table 2.3).

2.4.3 Combined traditional traps of all Coleoptera versus feces collections

Richness levels of traditional trap collections of all Coleoptera were significantly different than feces collections (est.: 0.91, P<0.001). Elevation and percent of open space within a 1k radius did not affect species richness (beta-estimates and P-values in Table 2.4).

2.4.4 Composition dissimilarity

The Jaccard dissimilarity index indicated that the species composition both within and among all collection types were distinctly different. Species composition within attraction traps had the least differences, although composition was still distinctly different (0.87 out of 1). All other trap within/among index results were above 0.92. Similar to the data that includes all Coleoptera, a Jaccard index examination showed very different compositions of saproxylic species within and among all collection types, with a dissimilarity index over 0.78 within attraction traps, and over 0.91 between and among all other collection type combinations. When all traditional collection results were combined into one variable, the Jaccard index continued to measure distinct species differences between and among all traditional collections combined and feces collections.

Twelve Coleoptera species were found in both traditional and feces collections (Table 2.2). Five of these 12 were saproxylic species (Table 2.2). Thirty-nine species were found in just feces collections and 141 were found in just traditional collections (Table 2.2). Of the 141 species found in only traditional collections, 112 are Andorran records, and nine Andorran record species were found in both types of collections (feces and traditional). Records are defined as previously uncollected in Andorra, according to the most recent editions of the Catalogue of Palearctic Coleoptera (Löbl & Smetana 2011; Löbl & Smetana 2013b; Löbl & Smetana 2013a, c; Löbl & Löbl 2015, 2016; Iwan & Löbl 2020). Some of the species found in traditional collections are unusual, such as *Axinotarsus tripatriae* Constantin 2013, a new species recently described (Constantin 2103) and *Curtimorda maculosa* (Naezen, 1794), rare in the Iberian Peninsula. Other rare species include *Atheta parapicipennis* Brundin, 1954 and *A. nigritula* (Gravenhorst, 1802) and *Pityophthorus glabratus* Eichhoff, 1878 and *Pityogenes conjuntus* (Reitter, 1887).

Fifty-seven percent of species found only in traditional collections were saproxylic, compared to 36.0% found only in feces collections (Table 2.2). Sixteen saproxylic species were found only in feces and 92 saproxylic species were found only by traditional traps (Table 2.2). Staphylinidae comprised 18.8% of species in traditional traps, versus 14.0% in feces collections (Fig. 2.2). Curculionidae comprised 12.5% of species found in traditional traps, compared to 24.0% in feces (Fig. 2.2).

2.4.5 Functional group analysis: Jaccard dissimilarity, modeling, and k-means cluster analysis

A Jaccard dissimilarity analysis showed distinct differences within and among collection types when species were partitioned into functional guilds; all dissimilarity measures were above 0.92. Indeed, collection types caught different functional guilds (Beta estimates and P values in Table 2.5) (Fig. 2.3A-D). Flight intercept traps harbored significantly higher richness of predators and wood-feeders than attraction collections (Fig. 2.3A,C). Richness of phytophagous guilds were significantly higher in malaise than flight intercept and feces collections, and feces collections were moderately higher than flight intercept collections (Fig. 2.3D). Too few phytophagous guild Coleoptera were found in attraction collections to analyze, and too few detritivore guild Coleoptera were found in bird feces collections to analyze (Beta estimates and P values in Table 2.5).

K-means hierarchical clustering results showed attraction and feces collections tended to cluster together when species were organized into functional guilds (Fig. 2.4). These results are similar to generalized linear model results in which both species richness (see Fig. 2.1A,B) and functional guild richness (see Fig. 2.3A-D) of attraction traps and feces trended together at lower richness levels than flight intercept and malaise.

2.4.6 Rarefaction and extrapolation curves

A rarefaction and extrapolation curve created by iNEXT estimated that 160 malaise, 175 attraction, 256 feces, and 417 flight intercept collections would be needed to approach 99% completeness sample coverage (Although 95% confidence intervals show flight intercept and malaise are the only collection types that do not overlap) (Fig. 2.5A). At 99% sample coverage, feces collections are expected to have higher diversity than attraction traps (29.2±6.4), and lower diversity than malaise and flight intercept traps. 256 feces collections would be needed to reach 99% sample coverage (Fig. 2.5B).

2.5 Discussion

Metabarcoding is a rapidly evolving technology with many untapped potential applications. Our study compared two methods of surveying saproxylic Coleoptera diversity (i.e., metabarcoding birds feces and deploying traditional traps), with the aim of adding metabarcoding bird feces as a viable addition to Coleoptera biodiversity surveys. The results showed 1) the four collection types (metabarcoding and three types of traditional traps) caught different species and different levels of functional guild richness, and 2) metabarcoding of bird feces successfully recorded a segment of taxonomic and functional diversity that both overlapped with and were distinct from traditional trap species lists in our plots. This approach is becoming less expensive but also is limited by disadvantages, including biases that can lead to incorrect data output (O'Rourke et al. 2020).

The collections of the four collection types had very little taxonomic overlap, and rarefaction and extrapolation curves show additional samples of all four collection types would be needed to fulfill the taxonomic diversity potential of each collection type (Fig. 2.5B). Dissimilarity within all collection types was high. The birds in our study also consumed a wide variety of prey, and high among-feces dissimilarity was also recorded in other dietary metabarcoding studies involving insectivorous Passerines (Shutt et al. 2020) and insectivorous bats (Vesterinen et al. 2016). Thus, the addition of metabarcoding would very likely add additional information to a traditional trap schema, but the results also suggest future studies deploying different collection types should take into account varying levels of collection type efficiency (see Alinvi et al. 2007 and Silva et al. 2018).

Collections of attraction traps and feces tended to cluster together in terms of functional guilds richness per collection type, probably due to their lower levels of species richness and functional diversity when compared to flight intercept and malaise traps (species diversity: Fig. 2.1; functional guild diversity: Fig. 2.3; cluster analysis: Fig. 2.4). However, the functional richness of phytophage guild Coleoptera in feces collections were moderately higher than flight intercept (Table 2.5, Fig. 2.3D), indicating future studies focusing on phytophage Coleoptera would be well served with additional metabarcoding feces surveys. The difference in functional diversity caught by fecal metabarcoding is unsurprising as the diets of many Passerine birds (especially during migration and nesting season) depend heavily on phytophagous insects (Tallamy & Shriver 2021). Flight intercept, malaise, and attraction traps are designed to capture mobile arthropods (Yi *et al.* 2012), while birds can actively search out sessile arthropods and less mobile larval stages of many phytophagous and saproxylic insects. For example, *Rhamphus pulicarius* (Herbst, 1795), a leaf miner captured in our study only in feces (Table 2.2), has no abdominal legs in larval form (Morris 2012). Another study comparing metabarcoding results of malaise and soil samples found numerous winged dipterans identified in the metabarcoding soil results (likely as eggs or larvae) but not the malaise samples, possibly due to the short flight season of these animals (Kirse *et al.* 2021).

It is also important to underline the fact that because our study was conducted from spring to fall (overlapping migration and nesting seasons), our dietary results could be very different than a Passerine dietary study conducted in winter; many resident omnivorous Passerine birds transition to a more herbivorous diet in the winter (Chamberlain et al. 2007; Renner et al. 2012). Furthermore, otherwise sedentary Passerines, such as the crested tit [*Lophophanes cristatus* (L., 1758)], can make short migration movements in especially harsh winters (Busse 1995). Examining the differences in diet between species of Passerine birds, however, is out of the scope of this paper, as the focus herein was to capture the most birds possible in a given site in order to explore the ways in which feces collections by mist netting could complement traditional insect traps.

In our plots, metabarcoding of bird feces successfully recorded a segment of taxonomic and functional diversity distinct from traditional trap species lists. The high level of species

richness recorded in the birds' feces is supported by other fecal metabarcoding studies involving insectivorous and/or omnivorous Passerines (Ribeiro et al. 2019; Rytkönen et al. 2019; Shutt et al. 2020; Silva et al. 2020). However, there was also significant overlap in our study; 12 species were shared between traditional and feces collections (9 of the 12 were country records). This proves the value of incorporating metabarcoding bird feces to document diversity as opposed to relying only on traditional traps, especially for projects designed to detect the presence of a rare or specific insect species or the presence of a forest pest. A database composed of DNA extracted from voucher specimens of a species of interest, with a mock community that also includes this extracted DNA, would allow for a focused study plan with fewer biases inherent in the metabarcoding pipeline. Furthermore, as metabarcoding becomes more widely used and less expensive, family or group experts would be unnecessary.

Nonetheless, there still exists certain disadvantages to dietary metabarcoding. Metabarcoding relies on characterizing DNA sequences. Transforming these sequence counts into individual abundance (the number of individual specimens per taxa in the feces sample) is thus far unattainable (Piper et al. 2019). Capturing relative abundance (the percentage of a taxa within a feces sample) is difficult due to technological and biological biases such as differential PCR amplification and DNA extraction efficiency (Deagle et al. 2013; Piñol et al. 2018; Deagle et al. 2019). Most metabarcoding studies including ours transform sequence counts into a presence/absence matrix (O'Rourke et al. 2020). However, PCR-free approaches show promise in attaining accurate abundance measures in biological assessments using metabarcoding methods (Liu et al. 2016). As metabarcoding is a novel and rapidly evolving technology, the rate of false positive errors can also be high due to potential biases including inappropriate filtering thresholds and chimeric fragments (Zinger et al. 2019). Furthermore, insects are still poorly represented in the NCBI reference databases (Schoch et al. 2020), which highlights the need for more comprehensive and curated databases. A complete reference database is especially import as other fecal adult Passerine metabarcoding studies have recorded surprisingly high species rich diets (Shutt et al. 2020). The dearth of insect DNA in reference databases can lead to taxonomic classification errors or record gaps (Geiger et al. 2016). The list of species from our metabarcoding study, for example, had high levels of uncertainty; for Coleoptera, the mock community showed a 33.3% uncertainty in the assignment of species, 33.3% uncertainty in the assignment of order, and 16.7% uncertainty in the assignment of class. Finally, the influence of digestion must be a consideration when regarding the recovery and detection of fecal DNA, as both physical and chemical processes will govern unequal DNA processing (Snider et al. 2021).

Parmain et al. (2013) reported a 20% mean assemblage dissimilarity between years in saproxylic Coleoptera. We acknowledge that our comparisons between our collecting methods could have been affected by normal population fluctuations. In our study, logistical reasons resulted in traditional sampling and feces sampling being conducted in subsequent years. However, the magnitude of difference in species richness and functional guilds

observed in the traditional traps compared to that found in the feces suggests these differences should not be solely due to annual variation. To our knowledge, our study is the first to compare adult Passerine bird feces to collections of traditional insect traps using metabarcoding. However, another recent study compared Passerine nestling feces to insect frass collected in different years and found the technique to have promising applications (Rytkönen et al. 2019).

Even with these limitations (abundance count issues, false positive or negative errors, digestion considerations, and database restraints), the ability to monitor Coleoptera populations while simultaneously performing a survey of the bird population highlights the potential usefulness of metabarcoding technology. Our study compared Coleoptera species lists obtained using morphological identification from insects collected from traditional insect traps and metabarcoding procedures performed on birds' feces and found distinct compositions of taxonomic and functional diversity. Numerous species, including several country records, were found in both collections. Upscaling taxa classification in bulk samples (such as feces collections) that would otherwise be impossible due to time and cost constraints, could be an effective, albeit unusual, application for this rapidly evolving technology.

2.6 Figures

Fig. 2.1 A,B Boxplot of Coleoptera species richness by A) trap and feces collection in all Coleoptera and B) trap and feces collection in only saproxylic Coleoptera. A=Attraction, B=Bird feces, F=Flight intercept, M=Malaise *graphics program used to create artwork: R, followed by MS powerpoint

Fig. 2.2 Number of Coleoptera species aggregated by family in species lists created from traditional collection types and metabarcoding bird feces, including species found on both lists *graphics program used to create artwork: MS Excel

Fig. 2.3 A-D Boxplot of larval functional guild richness per collection type. The four most common larval guilds are shown. Boxes under a common letter are not significantly different according to Tukey tests. A=Attraction, B=Bird feces, F=Flight intercept, M=Malaise *graphics program used to create artwork: R, followed by MS powerpoint

Fig. 2.4 Scatter plot of k-means hierarchical cluster analysis. Points represent each collection type (n=200). Points are clustered according to richness of each larval functional guild per collection type. A=Attraction, B=Bird feces, F=Flight intercept, M=Malaise *graphics program used to create artwork: R

 Fig. 2.5 A,B Sample-size based rarefaction (solid line) and extrapolation (dotted line) curves with 95% confidence intervals for Coleoptera diversity found in four collection types: attraction, feces, flight intercept, and malaise. A) Percent of sample coverage (i.e. total probability of occurrence of the species observed in the sample) with respect to number of

44

sample units (collections). B) Shannon diversity estimates in rarefied and extrapolated samples with respect to number of sample units (collections). Numbers in parentheses indicate number of sample units necessary to reach 99% coverage, and insect diversity at 99% coverage with 95% confidence intervals. Feces collections are expected to have higher diversity than attraction traps (29.2±6.4), and lower diversity than malaise and flight intercept traps. 256 feces collections would be needed to reach 99% sample coverage. A=Attraction, B=Bird feces, F=Flight intercept, M=Malaise *graphics program used to create artwork: R, followed by MS powerpoint
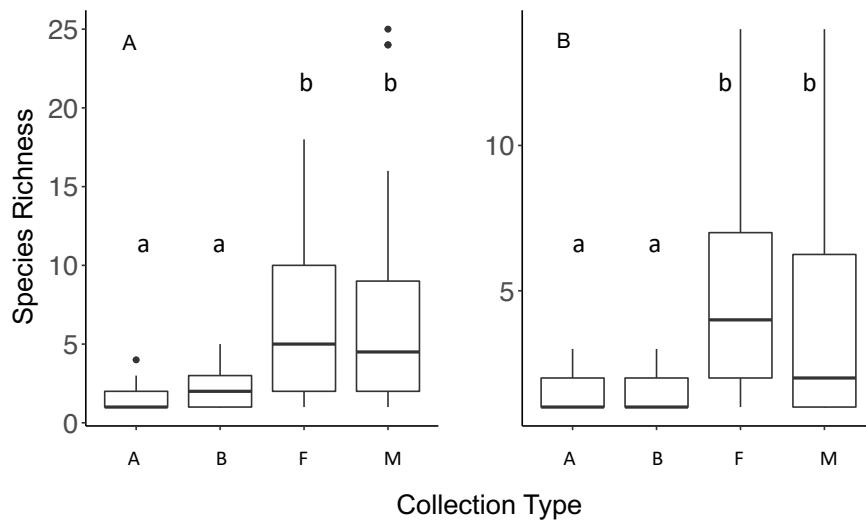
Fig. 2.1 A,B
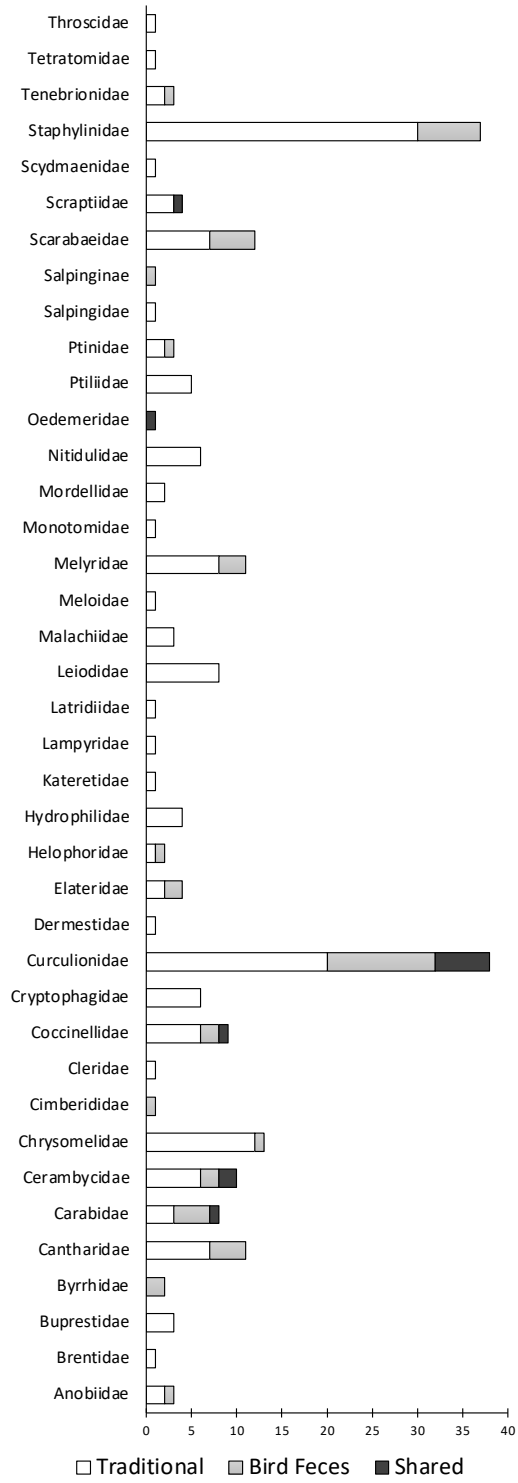
Fig. 2.2
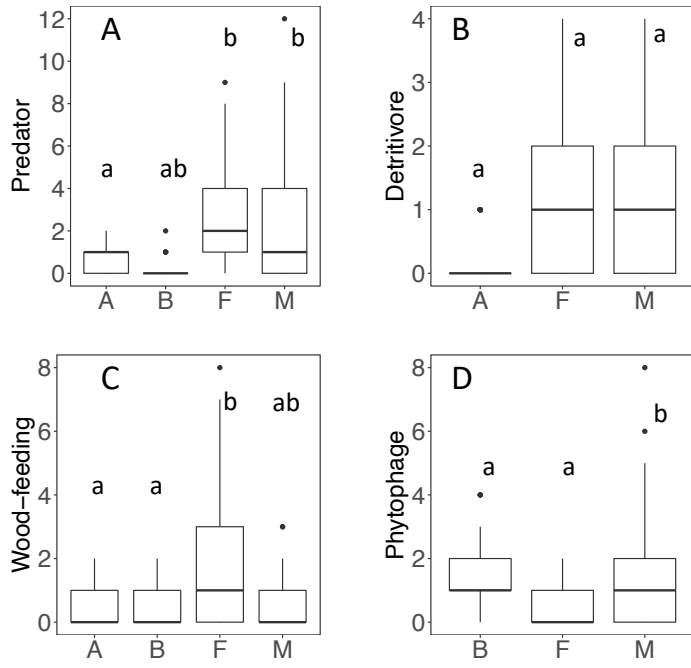
Fig. 2.3 A-D
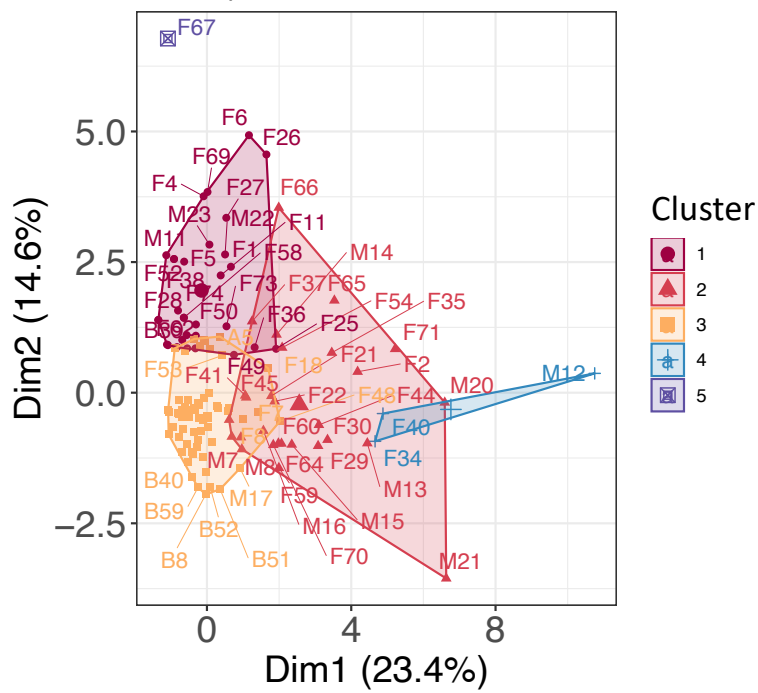
Fig. 2.4
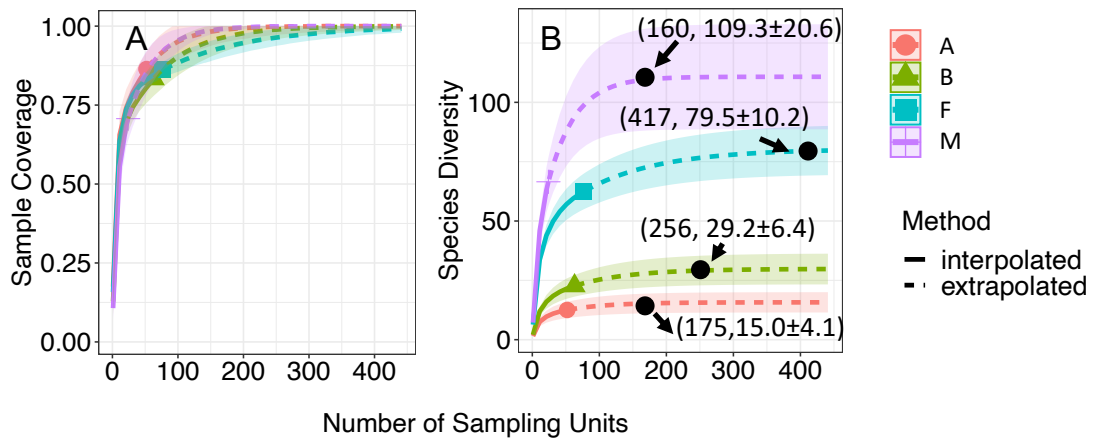
Fig. 2.5 A,B

2.7 Tables

Table 2.1 Table of experts involved with identifying specimens. Column labeled "Level of Assistance Provided" indicates if the expert in the respective row assisted with author J. Bookwalter's specimen identification of the specified family ("assisted"), or the expert in the respective row performed all identifications of the specified family ("identified").

Family	Expert	Level of assistance provided
Cantharidae	Fabrizio Fanti	identified
Carabidae	Benoit Dodelin	identified
Cerambycidae	Joan Bentanachs	assisted
Cerambycidae	Ulrich Bense	assisted
Chrysomelidae	Eduard Petitpierre Vall	assisted
Coccinellidae	Vincent Nicolas	identified
Cryptophagidae, Latridiidae	José Carlos Otero	identified
Curculionidae, Scarabaeidae	Jamie Bookwalter	identified
Dasytinae (Melyridae)	Gianfranco Liberti	assisted
Dermestidae	Jiri Háva	identified
Elateridae, Erotylidae, Meloidae, Nitilidae, Salpingidae	José Iñaki Recalde	identified
Hydrochidae, Hydrophilidae	Luis Valladares	identified
Hydrophilidae	Ayçin Yılmaz Akünal	identified
Kateretidae, Nitidulidae	Jose Manuel Pereira Martínez	identified
Leiodidae	Cédric Alonso	identified
Malachiidae	Gabriele Franzini	identified
Mordellidae	Dávid Selnekovič	identified
Ptiliidae	Mikael Sörensson	identified
Ptinidae	Amador Viñolas	identified
Scirtidae	Rafal Ruta	identified
Scolytinae (Curculionidae)	Miguel Alonso-Zarazaga	assisted
Scolytinae (Curculionidae)	Thierry Noblecourt	assisted
Scraptiidae	Brian Levey	identified
Staphylinidae	Benedikt Feldmann	identified
Tenebrionidae	Enrico Ruzzier	assisted
Throscidae	Cyrille van Meer	identified

Table 2.2 List of prey species found only in traditional collections, only in feces collections, and in both traditional and feces collection types. "List" indicates in which type of collection (only traditional= OT, only feces= OF, both traditional and feces= both) the species was found. Record= record for Andorra. Saproxylic: obligate= obligate saproxylic, facultative= facultative saproxylic, NS = non-saproxylic, no data = no information available. Saproxylic obligate and facultative are both treated equally as saproxylic in this study. Larval trophic guilds are predator, fungivore, herbivore (including pollen and nectar feeder), wood-feeding (including wood-boring and considered separate from herbivores), detritivore, multiple = species belonging to more than one guild, coprophagous, myrmecophilous, parasitic, rhizophagous= feeds on roots, no data = no information available.

Family	Species	Record	Saproxylic	Larval Guild	List
Carabidae	*Calodromius spilotus*	yes	facultative	no data	both
Cerambycidae	*Anastrangalia sanguinolenta*	no	obligate	wood-feeding	both
Cerambycidae	*Stictoleptura rubra*	yes	obligate	wood-feeding	both
Coccinellidae	*Myzia oblongoguttata*	yes	NS	predator	both
Curculionidae	*Anthonomus phyllocola*	yes	NS	herbivore	both
Curculionidae	*Brachonyx pineti*	yes	NS	herbivore	both
Curculionidae	*Brachyderes incanus*	yes	NS	rhizophagous	both
Curculionidae	*Magdalis memnonia*	yes	obligate	wood-feeding	both
Curculionidae	*Otiorhynchus singularis*	no	NS	rhizophagous	both
Curculionidae	*Rhamphus pulicarius*	yes	NS	herbivore	both
Oedemeridae	*Chrysanthia viridissima*	no	obligate	rhizophagous, wood-feeding	both
Scraptiidae	*Anaspis ruficollis*	yes	obligate	wood-feeding	both
Anobiidae	*Ernobius mollis*	NA	obligate	no data	OF
Byrrhidae	*Byrrhus pilula*	NA	NS	herbivore	OF
Byrrhidae	*Byrrhus sp.*	NA	NS	herbivore	OF
Cantharidae	*Cantharis figurata*	NA	NS	predator	OF

Family	Species	Record	Saproxylic	Larval Guild	List
Cantharidae	*Cantharis livida*	NA	NS	predator	OF
Cantharidae	*Cantharis tristis*	NA	NS	predator	OF
Cantharidae	*Rhagonycha fuscitibia*	NA	NS	predator	OF
Carabidae	*Amara bifrons*	NA	NS	predator	OF
Carabidae	*Calathus sp.*	NA	NS	herbivore, predator	OF
Carabidae	*Pterostichus pumilio*	NA	NS	herbivore, predator	OF
Carabidae	*Pterostichus sp.*	NA	NS	herbivore, predator	OF
Cerambycidae	*Monochamus sartor*	NA	obligate	wood-feeding	OF
Cerambycidae	*Oxymirus cursor*	NA	obligate	wood-feeding	OF
Chrysomelidae	*Donacia clavipes*	NA	NS	rhizophagous	OF
Cimberididae	*Doydirhynchus austriacus*	NA	facultative	herbivore	OF
Coccinellidae	*Anatis ocellata*	NA	facultative	predator	OF
Coccinellidae	*Calvia quatuordecimguttata*	NA	NS	predator	OF
Curculionidae	*Cleopomiarus graminis*	NA	NS	herbivore	OF
Curculionidae	*Hylastes brunneus*	NA	obligate	wood-feeding	OF
Curculionidae	*Hypera nigrirostris*	NA	NS	herbivore	OF
Curculionidae	*Magdalis linearis*	NA	obligate	wood-feeding	OF
Curculionidae	*Otiorhynchus pauxillus*	NA	NS	rhizophagous	OF
Curculionidae	*Phyllobius argentatus*	NA	NS	herbivore	OF
Curculionidae	*Pissodes validirostris*	NA	obligate	wood-feeding	OF
Curculionidae	*Polydrusus cervinus*	NA	NS	herbivore	OF
Curculionidae	*Polydrusus mollis*	NA	NS	herbivore	OF

Family	Species	Record	Saproxylic	Larval Guild	List
Curculionidae	*Strophosoma melanogrammum*	NA	NS	rhizophagous	OF
Curculionidae	*Trachyphloeus sp.*	NA	no data	no data	OF
Curculionidae	*Xylosandrus crassiusculus*	NA	obligate	fungivore	OF
Elateridae	*Limoniscus violaceus*	NA	facultative	detritivore	OF
Elateridae	*Melanotus villosus*	NA	obligate	wood-feeding	OF
Helophoridae	*Helophorus sp.*	NA	NS	no data	OF
Melyridae	*Dasytes sp. A*	NA	multiple	multiple	OF
Melyridae	*Dasytes sp. B*	NA	multiple	multiple	OF
Melyridae	*Enicopus pilosus*	NA	facultative	herbivore	OF
Ptinidae	*Anobium fulvicorne*	NA	obligate	wood-feeding	OF
Salpingidae	*Rabocerus gabrieli*	NA	obligate	predator, wood-feeder	OF
Scarabaeidae	*Amphimallon speciesx*	NA	NS	rhizophagous	OF
Scarabaeidae	*Aphodius abdominalis*	NA	NS	coprophagous	OF
Scarabaeidae	*Aphodius sp.*	NA	NS	no data	OF
Scarabaeidae	*Hoplia philanthus*	NA	NS	rhizophagous	OF
Scarabaeidae	*Phyllopertha horticola*	NA	NS	rhizophagous	OF
Staphylinidae	*Atheta bosnica*	NA	no data	no data	OF
Staphylinidae	*Atheta sp.*	NA	no data	no data	OF
Staphylinidae	*Ocypus aeneocephalus*	NA	no data	no data	OF
Staphylinidae	*Ocypus fulvipennis*	NA	no data	no data	OF
Staphylinidae	*Ocypus sp.*	NA	no data	no data	OF
Staphylinidae	*Stenus brunnipes*	NA	facultative	facultative	OF

Family	Species	Record	Saproxylic	Larval Guild	List
Staphylinidae	*Stenus impressus*	NA	no data	no data	OF
Tenebrionidae	*Isomira semiflava*	NA	obligate	obligate	OF
Anobiidae	*Ernobius nigrinus*	yes	obligate	no data	OT
Anobiidae	*Ernobius pini*	no	obligate	coprophagous	OT
Brentidae	*Protapion ruficroides*	yes	NS	no data	OT
Buprestidae	*Anthaxia carmen*	yes	obligate	wood-feeding	OT
Buprestidae	*Anthaxia quadripunctata*	no	obligate	wood-feeding	OT
Buprestidae	*Buprestis rustica*	no	obligate	wood-feeding	OT
Cantharidae	*Cantharis obscura*	no	NS	predator	OT
Cantharidae	*Malthodes atratus atratus*	yes	obligate	predator, wood-feeder	OT
Cantharidae	*Malthodes chelifer*	yes	obligate	predator, wood-feeder	OT
Cantharidae	*Malthodes group femoralis*	NA	obligate	predator, wood-feeder	OT
Cantharidae	*Malthodes guttifer*	yes	obligate	predator, wood-feeder	OT
Cantharidae	*Malthodes sp.A*	NA	obligate	predator, wood-feeder	OT
Cantharidae	*Malthodes sp.B*	NA	obligate	predator, wood-feeder	OT
Carabidae	*Dromius fenestratus*	yes	facultative	predator	OT
Carabidae	*Lebia cruxminor var. nigripes*	yes	no data	parasitic	OT
Carabidae	*Microlestes luctuosus*	no	no data	predator	OT
Cerambycidae	*Anastrangalia dubia*	no	obligate	wood-feeding	OT
Cerambycidae	*Lepturobosca virens*	yes	obligate	wood-feeding	OT
Cerambycidae	*Pogonocherus fasciculatus*	yes	obligate	wood-feeding	OT
Cerambycidae	*Stenurella melanura*	yes	obligate	wood-feeding	OT

Family	Species	Record	Saproxylic	Larval Guild	List
Cerambycidae	*Stictoleptura maculicornis*	yes	obligate	wood-feeding	OT
Cerambycidae	*Stictoleptura stragulata*	yes	obligate	wood-feeding	OT
Chrysomelidae	*Aphthona herbigrada*	yes	NS	herbivore, rhizophagous	OT
Chrysomelidae	*Calomicrus circumfusus*	yes	NS	rhizophagous	OT
Chrysomelidae	*Chaetocnema aerosa*	yes	NS	rhizophagous	OT
Chrysomelidae	*Clytra quadripunctata*	no	NS	myrmecophilous	OT
Chrysomelidae	*Cryptocephalus labiatus*	no	NS	herbivore	OT
Chrysomelidae	*Cryptocephalus pini*	yes	NS	detritivore	OT
Chrysomelidae	*Labidostomis humeralis*	yes	NS	rhizophagous	OT
Chrysomelidae	*Longitarsus ochroleucus*	no	NS	rhizophagous	OT
Chrysomelidae	*Longitarsus succineus*	no	NS	rhizophagous	OT
Chrysomelidae	*Luperus pyrenaeus*	yes	NS	rhizophagous	OT
Chrysomelidae	*Neocrepidodera melanopus*	no	NS	rhizophagous	OT
Chrysomelidae	*Smaragdina concolor*	yes	NS	myrmecophilous	OT
Cleridae	*Thanasimus formicarius*	yes	obligate	predator	OT
Coccinellidae	*Adalia decempunctata*	no	NS	predator	OT
Coccinellidae	*Coccinella hieroglyphica*	yes	NS	predator	OT
Coccinellidae	*Coccinella magnifica*	yes	NS	predator	OT
Coccinellidae	*Coccinella septempunctata*	no	NS	predator	OT
Coccinellidae	*Propylea quatuordecimpunctata*	no	NS	predator	OT
Coccinellidae	*Scymnus mimulus*	yes	NS	predator	OT
Cryptophagidae	*Cryptophagus cylindrellus*	yes	obligate	fungivore	OT

Family	Species	Record	Saproxylic	Larval Guild	List
Cryptophagidae	*Cryptophagus denticulatus*	yes	facultative	fungivore	OT
Cryptophagidae	*Cryptophagus jakowlewi*	yes	obligate	fungivore	OT
Cryptophagidae	*Cryptophagus saginatus*	yes	facultative	fungivore	OT
Cryptophagidae	*Cryptophagus scanicus*	yes	facultative	fungivore	OT
Cryptophagidae	*Cryptophagus sp.*	NA	facultative	fungivore	OT
Curculionidae	*Anthonomus rubi*	yes	NS	herbivore	OT
Curculionidae	*Curculio venosus*	yes	NS	herbivore	OT
Curculionidae	*Ips acuminatus*	yes	obligate	wood-feeding	OT
Curculionidae	*Magdalis duplicata*	yes	obligate	wood-feeding	OT
Curculionidae	*Magdalis frontalis*	yes	obligate	wood-feeding	OT
Curculionidae	*Magdalis rufa*	yes	obligate	wood-feeding	OT
Curculionidae	*Miarus campanulae*	yes	NS	herbivore	OT
Curculionidae	*Micrelus ericae*	yes	NS	herbivore	OT
Curculionidae	*Phloeotribus rhododactylus*	yes	obligate	wood-feeding	OT
Curculionidae	*Phyllobius alpinus*	no	NS	rhizophagous	OT
Curculionidae	*Phyllobius pomaceus*	yes	NS	rhizophagous	OT
Curculionidae	*Pityogenes bistridentatus*	yes	obligate	wood-feeding	OT
Curculionidae	*Pityogenes conjuntus*	yes	obligate	wood-feeding	OT
Curculionidae	*Pityogenes quadridens*	yes	obligate	wood-feeding	OT
Curculionidae	*Pityogenes trepanatus*	yes	obligate	wood-feeding	OT
Curculionidae	*Pityophthorus buyssoni*	yes	obligate	wood-feeding	OT
Curculionidae	*Pityophthorus glabratus*	yes	obligate	wood-feeding	OT
Curculionidae	*Pityophthorus pityographus*	yes	obligate	wood-feeding	OT

Family	Species	Record	Saproxylic	Larval Guild	List
Curculionidae	*Tomicus piniperda*	yes	obligate	wood-feeding	OT
Curculionidae	*Tychius sp.*	NA	NS	herbivore	OT
Dermestidae	*Anthrenus fuscus*	no	facultative	detritivore	OT
Elateridae	*Idolus picipennis*	yes	no data	no data	OT
Elateridae	*Pheletes aeroniger*	yes	NS	herbivore	OT
Helophoridae	*Helophorus glacialis*	yes	NS	no data	OT
Hydrophilidae	*Cercyon sp.*	NA	facultative	detritivore, predator	OT
Hydrophilidae	*Sphaeridium bipustulatum*	yes	NS	predator	OT
Hydrophilidae	*Sphaeridium lunatum*	yes	NS	predator	OT
Hydrophilidae	*Sphaeridium marginatum*	yes	NS	predator	OT
Kateretidae	*Brachypterolus longulus*	yes	NS	herbivore	OT
Lampyridae	*Lampyris noctiluca*	no	NS	predator	OT
Latridiidae	*Stephostethus lardarius*	yes	facultative	fungivore	OT
Leiodidae	*Agathidium sp.*	NA	facultative	fungivore	OT
Leiodidae	*Anisotoma humeralis*	yes	facultative	fungivore	OT
Leiodidae	*Catops sp.*	NA	multiple	detritivore, fungivore, necrophagous	OT
Leiodidae	*Catops tristis*	yes	NS	no data	OT
Leiodidae	*Leiodes dubia*	yes	facultative	fungivore	OT
Leiodidae	*Leiodes obscura*	yes	facultative	fungivore	OT
Leiodidae	*Leiodes sp.*	NA	facultative	fungivore	OT
Leiodidae	*Sciodrepoides sp.*	NA	multiple	detritivore, fungivore, necrophagous	OT
Malachiidae	*Attalus amictus*	yes	facultative	predator	OT
Malachiidae	*Axinotarsus tripatriae*	no	facultative	predator	OT

Family	Species	Record	Saproxylic	Larval Guild	List
Malachiidae	*Troglops cephalotes*	yes	facultative	predator	OT
Meloidae	*Mylabris flexuosa*	no	NS	parasitic	OT
Melyridae	*Aplocnemus alpestris*	yes	obligate	predator	OT
Melyridae	*Danacea pallipes*	no	obligate	predator	OT
Melyridae	*Dasytes gonocerus*	yes	obligate	predator	OT
Melyridae	*Dasytes niger*	no	obligate	predator	OT
Melyridae	*Dasytes nigropilosus*	no	obligate	predator	OT
Melyridae	*Dasytes subaeneus*	no	obligate	predator	OT
Melyridae	*Dasytes virens*	no	obligate	predator	OT
Melyridae	*Enicopus sp.*	NA	obligate	predator	OT
Monotomidae	*Rhizophagus depressus*	yes	obligate	predator	OT
Mordellidae	*Curtimorda maculosa*	yes	obligate	predator	OT
Mordellidae	*Mordella aculeata*	yes	obligate	fungivore	OT
Nitidulidae	*Brassicogethes viridescens*	no	NS	herbivore	OT
Nitidulidae	*Epuraea marseuli*	no	facultative	predator	OT
Nitidulidae	*Epuraea sp.*	NA	facultative	predator	OT
Nitidulidae	*Fabogethes nigrescens*	no	NS	herbivore	OT
Nitidulidae	*Meligethes sp.*	NA	NS	herbivore	OT
Nitidulidae	*Sagittogethes obscurus*	yes	NS	herbivore	OT
Ptiliidae	*Acrotrichis grandicollis*	yes	facultative	fungivore	OT
Ptiliidae	*Acrotrichis parva*	yes	facultative	fungivore	OT
Ptiliidae	*Acrotrichis rugulosa*	yes	facultative	fungivore	OT
Ptiliidae	*Ptiliidae sp.*	NA	facultative	fungivore	OT

Family	Species	Record	Saproxylic	Larval Guild	List
Ptiliidae	*Ptiliola brevicollis*	yes	facultative	fungivore	OT
Ptinidae	*Dryophilus anobioides*	yes	facultative	fungivore, wood-feeding	OT
Ptinidae	*Ptinus dubius*	no	facultative	fungivore, wood-feeding, coprophagous	OT
Salpingidae	*Sphaeriestes castaneus*	yes	obligate	predator	OT
Scarabaeidae	*Acrossus rufipes*	yes	NS	coprophagous	OT
Scarabaeidae	*Agoliinus satyrus*	yes	NS	coprophagous	OT
Scarabaeidae	*Amidorus obscurus*	no	NS	coprophagous	OT
Scarabaeidae	*Loraphodius suarius*	yes	NS	coprophagous	OT
Scarabaeidae	*Nimbus contaminatus*	yes	NS	coprophagous	OT
Scarabaeidae	*Omaloplia ruricola*	yes	NS	rhizophagous	OT
Scarabaeidae	*Trichius fasciatus*	yes	obligate	wood-feeding	OT
Scraptiidae	*Anaspis pyrenaea*	yes	obligate	wood-feeding	OT
Scraptiidae	*Anaspis rufilabris*	yes	obligate	wood-feeding	OT
Scraptiidae	*Anaspis varians*	yes	obligate	wood-feeding	OT
Scydmaenidae	*Scydmaenidae sp.*	NA	facultative	detritivore, predator, wood-feeding	OT
Staphylinidae	*Aleochara bilineata*	yes	NS	parasitic	OT
Staphylinidae	*Aleochara intricata*	yes	facultative	detritivore, coprophagous	OT
Staphylinidae	*Aleochara sparsa*	yes	NS	parasitic	OT
Staphylinidae	*Aleochara tristis*	yes	NS	parasitic	OT
Staphylinidae	*Anotylus nitidulus*	yes	obligate	coprophagous, necrophagous, wood-feeding	OT
Staphylinidae	*Anthophagus alpinus pyrenaeus*	yes	NS	predator	OT

Family	Species	Record	Saproxylic	Larval Guild	List
Staphylinidae	*Atheta ischnocera*	yes	no data	no data	OT
Staphylinidae	*Atheta nigritula*	yes	facultative	fungivore	OT
Staphylinidae	*Atheta parapicipennis*	yes	no data	no data	OT
Staphylinidae	*Atheta vaga*	yes	facultative	detritivore, predator	OT
Staphylinidae	*Eusphalerum umbellatarum*	yes	NS	predator	OT
Staphylinidae	*Leptusa pulchella*	yes	obligate	no data	OT
Staphylinidae	*Lordithon bimaculatus*	yes	facultative	fungivore, predator	OT
Staphylinidae	*Lordithon lunulatus*	yes	facultative	no data	OT
Staphylinidae	*Mycetoporus piceolus*	yes	no data	necrophagous	OT
Staphylinidae	*Mycetoporus punctus*	yes	facultative	predator	OT
Staphylinidae	*Omalium excavatum*	yes	facultative	coprophagous, detritivore	OT
Staphylinidae	*Philonthus cruentatus*	yes	NS	coprophagous, detritivore, necrophagous	OT
Staphylinidae	*Philonthus montivagus*	yes	no data	no data	OT
Staphylinidae	*Placusa tachyporoides*	yes	obligate	predator	OT
Staphylinidae	*Platystethus cornutus*	yes	NS	detritivore	OT
Staphylinidae	*Platystethus nitens*	yes	NS	coprophagous, detritivore, necrophagous	OT
Staphylinidae	*Proteinus cf_ovalis*	NA	facultative	coprophagous, detritivore, fungivore	OT
Staphylinidae	*Pselaphinae sp.*	NA	facultative	myrmecophilous, predator	OT
Staphylinidae	*Quedius anceps*	yes	no data	detritivore	OT
Staphylinidae	*Quedius boops*	yes	no data	detritivore	OT
Staphylinidae	*Tachinus fimetarius*	yes	NS	coprophagous	OT
Staphylinidae	*Tachinus marginellus*	yes	NS	coprophagous	OT
Staphylinidae	*Tachyporus nitidulus*	yes	NS	no data	OT

Family	Species	Record	Saproxylic	Larval Guild	List
Staphylinidae	*Xantholinus linearis*	yes	facultative	detritivore, myrmecophilous	OT
Tenebrionidae	*Cteniopus sulphureus*	yes	obligate	wood-feeding	OT
Tenebrionidae	*Isomira sp.*	NA	obligate	wood-feeding	OT
Tetratomidae	*Hallomenus sp.*	NA	obligate	fungivore	OT
Throscidae	*Trixagus leseigneuri*	yes	obligate	wood-feeding	OT

Table 2.3 Results of generalized linear mixed-effects modeling and subsequent Tukey tests: Effects of variables on richness of all Coleoptera collected and just saproxylic Coleoptera collected. Estimates and P values in bold font reflect a P value <0.05. (Estimate=Beta-estimate, ColType= Collection type, A= Attraction trap, B= Bird feces, F= Flight intercept, M= Malaise)

		All Coleoptera	Saproxylic Coleoptera
Elevation:Low	Estimate	0.72	-0.03
	P value	0.72	0.88
Open space	Estimate	-0.01	-0.1
	P value	0.34	0.37
ColType A:B	Estimate	0.26	-0.01
	P value	0.09	0.95
ColType A:F	Estimate	**1.4**	**1.29**
	P value	**<0.001**	**<0.001**
ColType A:M	Estimate	**1.57**	**1.15**
	P value	**<0.001**	**<0.001**
Season Spring:Summer	Estimate	**1.07**	**1.21**
	P value	**<0.001**	**<0.001**
Season Spring:Fall	Estimate	**0.77**	**0.93**
	P value	**<0.001**	**<0.001**

		All Coleoptera	Saproxylic Coleoptera
Season Summer:Fall		**<0.001**	**<0.001**
Season Spring:Fall	Tukey tests	**<0.001**	**<0.001**
Season Spring:Summer		**<0.001**	**<0.001**

		All Coleoptera	Saproxylic Coleoptera
ColType A:B		0.32	1
ColType A:F		**<0.001**	**<0.001**
ColType A:M		**<0.001**	**<0.001**
ColType F:B	Tukey tests	**<0.001**	**<0.001**
ColType M:B		**<0.001**	**<0.001**
ColType M:F		0.24	0.68

Table 2.4 Results of generalized linear mixed-effects modeling and subsequent Tukey tests: Effects of variables on Coleoptera richness when traditional collection types are combined and compared with bird feces results. Reference used: bird feces. Estimates and P values in bold font reflect a P value <0.05. (Estimate=Beta-estimate, ColType= Collection type, T= Traditional)

Elevation: Low	Estimate	-0.26
	P value	0.11
Open space	Estimate	-0.01
	P value	0.26
ColType: T	Estimate	**0.91**
	P value	**<0.001**
Season Spring:Fall	Estimate	**1.14**
	P value	**<0.001**
Season Summer:Fall	Estimate	**0.78**
	P value	**<0.001**
Season Summer:Spring	Estimate	**-0.35**
	P value	**<0.001**

Table 2.5 Differences of functional richness between collection type (i.e. species richness of each larval feeding guild) calculated using generalized linear mixed-effects modeling and subsequent Tukey tests. Estimates and P values in bold font reflect a P value <0.05. (Estimate=Beta-estimate, A= Attraction trap, B= Bird feces, F= Flight intercept, M= Malaise)

Collection Type		Wood-feeding	Phyto-phagous	Detritivore	Predator
B:A	Estimate	-0.08			1.64
	P value	1.00			0.60
F:A	Estimate	**2.30**		19.45	**3.71**
	P value	**0.00**		1.00	**0.00**
M:A	Estimate	1.37		19.66	**3.98**
	P value	0.17		1.00	**0.00**
F:B	Estimate	**2.38**	-1.08		2.07
	P value	**0.00**	0.05		0.12
M:B	Estimate	1.45	**0.86**		2.34
	P value	0.13	**0.00**		0.06
M:F	Estimate	-0.93	**1.94**	0.22	0.27
	P value	0.08	**0.00**	0.77	0.25

2.8 Acknowledgements

2.9 References

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CHAPTER 3

METABARCODING PASSERINE BIRD FECES AT TREE-LINE UNCOVERS LITTLE INTRA- AND INTER-SPECIES DIETARY OVERLAP

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3.1 Abstract

High elevation insectivorous birds are currently confronted with the reality of a changing climate, land use shifts, and the decline of many prey groups. The diet dynamics among many imperiled animals in this group are still unresolved. Examining the diets of tree-line Passerine birds to the species level of the prey allows for stronger population predictions. This study uses metabarcoding to identify prey insects from adult Passerine bird feces at and slightly below tree-line in a Pyrenean forest. Our objective was to quantify the intra-and inter-

species richness and overlap of Passerine bird diet over time and space. The results showed that adult Passerine diets had extremely low overlap between and among species, a finding dissimilar to many traditional Passerine dietary studies. The species with the highest captures showed higher diet richness in fall. The lack of association between dietary richness and open space and elevation, and lack of differences between dietary overlap and open space and elevation, suggest high elevation Passerine birds have very high dietary flexibility. The results also showed that aphids known to be pests to conifers, and other conifer pests, were prevalent in the birds' diets. While the metabarcoding approach used in this study allowed us to appreciate results that contrast findings from traditional dietary studies, the high percentage of taxonomic uncertainty for some orders in our mock communities suggests caution in their interpretation. Implications for the long-term projections relative to tree-line Passerine populations are discussed.

3.2 Introduction

Worldwide, insectivorous birds consume an estimated 400-500 million tons of insects a year (Nyffeler et al. 2018), which is more than the 350 million tons of flesh consumed annually by humans (Hicks et al. 2018). Insectivorous bird populations are especially vulnerable to climate change; in North America terrestrial insectivorous birds have declined 33% over the last 50 years, while other terrestrial bird groups have increased (Tallamy and Shriver 2021). Meanwhile, in Europe insectivorous and non-insectivorous birds have declined dramatically over the last 30 years (Inger et al. 2015).

Upper elevation birds are particularly at risk because mountains are expected to be more affected by climate change than lowland areas, due to faster and enhanced warming (Mountain Research Initiative EDW Working Group 2015). Several studies have documented a declining abundance of some high elevation birds. For example, high elevation populations of Canadian jays [*Perisoreus canadensis* (L., 1766)] declined 50% over a 30-year period, and the decline was attributed to warmer and more variable weather (Sutton et al. 2021). This change in weather pattern increased the number of freeze-thaw events, which caused an increase in the spoilage of cached food items. The survival of another high elevation Passerine, the white winged snowfinch [*Montifringilla nivalis* (L., 1766)] is in doubt because its foraging behavior is closely tied to snow retreat conditions which are becoming increasingly less consistent (Resano-Mayor et al. 2019). Finally, Barras et al. (2021) found that elevated ambient temperatures at the tree-line in the Swiss alps resulted in fewer nestling prey provisioning of the Alpine ring ouzel (*Turdus torquatus alpestris* L., 1758).

The flora and fauna of the Pyrenees mountains are especially threatened due to land use shifts and climate change (OPCC-CTP 2018). The snowpack is warmer and thus particularly sensitive to slight changes in ambient temperature (Lopez-Moreno et al. 2017), and the decline of agropastoral practices in the Pyrenees has led to transitions of open grassland into forest (Roura et al. 2005). The Pyrenean tree-line is also shifting upward

(Ameztegui et al. 2016). Tree-line dynamics globally are affected by a variety of factors, including precipitation, tree composition, and soil structure (Grace et al. 2002; Körner 2012). However, the upward shift and densification of the tree-line in the Pyrenees is generally linked to local agricultural abandonment (Batllori and Gutiérrez 2008), but there can be locally important factors such as slope morphometry and lithology (Feuillet et al. 2020).

It is within this context that we examined the diet of Passerines at elevations located below and at tree-line to better explain the decline of omnivorous and insectivorous birds. The diet of many European passerine birds, e.g., Paridae, has been examined closely, even though most studies were limited to estimating the diet of nestlings using either methods that are invasive [neck collars (Barba and Gil-Delgado 1990; Pagani-Núñez et al. 2011) and stomach flushing (Senécal et al. 2021)], noninvasive but results are less detailed [e.g., cameras (Currie et al. 1996)], or lethal [gizzard extraction (Sehhatisabet et al. 2008)]. Dietary studies of this group largely have focused on nestlings while fewer studies focused on the diet of adults. Recent advances in metagenomic technology have increased our ability to analyze the diet of adult Passerines in a non-invasive manner at a high level of taxonomic classification (see Ribeiro et al. 2019; Shutt et al. 2020; Silva et al. 2020).

Metabarcoding supports high-throughput (i.e. massively parallel) taxonomic classification within a sample (Bush et al. 2019). A short portion of a gene (barcode) from an environmental or biological sample is amplified by a primer designed to provide taxonomic resolution of a target organism or taxonomic group (Hajibabaei et al. 2007; Deagle Bruce E. et al. 2014). However, there are many fundamental limitations of fecal metabarcoding. For example, raw abundance cannot be determined from the number of reads in a similar DNA sequence, and relative abundance is difficult to recover because of technological and biological biases including primer mismatch and differences in PCR amplification due to primer sequence length (Deagle B. E. et al. 2013; Pinol et al. 2015; Krehenwinkel et al. 2017). Sample contamination and differing rates of DNA preservation in the gut can also present issues (Galan et al. 2018; Nielsen et al. 2018). With these limitations in mind, mock communities are useful in quantifying the sensitivity and taxonomic resolution of a study's protocol by assembling a pool of DNA extracts of sequences from representative target prey species, and sequencing this pool alongside sample (e.g., fecal) extractions (Braukmann et al. 2019).

Using metabarcoding, Shutt et al. (2020) uncovered 432 putative dietary items from 793 fecal samples of *Cyanistes caeruleus* (L., 1758) (blue tit), revealing a surprisingly diverse arthropod diet. However, to our knowledge, no study has included the diet of multiple adult Passerines using metabarcoding [although see Sottas et al. (2020)]. The goal of our study was to determine if and how open space, elevation, and season affects the diet of high-elevation Pyrenean Passerines. We expected higher niche differentiation (i.e. difference in diet composition) in morphologically and behaviorally similar species and higher dietary richness in and higher dietary overlap among species as spring progressed to fall. Passerines that have

similar traits often have competition-driven niche separation (Alatalo R. V. et al. 1986; Cowie and Hinsley 1988; Sottas et al. 2020), and higher abundance of prey is linked to less dietary partitioning (Davies et al. 2022). In some species, we expected diet richness to be positively linked with the percentage of open space because patchier habitats have been shown to benefit some species but not others (Suarez-Seoane et al. 2002). Finally, we expected higher overlap in below tree-line plots because we expected the conditions to be more favorable to Passerines. Higher overlap is common in more favorable habitats (Hou et al. 2021).

3.3 Methods

3.3.1 Study area and feces collection

Ten plots were selected in grid format within a black pine forest (*Pinus mugo* Turra) in Vall d'Ordino, a valley located within three km of Vall de Sorteny Natural Park in the parish of Ordino, Andorra. Plots were situated between 1729 and 2352 masl. Percent open space surrounding each plot (1000 m radius) was calculated using QGIS3.4 and the MCSA 2012 landcover map downloaded from the Institute of Andorran Studies (Centre de Biodiversitat de l'Institut d'Estudis Andorrans 2012). Plots were characterized as "below tree-line" or "at tree-line" depending on positioning above or below the median elevation of all plots (i.e., 2077 masl). In most of Andorra, tree-line occurs between 2200 and 2400 masl, and in a few areas between 2100 and 2500 masl (Carreras et al. 1996).

Birds were captured using Ecotone mist-nets (9 m and 6 m long and 2.5 m high, with 5 shelves and a mesh size of 16 mm^2) stretched between 4 m poles inserted perpendicularly in the ground. In each plot, mist nets were deployed every two weeks between May 15, 2018, and September 30, 2018. The start date for the field component of this study coincided with the date when snow historically has retreated from the Andorran tree-line. Mist nets were not set at a plot when there was precipitation or high winds to ensure good capture conditions and welfare of the bird. Once a bird was captured, it was placed in a single use individual paper bag. After defecation, feces were carefully removed from the paper bag using a single-use toothpick and stored in plastic vials. Vials were placed on ice in the field and transferred to long-term refrigeration as soon as possible. All birds caught were identified at species level, ringed, aged, sexed, and measured following standard ringing procedure. Birds were handled by certified ringers and all the procedures approved by the Environment and Sustainability Department of Andorran Government.

A total of 132 fecal samples was collected. No bird was captured twice. Samples collected in June were considered to be from the spring, July and August samples were considered to be from the summer, and September samples were considered to be from the fall.

3.3.2 DNA extraction and amplification

DNA in the collected fecal samples and in four DNA extraction blanks (i.e. vials with no fecal samples that served as control) was extracted using the QIAamp DNA Stool Mini Kit (QIAGEN) following the manufacture's protocol with modifications suggested by Davies et al. (2022). Each sample weighed approximately 3 mg. DNA concentration was quantified using a Qubit 3.0 Fluorometer (Thermo Fisher Scientific Inc.). PCR library preparation and sequencing was carried out by the Georgia Genomics and Bioinformatics Core (University of Georgia, Athens GA, USA) on the Illumina MiSeq platform (Illumina) using v3 chemistry with 600 cycles of 2x250 bp paired-end read lengths. DNA extracted from feces is often highly degraded and fragmented (Deagle Bruce E. et al. 2006). Initial plans called for the use of a primer amplifying a longer region, but a preliminary test (data not shown) indicated higher efficacy of a shorter primer, the mini-barcode mitochondrial primer (ANML). ANML amplifies a smaller 180 bp segment on the cytochrome oxidase C subunit 1 (COI) (Jusino et al. 2017).

3.3.3 Mock community

Five mock communities were created based on the results of malaise and other traditional insect traps deployed in the mist net plots, as described in Bookwalter et al. (2022). The composition of these communities is described in Appendix 3.1. These communities were used as positive control references to validate the molecular pipeline (Jusino et al. 2017; Braukmann et al. 2019). First, insects were identified to family using traditional morphology before their subsequent verification by Sanger sequencing. All specimens were dipped in a 1% concentration of detergent (Thermo Scientific Tween-80), placed in a sonicating water bath for 60 seconds, then moved to sterile distilled water. The head, wings, and legs of each specimen was removed, placed in a buffer solution and macerated with a sterile pestle. DNA from each insect specimen was extracted and quantified using the same kit and protocol used for the fecal samples. Conventional PCR was performed using the LCO1490 and HC02198 primers (Folmer O et al. 1994). The PCR contained a final concentration of 0.2 mM dNTP, 2.5 mM MgCl2, 0.4 uM of each primer, 1X buffer, Taq polymerase (Promega) and 1 ul of template DNA for a final volume of 25 ul. Reactions were run on a Mastercyler Gradient thermocycler (Eppendorf) following the conditions specified in Folmer et al. (1994) and amplicons were visualized in 1% agarose gel. Successfully amplified samples were Sanger sequenced (F verse) by Eton Bioscience Inc. (Raleigh, NC, USA) and results were queried using the NCBI BLAST algorithm tool (National Center for Biotechnology Information) and BOLD (Barcode of Life Data System) to identify the specimen to the species level. Species identity was assigned if the sequence with the highest percent identity had a value of 96% query cover or above (Jedlicka et al. 2013).

3.3.4 Bioinformatic analysis

Within the QIIME 2 2020.6 environment, tagged feces sequence reads generated from the Illumina MiSeq sequencer were demultiplexed and primers clipped to create fastq files (Bolyen et al. 2019). We then used the DADA2 pipeline for further downstream analysis, which created a table of amplicon sequence variants (ASVs) rather than traditional operational taxonomic units (OTUs), thereby improving reproducibility, comprehensiveness, and accuracy (Callahan et al. 2016). Potential contaminants in the ASV table were identified by the package Decontam (Davis et al. 2018). As the metabarcoding workflow introduces quantitative bias into results, ASV raw counts were transformed into a presence/absence matrix (Martoni et al. 2022).

ASVs were taxonomically classified by aligning sequences to those within the arthropod training database "tidybug" (O'Rourke et al. 2020) via the classy-sklearn naïve Bayes method implemented in QIIME 2's q2-feature-classifier plugin. The full QIIME script can be found in Appendix 3.2. Each taxonomic assignment was further examined individually using the following protocol: 1) The geographic range of the assignment was assessed, and species that do not occur in Europe were removed. 2) Species considered to be rare in Europe but are not known to occur in the Pyrenees were flagged. 3) Flagged assignments were verified by submitting query sequences to the NCBI BLAST tool and assignments that did not score at or above 98% identity were removed. All single flagged species composed of multiple ASV sequences were aligned to check for sequencing error, and sequences above an 80% sequencing error were kept in the analysis (Brandt et al. 2021; Ritter et al. 2022). 4) If an ASV showed multiple hits with the same max score on the NCBI BLAST tool, the ASV was removed from the analysis. Each separate taxonomic classification is referred hereafter as a "MOTU," or a molecular operational taxonomic unit (Powers et al. 2011).

Finally, the level of uncertainty (ratio of bias) according to taxonomic rank was calculated by comparing the taxonomic assignments given to the mock community ASVs by metabarcoding and Sanger sequencing/morphological assessment. We discarded orders and classes not successfully extracted and therefore not added to the composition of the mock community, including Arachnida. Therefore, only MOTUs in Coleoptera, Diptera, Hemiptera, Hymenoptera, Orthoptera, and Lepidoptera were kept in the analysis.

3.3.5 Statistical analyses

A Wilcoxon-Mann-Whitney test was performed to test for correlations between open space and elevation, using the percent of open space as a dependent variable. We calculated mean prey richness of all bird species and of the top two commonly collected bird species. Predictive roles of independent factors (season, open space, and elevation) affecting prey richness of these bird groups were calculated by fitting data to a negative binomial or Poisson model (GLMM) using the lme4 v.26 package (Bates et al. 2015) in R Version 1.3.1056 (R

Core Team 2021). Models were chosen through a combination of 1.) residual plotting with the DHARMa package (Hartig 2022); 2.) model performance testing using Pearson, Kendall, and Spearman correlation coefficients; and 3.) model accuracy evaluation by measuring the root mean square error and the mean-absolute deviation of each model. Plot was used as a random variable. Some models, however, did not accept a random factor and were thus run without one after numerical testing showed little differences between models with random factors and those without. After the richness model of each bird group was fit, post-hoc Tukey tests were carried out to investigate error rates of the categorical factor of season. Comparison of beta diversity (i.e. dissimilarity of diet) among and within bird species was determined by a Jaccard dissimilarity matrix using the R vegan package, with a value of 1 indicating there were no shared MOTUs and a value of 0 indicating complete sharing of MOTUs (Oksanen et al. 2020). Frequency of occurrence (number of times a prey item appeared in a fecal sample, divided by total number of samples) was calculated for each Insecta order. Predictive roles of season, open space, and elevation affecting MOTU richness within each prey family were calculated using the same model selection process as bird groups. Sample coverage was examined using the iNEXT package to create sample size-based rarefaction curves and extrapolation curves (Chao and Jost 2012; Chao et al. 2014).

3.4 Results

8.95 million sequence reads were produced in the feces samples, with ASV counts per feces sample ranging from 4 to 268999 (Appendix 3.3). ASVs were taxonomically classified as the MOTUs of 714 arthropod classifications, and then only MOTUS classified to genus or species were kept for a total of 594 arthropod MOTUs. Removing orders and classes not successfully extracted and therefore not comprising a section of the mock community lowered the MOTU count to 494 MOTUS.

3.4.1 Inter and intra-species dietary richness of Passerines

Fecal samples were collected and analyzed from 14 bird species (Table 3.1). *Periparus ater* (L., 1758) (coal tit), L*ophophanes cristatus* (L., 1758) (crested tit), and *Prunella modularis* (L., 1758) (dunnock) accounted for 69% of the bird species from which samples were collected. GLMM results showed that the richness of prey excreted by *P. ater* combined rose significantly from spring to fall (est. -0.65, Tukey test p-value: 0.04) (Fig. 3.1, Appendix 3.4). No significant seasonal differences were found when all bird species were combined (P values in Appendix 3.4). No captures of *P. modularis* were made in fall. Seventy-three and 59 birds were caught in the high elevation and low elevation plots, respectively.

The mean prey richness per bird capture was 11.5±5.7 MOTUs. *Phoenicurus ochrurus* (S. G. Gmelin, 1774) (black redstart) displayed the highest MOTU richness per bird capture, followed by *Sylvia atricapilla* (L., 1758) (blackcap) and *P. modularis*, although standard deviation bars do not show differences within these three species (Fig. 3.2).

According to Wilcoxon-Mann-Whitney tests, the percent of open space was significantly higher in the plots at tree-line (mean: 88±13.7) than the plots below tree-line (mean: 45.2±17.5) (Z = 4.25, P<0.001). However, GLMM results showed that the percentage of open space did not affect prey richness in the diets of *P. ater* and *L. cristatus*, or when all bird species were combined (Beta estimates and P values in Appendix 3.4). There were no elevational differences in prey richness when all bird species were combined, when *P. ater* and *L. cristatus* were combined, or when *P. ater* and *L. cristatus* were examined separately (Appendix 3.4).

3.4.2 Inter and intra-species dietary overlap of Passerines

Jaccard dissimilarity index showed very little overlap in the diet between and within bird species, and there was a mean dissimilarity of 0.90±0.05 in the diet of the 14 bird species. Beta diversity was very high among species. Compared to each other, *P. modularis /L. cristatus* and *P. modularis /P. ater* had a higher rate of dissimilarity (0.85, 0.81 respectively) than the rate between *P. ater /L. cristatus* (0.73). The dietary variability within *P. ater*, *L. cristatus*, and *P. modularis* was high as well: *P. ater* (0.81±0.02), *L. cristatus* (0.93±0.06), and *P. modularis* (0.92±0.05). Accordingly, sample size-based rarefaction curves indicated it would be necessary to capture over 100 more *P. ater* individuals than *P. modularis* and *L. cristatus* to reach 99% sample coverage (Fig. 3.3a). At 99% coverage, *P. modularis* is expected to have a higher diversity of diet than *P. ater* and *P. cristatus*, while *P. ater* is expected to have the lowest (Fig. 3.3b). The mean overlap among all individual birds captured was not different between the three seasons (spring, 0.94±0.06; summer, 0.93±0.07; fall, 0.92±0.07) (Appendix 3.5a). Similarly, when the mean overlap between the two most captured birds (*P. ater* and *L. cristatus*) was calculated by season, no difference in overlap was recorded (spring, 0.92±0.08; summer, 0.90±0.09; fall, 0.90±0.08) (Appendix 3.5a). The mean overlap between all birds and between *P.* ater and *P. cristatus* in the plots at tree-line and below tree-line were similarly low (see Appendix 3.5b).

3.4.3 Presence of MOTUs and MOTU trends

Most MOTUs were rare; over 60% of the MOTUs were collected only once (i.e. collected in one sample) (Table 3.2). However, eleven MOTUs were present in over 15% of samples (Appendix 3.6). Of these eleven MOTUs, seven were conifer pests. Aphid conifer pests (Hemiptera) were the two MOTUs most likely to be present (Appendix 3.6). Diptera and Lepidoptera represented 33% and 22% respectively of all taxonomically classified MOTUs (Fig. 3.4). GLMM results indicated that richness seasonality was varied among families, and elevation and open space did not drive richness of insect orders (Beta estimates, P values, and seasonality Tukey tests in Appendix 3.7).

Arachnida accounted for 12.5% (73 MOTUs) of the total number of MOTUs recovered in the feces, and a further 2.9% (17 MOTUs) were other arthropod species not

included in mock community. These MOTUs were removed from our results because we had no percent uncertainty for which to compare. The comparison between taxonomic assignments given to the mock community samples by metabarcoding and a combination of morphological assessment with sanger sequencing displayed a high percentage of uncertainty in some orders. For example, mock community results displayed a 16.67% uncertainty in the assignment of Coleoptera to order, an additional 33.3% uncertainty in the assignment of Coleoptera to family, an 83.33% uncertainty in assignment of Hemiptera to family, and a 35.71% uncertainty of assignment of Lepidoptera to order. The mock community results showed a lower uncertainty (13.33% and 17.65%) in assignment of Hymenoptera and Diptera to family.

3.5 Discussion

The difficulty in accurately defining species-level richness and identifying dietary components of adult insectivorous Passerines in a non-lethal manner is a quandary that has long confounded ornithologists. Using metabarcoding to study the composition of Passerine feces is a promising technology that can address this previously unfeasible task. With this technique, we were able to determine that: 1) There was extremely high variability in the diet of captured Passerines, a result that contrasts with many Passerine nestling studies; 2) The most captured bird species displayed higher dietary richness in the fall; 3) Open space did not affect dietary richness, and there was no difference in dietary overlap relative to open space or elevation which suggests that high elevation Passerine birds have high dietary mobility; 4) Composition of passerine feces was dominated by conifer pests. Even though metabarcoding is transforming dietary studies, we found a high percentage of uncertainty in the taxonomic classification of ASVs, suggesting taxonomic inferences may be problematic.

There was high biological richness in our analysis of the 132 fecal samples: over 594 taxonomically classified arthropod MOTUs were identified. Despite a mean±SE MOTU richness per bird capture of only 11.5±5.7, the dietary overlap among and within bird species was very low. We expected lower overlap (higher niche differentiation) between closely related bird species as it is well established that segregated foraging behavior occurs between closely related European Passerines that are insectivorous and hole-nesting. When a potential niche is left unoccupied by a Passerine bird species, the species that most resembles the absent species in body size is the species most likely to fill it [for a review of geographic niche changes in insectivorous hole-nesting Passerines, see Alatalo R. V. et al. (1986)]. Segregated foraging behavior makes sense in light of a study showing negative impacts upon a less dominant but closely related species sharing geographic space; *Parus major* (L. 1758) (great tit) nestlings raised sympatrically with *C. caeruleus* weighed less than those raised allopatrically, suggesting that a large overlap of resource utilization exists between the two closely related species (Torok and Tóth 1999). Most dietary studies comparing insectivorous hole-nesting Passerines (mainly some combination of *P. ater*, *P. major*, *L. cristatus*, and *C. caeruleus*) have historically examined nestling diets and reported a high overlap when dietary components are classified

to a combination of class and family (Nour et al. 1998; Michalski et al. 2011; Grzędzicka 2018). One study that classified Passerine prey of Lepidoptera and Arachnida to species also found high overlap (Atiénzar et al. 2013). However, studies that have examined dietary overlap in metabarcoding have encountered low overlap among and within European Passerines that are insectivorous and hole-nesting (Rytkönen et al. 2019; Shutt et al. 2020). Shutt et al. (2020) postulated that the high intraspecific dietary variation in *C. caeruleus* was likely due to prey availability and dietary flexibility. However, at least two studies have shown *P. major* and C. *caeruleus* feed differing sizes of caterpillar prey to nestlings (Torok and Tóth 1999; Ceia et al. 2016), a result that would not be observable in a DNA-based study such as ours. In Ceia et al. (2016) the authors showed composition of prey (classified to family) between the two bird species were similar, and they postulated that difference in prey size resulted from either interspecific competition between *P. major* and *C. caeruleus* or the segregation of bird foraging guilds; *C. caeruleus* is primarily a foliage-gleaner and more likely to come in contact with smaller instar caterpillars, while *P. major* are bark–foliage gleaners and therefore more likely to come in contact with later instar caterpillars.

While inter- and intraspecies overlap was very low in our study, the Jaccard dissimilarity indexes displayed slightly higher index values between *P. modularis*/*L. cristatus* and *P. modularis*/*P. ate*r than between the more closely related *P. ater* and *L. cristatus*. These results are likely due to diverging foraging habits. *Periparus ater* and *L. cristatus* both forage in trees (Alatalo Rauno V. 1981; Hartley 1987; Lens 1996) while P. modularis are mainly ground feeders (Bishton 1986).

Historical data reports divergent timing of clutch laying of closely related insectivorous hole-nesting Passerines (Sanz et al. 2010), and a more recent study reported that many Passerine nestlings are provisioned with differing prey types depending on the nestling's development stage (Orłowski et al. 2017). Historical data also suggest that resident insectivorous hole-nesting Passerines in many temperate forests are less segregated in both foraging sites and dietary components in summer when insect prey is more abundant. Insect prey in fall and winter is less abundant, leading to resource partitioning and interspecies competition (Gibb 1954; Betts 1955; Lister 1980). There has been disagreement over seasonal segregation and diet. For example, Ulfstrand S. (1977) found special segregation in summer compared to fall, whereas Wagner (1981) and Almeida and Granadeiro (2000) found no significant seasonal spatial differences. Obeso (1987) found no spatial difference but did find significantly different dietary components. Finally, a recent study found high dietary overlap between communities of insectivorous Passerines during times of limited insect availability, in contrast to many studies that show high dietary overlap during times of high resource availability. The researchers postulated this result indicated that during times of very low food availability, bird species were unable to avoid competition (Kent et al. 2022).

Our results did not show dietary differences among all individual birds captured across the three seasons. However, we did find increased dietary richness in our most commonly

caught bird, *P. ater*, from spring to summer. Richness of *L. cristatus*, the second most commonly caught bird species, increased from spring to summer and summer to fall, however these differences were not significant after post hoc Tukey tests were administered (Appendix 3.4). Our seasonal results are similar to the findings of an adult barcoding study done in a deciduous forest (Shutt et al. 2020). In that study, Shutt et al. (2020) linked seasonal dietary richness of *C. caeruleus* to rising herbivorous insect abundance and availability. Although few studies have examined seasonal richness trends of general insect diversity in conifer forests in the Pyrenees, studies examining montane and/or northern-European distributed Coleoptera associated with bark beetles (Scolytinae) found maximum densities in September (Tykarski 2006). These results (lack of seasonal prey overlap among bird species, increased dietary richness of *P. ater* from spring to summer, and no difference in richness levels among all bird species) likely indicate that the birds captured in our study have a high level of dietary flexibility, and Passerine dietary richness follows availability and abundance of prey.

We expected open space to affect dietary richness levels of ground and shrub foraging species, such as *P. modularis*, as structure and composition of vegetation can be very influential in nestling success of some hole-nesting Passerine species (Orłowski et al. 2017). We also expected higher dietary overlap in plots below tree-line; while abundance and diversity of insects is species specific (Hodkinson 2005), many montane fauna either decrease with elevation or have a humped shaped distribution along an elevational gradient (Rahbek 2005; McCain 2009). Temperature is known to be a major driver in insect community structure (Bale et al. 2002), and temperature swings are wider at higher elevations in the Pyrenees (Navarro-Serrano et al. 2020). Therefore, insects are likely more abundant in plots below tree-line, and thus insectivorous Passerines might be less segregated at these lower elevations. However, in our study percent of open space and/or elevation had no effect upon the richness of the bird diets, or when the most common birds were examined separately. While more birds were caught in plots below tree-line than in plots at tree-line, the diet composition within both these groups showed low overlap, i.e., the diet among birds in plots at tree-line had as much overlap as the diet of birds in plots below tree-line. More data would be needed to document and compare the diet of each 14 bird species we studied, but this lack of link between open space and elevation may indicate high mobility of the more common generalists that occupy high elevation Pyrenean landscapes.

Our study was performed in *P. mugo* forests, so it is unsurprising that bird diets from this habitat produced conifer aphids (Hemiptera) in six of the ten insect MOTUs. Over 53% of the samples contained *Cinara pini* (L., 1758) and 37% contained *Eulachnus rileyi* (Williams, 1911) (Appendix 3.6). *Cinara pini* is a common and native conifer pest in Europe. *Eulachnus riley*, however, is considered rare to uncommon in its native range in Europe and is considered a pest outside its native range (Blackman and Eastop 1994), so it is interesting to find this species to be common in our study. Even though, as a group, the hole-nesting European Paridae are some of most intensely studied birds in the world (Gibb 1954; Betts 1955; Lack 1964; Ulfstrand Staffan 1976; Cowie and Hinsley 1988), data are limited relative

to their adult diets. However, the abundance of aphids in adult diets in our study is similar to two other studies: Shutt et. al (2020) reported aphids comprised three of the top ten insect MOTUs and had the highest presence incidences, and Betts (1955) found aphids comprised over 50% of the adult diet of three species of hole-nesting European Paridae in June. Birds play important roles in top-down control of forest arthropod populations (Gunnarsson 1995, 1996; Philpott et al. 2004; Fayt et al. 2005; Schwenk et al. 2010). It is possible the Passerines in our study are shaping arthropod communities and causing a trophic cascade by affecting tree growth. Research examining trophic cascade affects by bird predation, however, have revealed complex interactions or mixed results (Gruner 2004; Schwenk et al. 2010).

Our taxonomic classifications given to MOTUs should be tempered with our mock community comparisons. Hemiptera, in particular, had a high percent uncertainty (83.33%) in assignment to family. This could be a result of the low number of Hemipteran species (two) in our mock community (Appendix 3.1), but it is also possible that some of our taxonomic classifications are inaccurate. Sequencing a mock community of likely or known components alongside the sample of interest is one of the only ways to benchmark taxonomic validity of metabarcoding studies (Braukmann et al. 2019; Elbrecht et al. 2019). However, numerous studies of Passerine diet have not utilized them (Jedlicka et al. 2017; Ribeiro et al. 2019; Rytkönen et al. 2019; Shutt et al. 2020). Despite the uncertainty, we still believe our results are of interest, as we do not know the level of taxonomic certainty of previous studies.

Regardless, we now know many high elevation species are under pressure (Öztürk et al. 2015), and alpine Passerines such as *Anthus spinoletta* (L. ,1758) (water pipit) are precipitously declining in some areas (Flousek et al. 2015). We caught only one specimen of *A. spinoletta*; the remainder of our species are elevational generalists and not confined to high elevation. While our data does not shed light on alpine specialists, the lack of differences in dietary overlap and diversity relative to open space and elevation, not to mention the extremely high levels of intra-species dietary overlap, suggest that adult diet may not be a constraining factor in populational growth of some generalist insectivorous hole-nesting Passerines in and around the Pyrenean tree-line. At least one European generalist insectivorous Passerine bird seems to display extreme plasticity in timing of egg-laying (Wesołowski et al. 2016)], and other ecological requirements such as suitable nesting sites and the provisioning needs for nestling could be more plausible population constraints. The upward migration of the tree-line in the Pyrenees, a phenomenon likely caused by both land-use shifts and climate change (Batllori and Gutiérrez 2008; Batllori et al. 2010), may therefore not be a significant factor affecting the diet of some adult generalist insectivorous Passerines.

Much remains to be discovered regarding the diets of adult European insectivorous Passerines (Cholewa and Wesołowski 2011). In the future, metabarcoding will undoubtably continue to elucidate the relationship between birds, insects, and landscape and has the potential to reveal vast quantities of dietary data. Our results showed very high prey diversity and very little overlap within and among hole-nesting Passerines. Spatial trends (open space

and elevation) had little effect on prey diversity and overlap. While these data indicate that the dietary plasticity of the more common birds is high, more studies are needed to reveal dietary components of rare species, such as *A. spinoletta*.

3.6 Figures

Fig. 3.1 MOTU richness of *Periparus ater* bird capture over season. Plot made with R version 1.3.1056 and MS Office

Fig. 3.2 MOTU richness per bird capture with standard deviation bars. Plot made with R version 1.3.1056 and MS Office

Fig. 3.3 Sample size-based rarefaction curves (solid line) and extrapolation curves (dotted line) with 95% confidence intervals. a) Number of birds caught per sample coverage. Numbers in parentheses indicate bird species and number of individual birds caught at 99% sample coverage. To reach 99% coverage, it would be necessary to capture over 100 more *P. ater* individuals than *P. modularis* and *L. cristatus*. b) Prey diversity of bird species per number of individual birds sampled. Numbers in parentheses indicate number of captures of birds per species necessary to reach 99% coverage, and insect diversity at 99% coverage with 95% confidence intervals. *Prunella modularis* is expected to have a higher diversity of diet at 99% sample coverage while *P. ater* is expected to have lowest diversity. Plot made with R version 1.3.1056 and MS Office

Fig. 3.4 Relative species richness by order, i.e. number of MOTUs classified to species belonging to the Insecta orders of Coleoptera, Diptera, Hemiptera, Hymenoptera, Orthoptera, and Lepidoptera as a percentage of total number of MOTUs classified to species. Plot made with R version 1.3.1056 and MS Office
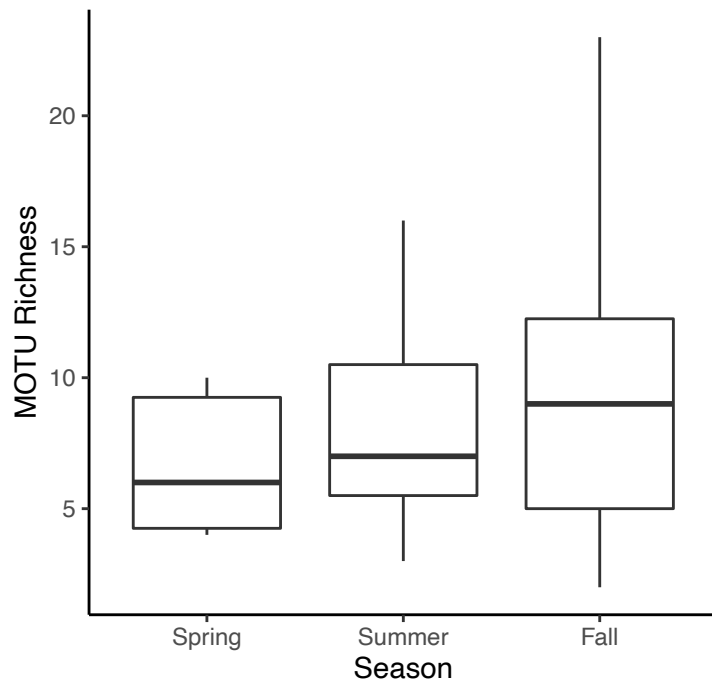
Fig. 3.1
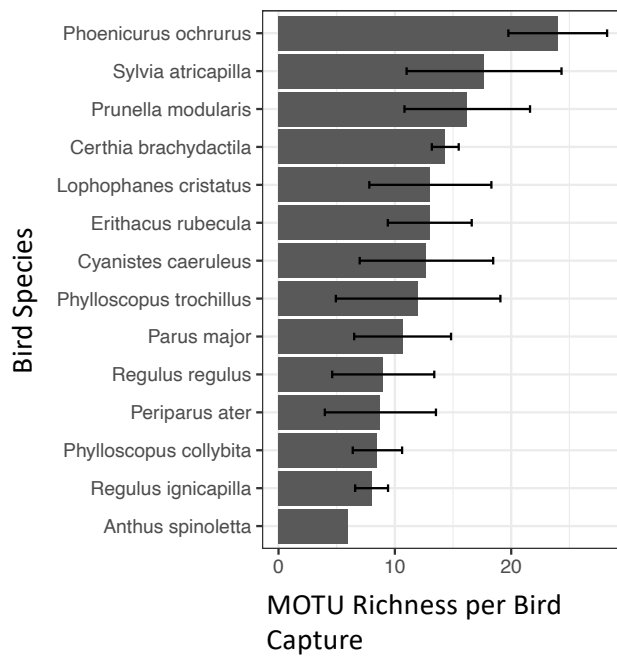
Fig. 3.2
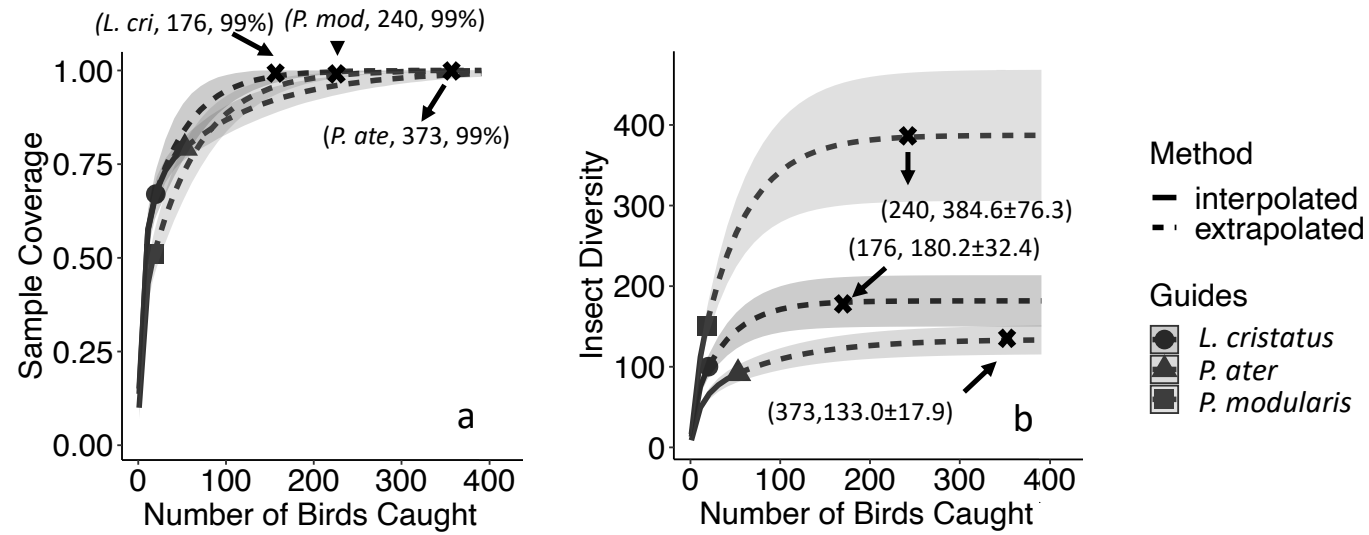
Fig. 3.3
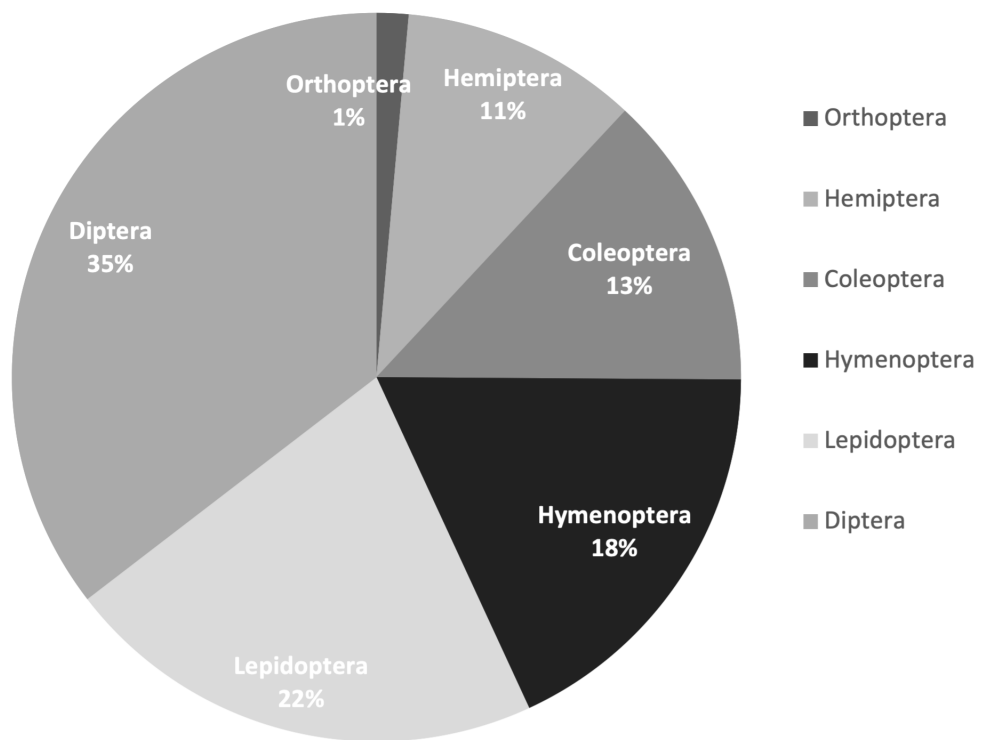
Fig. 3.4

3.7 Tables

Table 3.1 Number of Passerines caught.

num. captures	Scientific name	Common name
53	*Periparus ater* (L., 1758)	coal tit
20	*Lophophanes cristatus* (L., 1758)	European crested tit
18	*Prunella modularis* (L., 1758)	dunnock
9	*Regulus regulus* (L., 1758)	goldcrest
7	*Cyanistes caeruleus* (L., 1758)	blue tit
7	*Erithacus rubecula* (L., 1758)	European robin
3	*Certhia brachydactyla* Brehm, 1820	short-toed treecreeper
3	*Parus major* L., 1758	great tit
3	*Sylvia atricapilla* (L., 1758)	Eurasian blackcap
2	*Phoenicurus ochrurus* (S. G. Gmelin, 1774)	black redstart
2	*Phylloscopus collybita* (Vieillot, 1817)	common chiffchaff
2	*Phylloscopus trochillus* (L., 1758)	willow warbler
2	*Regulus ignicapilla* (Temminck, 1820)	common firecrest
1	*Anthus spinoletta* (Linnaeus, 1758)	water pipit

Table 3.2 MOTU Distribution among bird feces collections. Number in parentheses is tally of an individual MOTU recorded among the bird feces collection. For example, of the 494 MOTUs classified in the study, five (or 1.01% of all MOTUs recorded) were recorded in six feces. Most MOTUs were rare; 58.91% of the MOTUs (or 291) were recorded in only one feces collection.

Number of Feces Recorded	(Number of MOTUs) % of MOTU Recorded
1	(291) 58.91
2	(85) 17.21
3	(37) 7.49
4	(18) 3.64
5	(5) 1.01
6	(5) 1.01
7	(9) 1.82
8	(4) 0.81
9	(5) 1.01
10	(5) 1.01
11	(5) 1.01
12	(3) 0.61
13	(4) 0.81
14	(2) 0.4
16	(2) 0.4
18	(1) 0.2
19	(2) 0.4
21	(1) 0.2
22	(1) 0.2
23	(2) 0.4
25	(1) 0.2
26	(1) 0.2
33	(1) 0.2
37	(1) 0.2
39	(1) 0.2
50	(1) 0.2
70	(1) 0.2

3.8 Acknowledgements

3.9 References

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CHAPTER 4

THE COLEOPTERA COMMUNITY AT TREE-LINE IS EXPLAINED BY DIVERGENT DRIVERS: TAXONOMIC AND FUNCTIONAL GUILD APPROACHES

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4.1 Abstract

1. Mountain species are on the forefront of climate change disruption, and montane saproxylic Coleoptera are facing large- and small-scale changes in their surroundings. Saproxylic Coleoptera are both functionally and taxonomically diverse and are representative

of an imperiled fauna confronted with the realities of a changing landscape. Understanding the effects of elevation and other forest characteristics on saproxylic and non-saproxylic Coleoptera is a step toward predicting the future of functional group and taxonomic biodiversity at tree-line and on mountains.

2. The objective of this study was to examine the effect of elevation and other forest characteristics on the biodiversity of montane Coleoptera at tree-line using both taxonomic and functional feeding guild classifications.

3. Our results suggest that abundance of saprophytes is closely linked to density of large trees rather than volume of wood. Edge effects and elevation seem to drive abundance patterns of some species and also influence taxonomic and functional guild community patterns differently. Finally, we discuss the implications of climate change and land abandonment to future Coleoptera community structure.

4.2 Introduction

The decline of insects in the last 50 years is well-documented (Kotze & O'Hara 2003; Hallmann *et al.* 2017; Wendorff & Schmitt 2019), driven at least in part by climate change and loss of habitat (Wagner *et al.* 2021). Montane insect species in particular are in peril (Dirnbock *et al.* 2011; Sánchez-Bayo & Wyckhuys 2019) and climate change can trigger a loss of habitat. Studies have shown contractions of lower elevation ranges may not correspond to an upward shift of higher elevational ranges (Moret *et al.* 2016; Dahlhoff *et al.* 2019). Other montane insects may "run out of mountain," i.e., there may be no habitable terrain above where they currently exist that is available for colonization (Wilson *et al.* 2005; Dieker *et al.* 2011). Montane saproxylic Coleoptera, or beetles that depend in some part of their lifecycle on dead or dying wood (*sensu* Speight 1989) and are of particular interest because they are ecologically important and taxonomically and functionally diverse (Nieto & Alexander 2010). Saproxylic Coleoptera not only play important roles in nutrient recycling, they also include many feeding guilds, including predators, parasites, fungivores, detritivores, myxomycophages (slime mold feeders), wood-consumers, and omnivores (Gimmel & Ferro 2018). Saproxylic Coleoptera are often used as biodiversity indicators for wider forest ecosystem functioning (Burns *et al.* 2014; Karpiński *et al.* 2021). Therefore, understanding the spatial dynamics of montane saproxylic Coleoptera communities at tree-line is fundamental to forecasting the change in biodiversity patterns in a shifting landscape.

Following the trend of many montane flora and fauna (Rahbek 2005), Coleopteran biodiversity generally decreases with increasing elevation (Franc *et al.* 2007; Corcos *et al.* 2018) or displays a humped shaped distribution along an elevational gradient (Tykarski 2006). Coleoptera biodiversity can increase with increasing elevation, but this is rare (Dolson *et al.* 2021). These trends vary among taxonomic and functional groups and across geographic areas and depend on spatial scale and elevational gradient range (Colwell *et al.* 2004; McCain

2009; Chamberlain *et al.* 2016). Numerous variables are known to drive community structure along an elevational gradient, especially temperature, which can delay timing of flight and elongate life cycles of bark beetles and other herbivorous insects (Bale *et al.* 2002; Reymond *et al.* 2013). Rising temperatures are expected to shorten generation times of some pest bark beetles, such as *Ips typographus* (Linnaeus, 1758), at higher elevations (Jakoby *et al.* 2019). However, little is known about saproxylic Coleoptera community structure at tree-line, an important eco-tone.

In most mountains, the delineation between a forest margin and shrub-only terrain is a matter of scale, as canopies can open gradually or with a sharp transition depending on slope and other environmental factors (Holtmeier & Broll 2007). Tree-line is generally defined as the point in which the dominant stem of a tree no longer grows above 3m (Körner 2012) and in the last 100 years the tree-line delineation in some mountains has migrated upward (Harsch *et al.* 2009). The relationship between tree-line and climate change is difficult to untangle from other biotic and abiotic variables; soil temperature, local, current, and historic land use, and abiotic site conditions can all play a role in limiting tree growth at a specific elevation (Hofgaard 1997; Holtmeier & Broll 2005; Körner 2012). Research shows that the rise in the tree-line in the Pyrenees mountains is likely influenced more strongly by land abandonment rather than climate change (Batllori & Gutiérrez 2008; Ameztegui *et al.* 2016). There is little debate, however, that the eastern Pyrenean tree-line is migrating upward and the population of the dominant tree at the Pyrenean tree-line, *Pinus mugo* Turra, has become denser and less patchy over the last 50 years (Batllori & Gutiérrez 2008; Batllori *et al.* 2010), although these two spatial phenomena are driven by different factors (Feuillet *et al.* 2020)

In this work we examined taxonomic and functional saproxylic and non-saproxylic Coleoptera community responses to stand and landscape characteristics at tree-line and 200-300 meters below tree-line in a forest in the eastern Pyrenees. Other studies have examined saproxylic Coleoptera community responses to stand level characteristics in Mediterranean mountains (Parisi *et al.* 2020), Scandinavian forests (Gibb *et al.* 2006; Brunet & Isacsson 2009a, b), boreal Canadian forests (Saint-Germain *et al.* 2006), and the Swiss Alps (Schiegg 2000, 2003), but to our knowledge, this is the first study to examine saproxylic Coleoptera community responses to landscape and stand characteristics specifically at tree-line. In this study, we expected the following outcomes: 1) Abundance and richness of saprophytes and saproxylic Coleoptera closely linked to the volume of dead wood and large trees and 2) forest characteristics that are related to higher levels of sunlight, volume of dead wood, and density of larger trees predict higher taxonomic abundance, taxonomic richness, functional feeding guild abundance, and functional feeding guild richness. This study was conducted as part of a larger research program monitoring climate change in high elevation Andorra (Bookwalter *et al.* 2022)

4.3 Methods

4.3.1 Study area and sample collection

The study spanned one year in a 20km2 section of Vall d'Ordino, Ordino Parish, Principality of Andorra and included Vall d'Ordino and Vall de Sorteny Natural Park. We selected paired 0.1km2 plots at five locations (N = 10 plots) in black pine (*P. mugo*) forest and installed seven traditional insect traps (three attraction, three flight intercept, and one malaise) in each plot (Fig. 4.1).

Each attraction trap consisted of a 1L plastic soda bottle with a single 3cm diameter hole in the side, hung upright from a tree branch and positioned 30cm from the trunk. The soda bottle was filled to just below the hole with ~250g of a bulk bait mixture containing the following ratio: 7L sangria (Don Simon):2L peach juice (Spar):1kg salt: 1kg sugar (Viñolas et al. 2009). Flight intercept traps consisted of two transparent laminate plastic PVC panes perpendicularly crossed below a 14cm diameter white hard plastic disk attached to a 13cm diameter white plastic funnel. Each white malaise trap measured 120 x 100 x 150cm (Entosphinx S.R.O). A collection bottle containing 70% propylene glycol (VWR Chemicals) and a few drops of dish detergent (Fairy) (to lower surface tension) was attached to each flight intercept and malaise trap. Hymenoptera and Diptera are traditional targets of malaise traps (Karlsson et al. 2020) but Coleoptera can be captured as well (Skvarla & Dowling 2017). Traps were suspended 1.8 to 1.9m above the ground and were spaced at least 30m apart within each plot. Traps were installed May 23-28, 2017, and their contents removed and baits refilled every 13-15 days until September 30-31, 2017. All specimens captured in the traps were kept in 70% ethanol until processed and deposited in the Museu de Ciències Naturals de Barcelona.

For each pair of plots, one was established at the elevation that generally is associated with tree-line and the other was established well below tree-line. To the degree possible, each tree-line plot was directly upgradient from its paired below tree-line plot. Distance between the two plots that formed a pair to other pairs of plots ranged from 430 to 1000m. Plots at tree-line ranged from 2055masl to 2217masl and plots below tree-line from 1719 to 1998masl. In Andorra, tree-line is considered to be positioned from 2200 to 2400masl, with local isolated boundaries from 2100 to 2500masl (Carreras et al. 1996).

At each trap location, percentage of open space (1k radius), aspect, and slope were calculated using QGIS3.4 and the MCSA 2012 landcover map downloaded from the Institute of Andorran Studies (Centre de Biodiversitat de l'Institut d'Estudis Andorrans 2012). Leaf Area Index (LAI) at each trap location was measured with an AccuPAR PAR/LAI ceptometer LP-80 (METER Group). Basal area, volume of dead wood above 7.5cm diameter, and density of live *P. mugo* at or above 30cm diameter breast height (dbh) (medium tree density), and 50cm dbh (large tree density) were measured at each trap location (Table

4.1). Volume of dead standing stems above 30cm diameter within a 20m radius of trap were initially calculated but we chose to discard this variable due to extremely low inventories. Percentage of open space was considered a landscape-scale forest characteristic, and all the other forest characteristics were considered stand-scale.

4.3.2 Species identification

All Coleoptera specimens were morphologically identified to the species level (list of experts involved in identification in Table 4.2) with the exception of Scydmaeninae. Morphotypes of Staphylinidae were sent to a Staphylinidae expert for species identification and were assigned to functional larval and adult guilds based on the literature currently available regarding each species' lifecycle as well as the FRISBEE database (Bouget et al. 2008). Species were assigned to one of three functional groups: phytophage, saprophyte (including wood-feeding, detritivore, and fungivore), or predator (including parasitoid). Taxa collected in this study, including status as saproxylic versus non-saproxylic and larval and adult guild, are listed in Appendix 4.1.

4.3.3 Statistical analyses

Median species diversity, richness, and abundance (total number of individual specimens caught per collection per trap) among plots at tree-line and below tree-line were calculated for two groups of Coleoptera: saproxylic and non-saproxylic species combined (hereafter referred to as "combined Coleoptera") and saproxylic only species (hereafter referred to as "saproxylic Coleoptera"). To understand which forest characteristics drive species richness and abundance, models were fit to a generalized linear mixed-effects model using the glmmTMB package (Brooks et al. 2017) in R (R Core Team 2021) using zero-truncated poisson, zero-truncated generalized poisson, or zero-truncated non-binomial depending on residual plots created by the DHARMa package (Hartig 2022). Model performance was tested by calculating the Pearson, Kendall, and Spearman correlation coefficients. Model accuracy was evaluated by measuring the root mean square error and the mean-absolute deviation of each model. Eight landscape and stand explanatory variables listed and described on Table 4.1 were used as explanatory factors. Moreover, a visual inspection of the datasets revealed that month had a parabolic-shaped response for abundance and richness, which led us to also include month-squared as a predictor (in R syntax, I(Month2)) in those analyses. These landscape and stand variables were chosen as they are characteristics often found to be influential in saproxylic species distributional patterns (Schiegg 2000; Müller & Bütler 2010; Thorn et al. 2016; Oto et al. 2022). Plot was inserted as a random factor. Potential multicollinearity between explanatory variables was tested using the both cor() function of the R package corrplot (Wei & Simko 2021) and the collin.diag() function of the R package misty (Yanagida 2022). The results of these analyses examining species richness and abundance of combined Coleoptera and saproxylic

Coleoptera are referred to as "taxonomic classification" results. The mean and standard error of the top five most abundant Coleoptera species are plotted in Fig. 4.2.

The above analyses were also performed on Coleoptera species partitioned into functional feeding guild groups, using guild richness and abundance as response variables, and forest characteristics, month, and trap type as explanatory variables. In the models examining richness and abundance of secondary consumers (parasitoids combined with predators, hereafter referred simply as predators), the richness or abundance of primary consumers was also tested as an explanatory variable, as primary consumers can serve as prey or host (Caballero-López et al. 2016). When species exhibited different guild behavior in larval and adult stages, abundances of that species were counted into both guilds, following Caballero-López et al. (2016). The results of these analyses are referred to as "functional feeding guild classification" results.

Differences in forest characteristic variables consisting of continuous values were examined using Wilcoxon-Mann-Whitney tests following methods performed in Parisi et al. 2020. To examine similarity among these continuous variables, a Principal Component Analysis (PCA) was also performed on the data using the R package ggfortify (Tang et al. 2016).

4.4 Results

4.4.1 Forest characteristic variables

Forest characteristic variables varied between the two elevations, with slope, percent of open space, and medium tree density comprising the largest differences (see r values, Table 4.3). Basal area and medium tree density were among the variables that were larger in plots below tree-line. Slope was steeper and plots were more open at tree-line. There was no difference between volume of dead wood between the two elevations (Table 4.3). These results are reinforced by a PCA displaying 1) basal area and open space were strongly negatively correlated and 2) higher elevation plots tended to be more open (Fig. 4.3). No multicollinearities between forest characteristic variables were found.

4.4.2 Combined Coleoptera

A total of 8995 specimens was collected, representing 237 species (146 saproxylic) and 41 families (Appendix 4.1). 171 of these species found in this study were new records for Andorra. (112 of these records were published in Bookwalter *et al.* 2022, as the data in these studies partly overlapped). The three most abundant species were found in higher elevation plots, although error bars overlapped (Fig. 4.2). Basal area and percent open were found to negatively affect abundance (est.: -0.61, P: 0.00; est.: -0.47, P: 0.01, respectively) while elevation positively affected abundance (est.: 0.57, P: 0.00) (Fig. 4.4). Elevation, basal area, dead wood volume, tree density, and percent open were not associated with combined

Coleoptera richness. Aspect was also found to influence abundance; however a boxplot did not show clear affiliation between abundance and a particular aspect level (Appendix 4.2). Levels of month and trap type were found to influence abundance and richness relative to their reference levels (see Table 4.4 for estimates and P values).

4.4.3 Saproxylic Coleoptera

Model results describing abundance and richness patterns of saproxylic Coleoptera were similar to combined Coleoptera. Two divergent results were found: 1) saproxylic Coleoptera showed a moderately positive dependence on elevation (est.: 0.34, P: 0.07), and 2) saproxylic abundance and richness depended negatively on medium tree density (est.: -0.48, P: 0.02; est.: -0.22, P: 0.04 respectively), unlike combined Coleoptera results (Table 4.4).

4.4.4 Functional feeding guild classification results

Similar to taxonomic abundance and richness results, month and trap type significantly explained variability in abundance and richness of phytophage, saprophyte, and predator Coleoptera (Table 4.5 and Table 4.6).

Higher abundance of phytophage Coleoptera was associated with higher levels of dead wood volume and large-tree density (est.: 0.31, P<0.0001; est.: 0.78, P: 0.01). Higher saprophyte abundance was also related to higher large-tree density (est.: 0.14, P: 0.03). Predator abundance depended positively on abundance of primary consumers (est.: -0.26, P<0.001) (Table 4.5).

Higher basal area and dead wood volume and lower medium tree density and elevation drove higher phytophage Coleoptera richness (est.: 0.06, P<0.001; est.: 0.05, P: 0.04; est.: -0.14, P: 0.00; -0.88, P<0.0001, respectively). Aspect significantly affected richness of phytophage and saprophyte Coleoptera. No forest characteristic besides aspect was significant in the saprophyte richness model. Finally, richness of predator families moderately and negatively depended upon dead wood volume (est.: -0.08, P: 0.07) and large tree density (est.: -0.13, P: 0.05), and depended positively upon richness of primary consumers (est.: 0.14, P<0.0001) (Table 4.6).

4.5 Discussion

Saproxylic Coleoptera are integral to a healthy ecosystem, and research in the last twenty years has focused upon describing habitat connectivity and relationships with forest variables at large and small scales (see Gibb *et al.* 2006; Saint-Germain *et al.* 2006; Brunet & Isacsson 2009a; Brin *et al.* 2011, 2016). The relationship between saproxylic Coleoptera functional and taxonomic diversity and forest variables at tree-line, however, is a topic both unexamined and pertinent, as land use shifts and climate change are driving abiotic and biotic transformations in mountains across the world. We found 1) saprophyte abundance

and density of large trees are closely linked, and dead wood correlations are not easily untangled, 2) edge effects and elevation drive abundances of some species, and 3) edge effects and elevation influence taxonomic and functional guild community patterns differently.

4.5.1 Abundance of saprophytes was more closely linked to density of large trees rather than volume of wood.

Large trees are a keystone feature in many habitats and can play an integral role in supporting forest biodiversity because they provide unique microhabitats (e.g., tree hollows, areas of dead wood within living trunks, and epiphytic lichens and mosses) (Hall & Bunce 2011; Lindenmayer *et al.* 2014). Numerous studies have linked large, veteran trees to Coleoptera abundance (Müller *et al.* 2014; Horak 2017; Ranius & Jansson 2000; Wetherbee *et al.* 2021), therefore it was unsurprising that saprophyte abundance was positively linked to large trees in our study.

The volume of dead wood is also known to be a predictor of saproxylic Coleoptera biodiversity (Karpiński *et al.* 2021), especially in cooler sites (Lachat *et al.* 2012), however it was not associated in our study with the abundance or richness of saproxylic Coleoptera or saprophyte functional group Coleoptera (Table 4.4). The lack of correlation between saproxylic Coleoptera and volume of dead wood agrees with studies that posit that this connection could be more muted and complex than previously hypothesized (Franc *et al.* 2007; Vodka *et al.* 2009; Lassauce *et al.* 2011). Furthermore, the volume of dead wood measured in our sites ($10.1\pm32.2m^3$ ha^{-1}) is lower compared to other European alpine coniferous forests ($26.0\pm5.7m^3$ ha^{-1}) (Puletti *et al.* 2019) as well as to other Spanish alpine coniferous forests ($21.04\pm30.50m^3$ ha^{-1}) (Alberdi *et al.* 2020). A volume of $10.1\pm32.2m^3$ ha^{-1} is probably lower than the dead wood thresholds needed to sustain many rare species. For example, Müller & Bütler (2010) found that an average threshold of 24 to >70 m^3 of dead wood was necessary to sustain a variety of saproxylic Coleoptera in a boreal Scandinavian coniferous forest. Some studies have suggested that because early-successional species like bark beetles can be highly mobile, larger volumes of dead wood within a 100m local might be less important than temperature when predicting abundance of saproxylic Coleoptera (Gibb *et al.* 2006). Finally, it is possible that spatial arrangement of the dead wood, or connectivity, is more important to saproxylic Coleoptera abundance than the total volume of dead wood, as suggested by Schiegg (2000).

Phytophage functional group abundance and richness, however, was driven by volume of dead wood. Other studies have found positive correlations between non-saproxylic Coleoptera groups and higher amounts of dead wood (Seibold et al. 2016). For example, an experiment in North American loblolly pine (*Pinus taeda*) forests found only positive correlations between volume of dead wood and ground beetles (Carabidae), a family with many non-saproxylic members. Similar to our study, no correlations between volume of dead wood and saproxylic Coleoptera as a group were found (Ulyshen & Hanula 2009).

Mechanisms that drive positive responses to dead wood by non-saproxylic Coleoptera and other arthropods include the addition of structural components, especially large logs. Large logs and other dead wood add structural and chemical complexity, surface area, and moisture refugia (Marra & Edmonds 1998; Castro & Wise 2010). Dead wood can also increase leaf litter, which can insulate animals including non-saproxylic arthropods from extreme temperatures (Langlands *et al.* 2011).

4.5.2 Edge effects and elevation seem to drive abundance of some species.

Higher levels of basal area and percent openness drove lower abundance of both combined Coleoptera and saproxylic Coleoptera. Taken together, these data could indicate association of some species of Coleoptera with edge effects. In other words, some species of Coleoptera are more successful in areas with higher landscape-level large scale tree coverage (i.e. lower percentage of openness) and lower stand-level smaller scale basal area. Forest edges have been found to harbor greater diversity and richness of saproxylic Coleoptera compared to closed, interior forest habitats (Wermelinger *et al.* 2007; Vodka *et al.* 2009)

Higher elevations also drove higher abundance of combined Coleoptera, and the abundance of saproxylic Coleoptera was moderately affected by higher elevations. Plots at higher elevations had lower levels of basal area and were more open (Table 4.3). While the mechanisms driving higher abundance at higher elevations do not necessarily include the higher levels of open space at higher elevations, the affiliation of saproxylic Coleoptera to both open space and edge habitat is supported by other studies (Wermelinger *et al.* 2007; Seibold *et al.* 2016; Oto *et al.* 2022). Open space and edge habitat can be a proxy for sunlight, and higher amounts of sunlight-exposed substrate are known to be important predictors of saproxylic biodiversity (Jonsell *et al.* 1998; Lindhe & Lindelöw 2004; Thorn *et al.* 2016; Vogel *et al.* 2020). It is hypothesized that higher amounts of sunlight could play a role in warming the substrate and the organisms within, leading to direct and indirect effects on saproxylic biodiversity. Indirectly, warmer temperatures could provoke changes in the type of wood-eating fungi available to saproxylic Coleoptera, e.g., higher abundance of certain wood-inhabiting fungi on sun-exposed logs (Bässler *et al.* 2010; Vogel *et al.* 2020) and directly through faster reproductive and growth rates of saproxylic Coleoptera (Brown *et al.* 2004). Open space can promote a complex architecture of forest with more sublevels, a positive influence upon abundance of the forest-associated taxa (Ampoorter et al. 2019)

4.5.3 Edge effects and elevation influence taxonomic and functional guild community patterns differently.

While elevation negatively affected abundance in combined Coleoptera and moderately and positively affected abundance of saproxylic Coleoptera, elevation also significantly positively affected abundance of phytophage functional group Coleoptera. Phytophage richness, however, was negatively affected by elevation. It's likely that a few very successful

species could be driving the greater abundance found at higher elevations (Fig. 4.2). Furthermore, the lower richness of phytophage Coleoptera at higher elevations could be linked to colder temperatures more likely to be found at the higher elevations. Temperature is most often the dominant abiotic factor affecting herbivorous insects (Reymond *et al.* 2013). Temperature fluctuation is also wider at higher elevations in the Pyrenees (Navarro-Serrano *et al.* 2020). Although Steven's extension to Rapaport's rule (i.e. the elevational range of a species is wider at higher elevations) is controversial (McCain & Bracy Knight 2013; Kim *et al.* 2019), Rasmann *et al.* (2014) found evidence for less host specialization of Buprestidae species (a saproxylic Coleoptera family) and Apiformes (a bee group) at higher elevations in the Swiss Alps. Thus, the greater abundance of herbivorous insects in the higher elevation plots may be strongly influenced by families that are less specialized and able to adapt to more variable conditions.

Phytophage richness was also positively affected by both landscape-level open space and stand-level basal area and negatively affected by medium tree density, indicating that as a group, phytophage Coleoptera are also more successful in edge habitat, albeit in edge habitat with more open space and smaller copse of trees. This is in contrast to the edge affects found in our study linked to taxonomic abundance, i.e. larger areas of higher landscape-level tree cover (low percent openness) interspersed with interior gaps in the stand-level tree cover (low basal area). Our results show the importance of testing Coleoptera data using both taxonomic and functional feeding guild focused approaches as well as landscape- and stand-level variables for a fuller understanding of saproxylic community data.

4.5.4 Climate change and land use shifts prediction

Land use shifts and climate change are driving the densification and upward migration of the Pyrenean tree-line (Batllori & Gutiérrez 2008; Batllori *et al.* 2010). In the Alps, it is likely that Coleoptera endemic to habitats above the tree-line will face disproportionate species loss in even the most conservative climate change and abandoned pasture scenarios (Dirnbock *et al.* 2011). These dynamic processes will play different roles in future saproxylic Coleoptera assemblages, as these communities are dependent on wood. In the Iberian eastern range of Pyrenees, Batllori & Gutiérrez (2008) found only 50% of forest densification at the tree-line co-occurred with tree-line upward migration. Our study indicates a closed forest without exterior or interior edges negatively affects the abundance of montane Coleoptera, montane saproxylic Coleoptera, and phytophage Coleoptera, suggesting that further forest densification without an upward migration tree-line shift could be detrimental for many tree-associated Coleoptera populations. While it is possible that the future forest densification and tree-line upward migration shift could be mitigated by forecasted climate change-triggered drought-stress, these processes are difficult to untangle (Galván *et al.* 2015). Poikilothermal animals such as arthropods are particularly sensitive to temperature (Roitberg & Mangel 2016), and climate change and land use shifts could elicit phenological desynchronization of species interactions, further complicating community functioning (Konvicka *et al.* 2016).

4.5.5 Conclusions

Our results are generally consistent with those of Wermelinger *et al.* (2007), Seibold *et al.* (2016), Vogel *et al.* (2020), and Oto *et al.* (2022). These studies showed high affiliation between Coleoptera and open space, forest edges, and/or sunlight. Saproxylic Coleoptera or saprophyte-group Coleoptera did not show marked differences in richness as a function of elevation in our study. However, some Coleoptera groups, especially phytophage Coleoptera, were richer and more abundant at plots closer to tree-line, which were more open. Ecological processes initiated and mediated by climate change and land use shifts are driving changes in biodiversity in mountains, especially within the Pyrenees range (OPCC-CTP 2018). Some of these changes include densification of the tree-line. The strong biodiversity correlations to edge effects and open space at tree-line indicate landscape and climate change effects on Pyrenean tree-line could greatly affect spatial patterns of montane and saproxylic montane Coleoptera in the future.

4.6 Figures

Fig. 4.1 Map of study site

Fig. 4.2 Abundance of five most common species found in high and low elevations.

Fig. 4.3 Principal component analysis of continuous forest characteristic values.

Fig. 4.4 Abundance of combined Coleoptera at high elevation and low elevations.
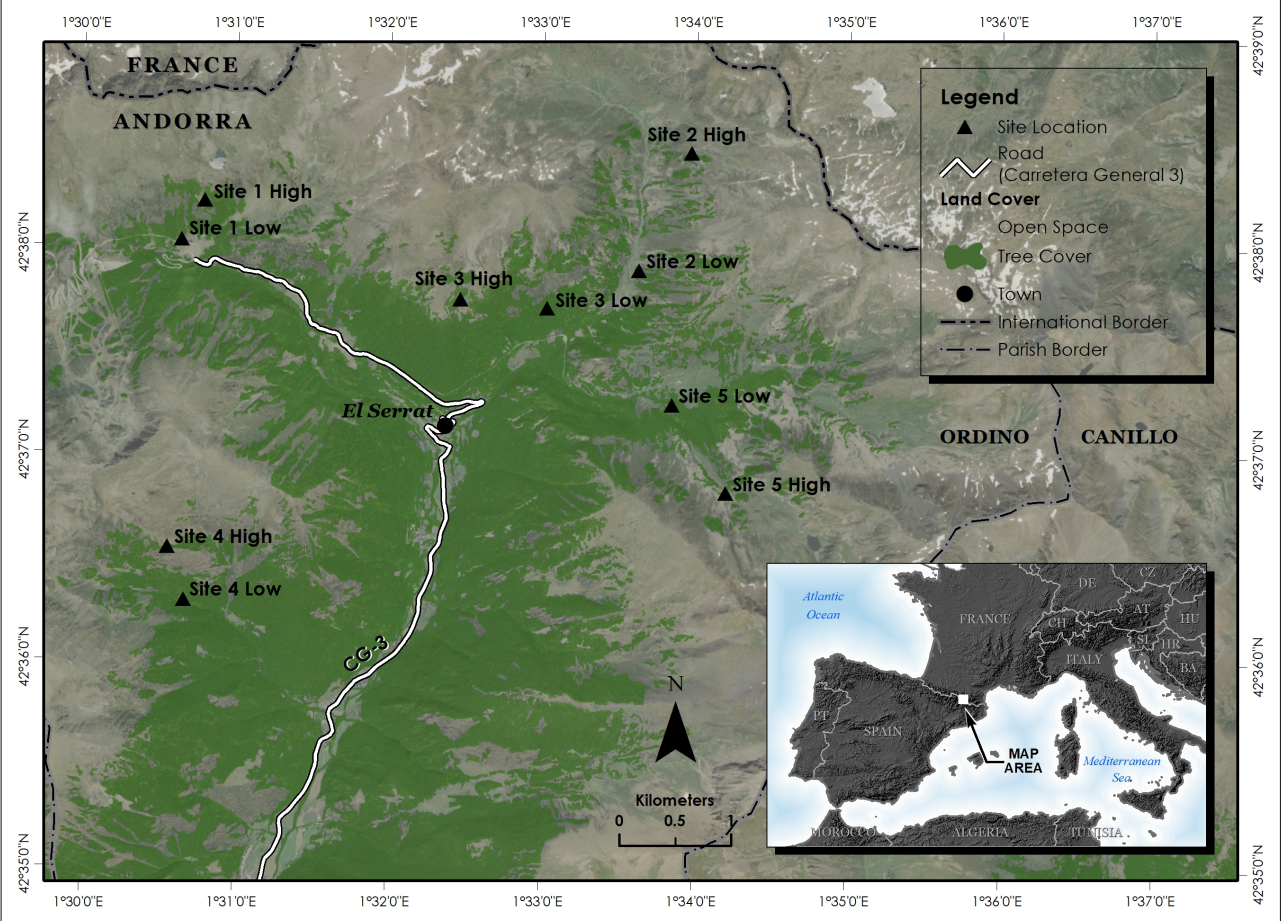
Fig 4.1
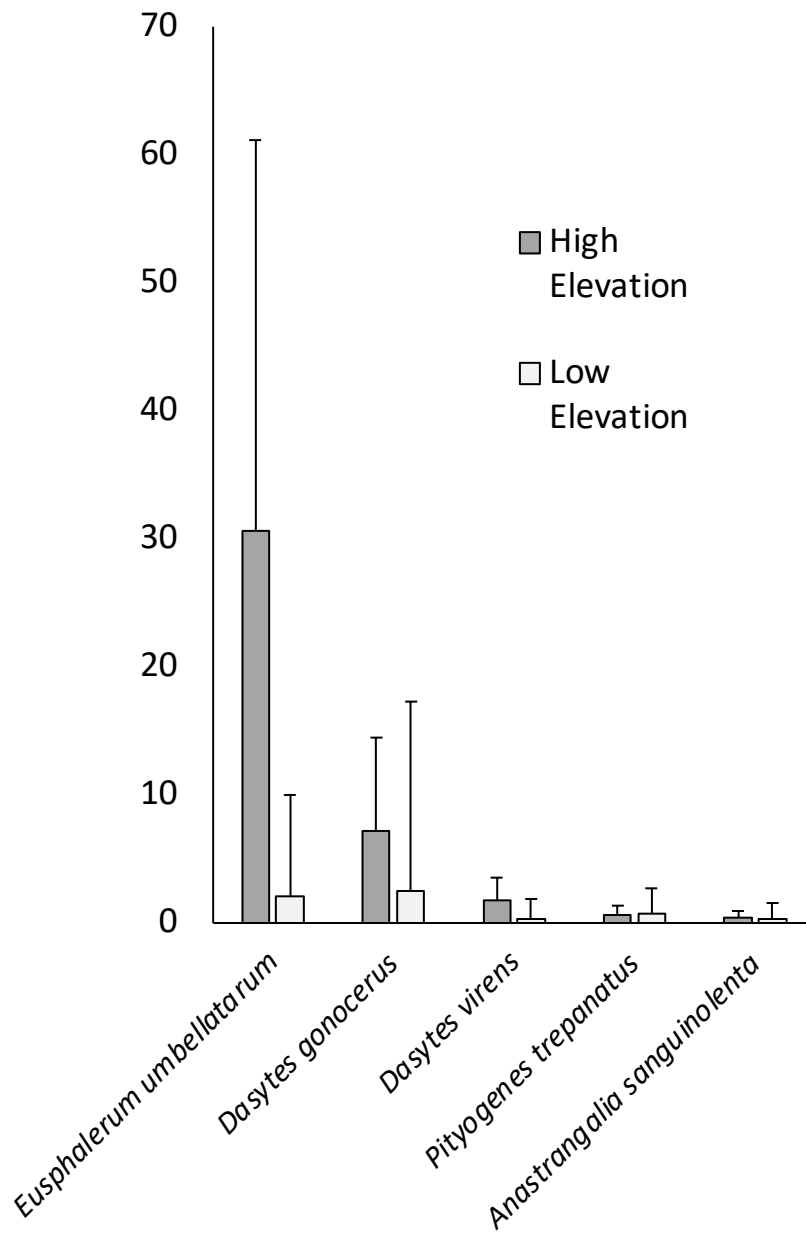
Fig. 4.2
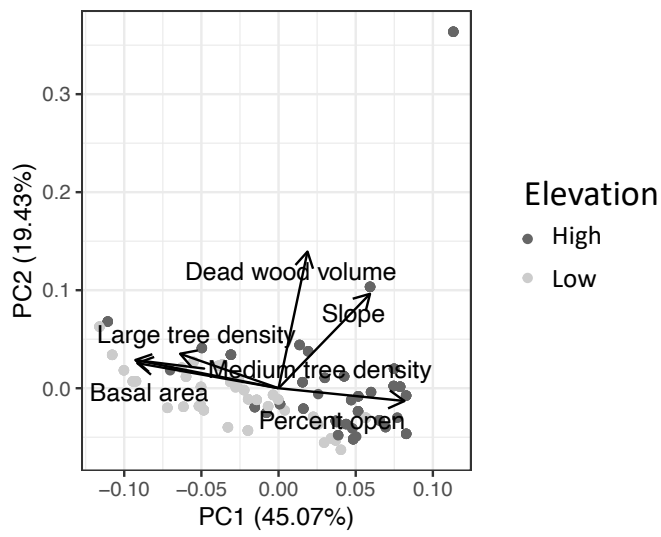
Fig 4.3
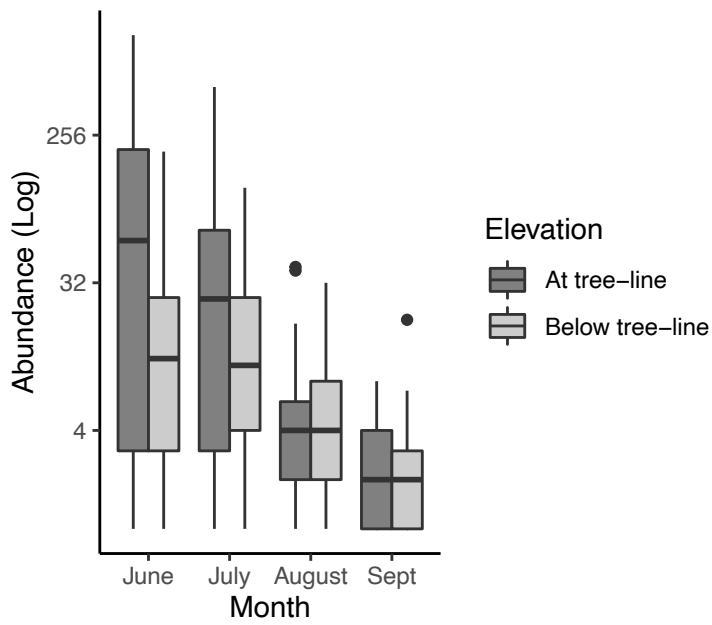
Fig 4.4

4.7 Tables

Table 4.1. Description of forest characteristic variables, month, and trap type.

Variable	Description	Scale
Elevation	At tree-line (2055-2217masl) or Below tree-line (1719-1998masl)	stand
Aspect	Flat, South, North, East, West	stand
Basal Area	Combined basal area (area of stem) of all *Pinus mugo* at or over 7.5cm diameter at breast height within 10m radius of trap (m^2 ha^{-1})	stand
Dead wood volume	Volume of dead wood with circumference at or over 7.5cm within 10m radius of trap (m^3 ha^{-1})	stand
Large tree density	Density of *Pinus mugo* at or over 50cm dbh within 20m radius of trap (ha)	stand
Medium tree density	Density of *Pinus mugo* at or over 30cm dbh within 20m radius of trap (ha)	stand
Month	June, July, August, September	NA
Percent open	Percent of open space (land without tree cover) within 1k radius of trap (1=completely without tree cover, 0=completely closed)	landscape
Slope	Gradient of land	stand
Trap type	Malaise, Flight intercept, Attraction	NA

Table 4.2 Table of experts involved with identifying specimens. Column labeled "Level of Assistance Provided" indicates if the expert in the respective row assisted with author J. Bookwalter's specimen identification of the specified family ("assisted"), or the expert in the respective row performed all identifications of the specified family ("identified").

Family	Expert	Level of assistance provided
Cantharidae	Fabrizio Fanti	identified
Carabidae	Benoit Dodelin	identified
Cerambycidae	Joan Bentanachs	assisted
Cerambycidae	Ulrich Bense	assisted
Chrysomelidae	Eduard Petitpierre Vall	assisted
Coccinellidae	Vincent Nicolas	identified
Cryptophagidae, Latridiidae	José Carlos Otero	identified
Curculionidae, Scarabaeidae	Jamie Bookwalter	identified
Dasytinae (Melyridae)	Gianfranco Liberti	assisted
Dermestidae	Jiri Háva	identified
Elateridae, Erotylidae, Meloidae, Nitilidae, Salpingidae	José Iñaki Recalde	identified
Hydrochidae, Hydrophilidae	Luis Valladares	identified
Hydrophilidae	Ayçin Yılmaz Akünal	identified
Kateretidae, Nitidulidae	Jose Manuel Pereira Martínez	identified
Leiodidae	Cédric Alonso	identified
Malachiidae	Gabriele Franzini	identified
Mordellidae	Dávid Selnekovič	identified
Ptiliidae	Mikael Sörensson	identified
Ptinidae	Amador Viñolas	identified
Scirtidae	Rafal Ruta	identified
Scolytinae (Curculionidae)	Miguel Alonso-Zarazaga	assisted
Scolytinae (Curculionidae)	Thierry Noblecourt	assisted
Scraptiidae	Brian Levey	identified
Staphylinidae	Benedikt Feldmann	identified
Tenebrionidae	Enrico Ruzzier	assisted
Throscidae	Cyrille van Meer	identified

Table 4.3 Differences in forest characteristics between plots below and at tree-line analyzed using Wilcoxon-Mann-Whitney z scores and associated P values. Extent of effect size is described using r scores (Parisi et al. 2020).

Independent Variable	Low elevation		High elevation		Wilcoxon-Mann-Whitney z	P	Effect size (r)
	mean value	SD	mean value	SD			
Basal area	38.7	19.8	23	11.2	-11	<0.001	-0.41
Dead wood volume	7.28	11.3	13.3	45.5	-0.61	0.54	-0.02
Medium tree density	107	58.8	47.7	32.5	-13.57	<0.001	-0.51
Large tree density	10.5	9.65	9.93	16.3	-4.43	<0.001	-0.16
Percent open	51.2	7.81	68.5	16	14.1	<0.001	0.53
Slope	12.9	10.3	30.4	18.3	15	<0.001	0.56

Table 4.4 Results of generalized linear mixed-effects modeling: Effects of independent variables on abundance and richness of combined Coleoptera and saproxylic Coleoptera. (Est.=Beta-estimates). *P value < 0.05, **P value < 0.01, ***P value < 0.001, and ****P value < 0.0001

Independent Variable	Abundance		Richness		Saproxylic Abundance		Saproxylic Richness	
	Est.	P value	Est.	P value	Est.	P value	Est.	P value
Elevation	0.57	0.00**	-0.29	0.53	0.34	0.07.	-0.11	0.44
Basal area	-0.61	0.03*	0.13	0.13	-0.9	0.00**	0.09	0.36
Dead wood volume	-0.06	0.49	-0.03	0.59	-0.08	0.38	-0.01	0.86
Medium tree density	-0.29	0.16	-0.18	0.08.	-0.48	0.02*	-0.22	0.04*
Large tree density	0.27	0.24	-0.05	0.65	0.09	0.63	-0.16	0.2
Percent open	-0.47	0.01*	0.31	0.44	-1.13	<0.0001***	-0.02	0.9
Slope	0.17	0.18	-0.03	0.74	0.37	0.00**	0.01	0.94
Month	1.36	<0.0001***	0.37	<0.0001***	1.23	<0.0001***	0.36	<0.0001***
$I(Month^2)$	-1.41	<0.0001***	-0.62	<0.0001***	-1.52	<0.0001***	-0.71	<0.0001***
Aspect N	0.27	0.74	-0.72	0.05*	0.5	0.55	-0.59	0.11
Aspect NE	3.21	0.00***	0.05	0.9	3.85	<0.0001***	0.32	0.48
Aspect E	0.54	0.5	-0.73	0.05.	0.37	0.66	-0.64	0.09.
Aspect SE	0.49	0.55	-0.53	0.19	0.32	0.72	-0.51	0.2
Aspect S	0.95	0.23	-0.56	0.12	1.56	0.06.	-0.3	0.39
Aspect SW	0.29	0.78	-0.66	0.10.	0.92	0.34	-0.6	0.17
Aspect W	0.19	0.81	-0.72	0.02*	0.62	0.42	-0.47	0.16
Aspect NW	-0.42	0.66	-0.86	0.00**	-0.19	0.84	-0.53	0.11
Trap Type: FI	7.16	0.01*	2.56	<0.0001***	5.82	<0.0001***	2.69	<0.0001***
Trap Type: M	5.31	0.07.	2.3	<0.0001***	4.55	<0.0001***	2.27	<0.0001***

Table 4.5 Results of generalized linear mixed-effects modeling: Effects of forest characteristics variables and PCAbun (Primary consumer abundance) on abundance of functional groups. (Est.=Beta-estimates). *P value < 0.05, **P value < 0.01, ***P value < 0.001, and ****P value < 0.0001

Independent Variable	Predator		Saprophyte		Phytophage	
	Est.	P value	Est.	P value	Est.	P value
Elevation	0.37	0.32	-0.07	0.59	2.66	0.03*
Basal area	-0.11	0.61	0.1	0.21	0.6	0.01**
Dead wood volume	0.01	0.84	-0.05	0.36	0.31	<0.0001***
Medium tree density	-0.08	0.73	-0.17	0.07.	0.17	0.68
Large tree density	-0.19	0.38	0.14	0.03*	0.78	0.01**
Percent open	-0.07	0.86	0.06	0.6	0.18	0.86
Slope	0.09	0.52	0.07	0.36	-1.5	<0.0001***
Month	1.31	<0.0001***	0.09	0.10.	3.7	<0.0001***
I(Month^2)	-1.34	<0.0001***	-0.18	<0.0001***	-2.9	0.00**
PCAbun	0.27	<0.0001***	NA		NA	
Aspect N	0.37	0.75	-0.34	0.21	-2.5	0.01
Aspect NE	2.01	0.10.	-0.32	0.31	-3.99	<0.0001***
Aspect E	0.08	0.95	-0.46	0.12	-1.43	0.15
Aspect SE	0.57	0.65	-0.01	0.97	-0.68	0.56
Aspect S	0.81	0.49	-0.06	0.84	-1.67	0.10.
Aspect SW	-0.34	0.79	-0.34	0.34	-1.25	0.23
Aspect W	0.49	0.65	-0.66	0.02*	-0.73	0.31
Aspect NW	-0.79	0.57	-0.5	0.08.	-4.01	0.000***
Trap Type: FI	3.81	0.00***	0.5	<0.0001***	5.87	<0.0001***
Trap Type: M	2.4	0.02*	0.22	0.2	5.09	<0.0001***

Table 4.6 Results of generalized linear mixed-effects modeling: Effects of forest characteristics variables and PCRich (Primary consumer richness) on richness of functional groups. (Est.=Beta-estimates). *P value < 0.05, **P value < 0.01, ***P value < 0.001, and ****P value < 0.0001

Independent Variable	Predator		Saprophyte		Phytophage	
	Est.	P value	Est.	P value	Est.	P value
Elevation	0.04	0.73	-0.06	0.38	-0.88	<0.0001***
Basal area	-0.02	0.77	0.04	0.51	0.06	0.04*
Dead wood volume	-0.08	0.07.	-0.04	0.28	0.05	0.01*
Medium tree density	-0.01	0.95	-0.07	0.31	-0.14	0.00***
Large tree density	-0.13	0.05.	0.04	0.48	0.04	0.25
Percent open	-0.03	0.79	0.05	0.38	0.92	<0.0001***
Slope	0.06	0.34	0.07	0.17	-0.17	<0.0001***
Month	0.22	<0.0001***	0.08	0.07.	0.33	<0.0001***
I(Month^2)	-0.26	<0.0001***	-0.13	<0.0001***	-0.52	<0.0001***
PCRich	0.14	<0.0001***	NA		NA	
Aspect N	-0.17	0.49	-0.35	0.06.	-0.44	<0.0001***
Aspect NE	-0.07	0.8	-0.26	0.25	0.44	<0.0001***
Aspect E	-0.3	0.22	-0.63	.00**	0.33	0.01**
Aspect SE	-0.17	0.5	-0.39	.04*	0.44	0.00**
Aspect S	-0.12	0.63	-0.21	0.25	-0.12	0.36
Aspect SW	-0.28	0.35	-0.27	0.32	-0.11	0.42
Aspect W	-0.4	0.12	-0.38	0.05	-0.23	0.02*
Aspect NW	-0.22	0.41	-0.27	0.16	-0.4	<0.0001***
Trap Type: FI	0.52	<0.0001***	0.56	<0.0001***	1.71	<0.0001***
Trap Type: M	0.62	<0.0001***	0.32	0.01**	1.71	<0.0001***

4.8 Acknowledgements

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CHAPTER 5

LADYBIRDS (COLEOPTERA, COCCINELLIDAE) OF THE PRINCIPALITY OF ANDORRA: NEW RECORDS AND ASSESSMENT OF KNOWLEDGE

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Ladybirds (Coleoptera, Coccinellidae) of the Principality of Andorra: new records and assessment of knowledge

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Résumé : La famille des Coccinellidae (coccinelles) est l'une des familles de coléoptères les plus emblématiques, mais très peu d'informations ont été publiées sur ce groupe dans la Principauté d'Andorre. Cette étude vise à combler certaines des lacunes dans les connaissances, avec l'ajout de cinq espèces nouvelles pour la dition. Les méthodes de collecte, la description de l'habitat et la bibliographie précédente concernant les anciennes collections de Coccinellidae en Andorre sont décrites.

Abstract : The Coccinellidae (ladybird) family is one of the most iconic Coleopteran families, yet little has been published about this group in the Principality of Andorra. This study aims to fill in some of the gaps in knowledge regarding this group in the country with the addition of five country records. Methods of collection, description of habitat, and previous bibliography concerning past Coccinellidae collections in Andorra are described.

Mots-clefs : Coleoptera, Coccinellidae, Andorre, Pyrénées, écologie, répartition altitudinale.

Keywords : Coleoptera, Coccinellidae, Andorra, Pyrenees, ecology, altitudinal distribution.

Introduction

The fauna of Andorra's ladybirds (Coleoptera, Coccinellidae) is particularly poorly understood, with few publications on the subject. In 2011, Diéguez-Fernández and Pujade-Villar reported 6 historically known taxa, bringing the total of species recorded in this territory to 22. The recent discovery of *Harmonia axyridis* (Pallas, 1773) somewhat completes the list (Sesma, 2015). This discovery, a relatively late addition in terms of the European extension of the species, is further proof of the weakness of the ladybird census in the Principality of Andorra. This study, as part of a larger study examining faunal diversity in Andorra, investigates the species list of ladybugs captured in a valley in Andorra and adds 5 new country records of ladybugs to the Principality.

Methods

Collections of coleoptera were made from mid-June through mid-September 2017 in the Vall d'Ordino, in the Ordino Parish, NW Andorra. Ten sites were established with one malaise, three attraction, and three flight intercept traps each for a total of 110 traps. The attraction traps consisted of a simple plastic soda bottle with a hold cut in the side, hung 2 feet from the trunk of a tree, and baited with a mixture of sangria, peach juice, salt, and sugar. Capture jars in the malaise and flight intercept traps were filled with propylene glycol. Traps were

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located within groves of pure black pine (*Pinus mugo* subsp. *uncinata* Turra) or a mixture of black pine and Scots pine (*Pinus sylvestris* L.), and in elevations ranging from 2216.6m to 1719.6m. Traps were emptied every two weeks and the insect collections were transferred to 97% alcohol for long term storage at Museu de Ciències Naturals de Barcelona and in that of the determinator (VN).

No additional active prospecting has been carried out.

Flight intercept – Rialb, El Serrat / Malaise – Besalí, El Serrat (photos: J. Bookwalter)

Results

25 specimens belonging to 11 distinct species were identified. In the following list, the nomenclature used for subfamilies and tribes is that proposed by Seago *et al.* (2011). The species names are taken from the reference work for the territory studied, i.e. the Iberian fauna (Eizaguirre, 2015).

Subfamily Coccinellinae Latreille, 1907

➤ **Tribe Chilocorini** Mulsant, 1846

Exochomus quadripustulatus (Linnaeus, 1758)

Two specimens were caught in an interception trap between mid-August and mid-September, at an altitude of ~2000m.

➤ **Tribe Coccidulini** Mulsant, 1846

Scymnus mimulus Capra & Fürsch, 1967

Two females were captured by a Malaise tent between 2100m and 2200m, in two locations and at different times (first half of July and second half of August).

> ➢ **Tribe Coccinellini** Latreille, 1807

Adalia decempunctata (Linnaeus, 1758)

Two individuals were trapped by flight interception in July at an altitude of ~2200m in two localities.

Calvia quatuordecimguttata (Linnaeus, 1758)

A singleton was captured by a Malaise tent at an altitude of 1876m during the second half of July.

Coccinella hieroglyphica Linnaeus, 1758

Between mid-July and mid-August, two specimens were caught in an interception trap in two localities located between 1900m and 2200m altitude.

Coccinella magnifica Redtenbacher, 1843

One individual was among a small series of the closely related species *C. septempunctata* Linnaeus collected by a Malaise tent during the first half of July, at an altitude of ~2200m.

Coccinella septempunctata Linnaeus, 1758

With five specimens, it is one of the two most represented ladybugs in this study. All were captured in the first half of July, in three different localities between 2060m and 2210m altitude, with a roughly identical representation in the interception traps and in the Malaise tents.

Halyzia sedecimguttata (Linnaeus, 1758)

A single specimen of this species was found in the company of *C. quatuordecimguttata*.

Myrrha octodecimguttata (Linnaeus, 1758)

During the first half of August, an individual of this pine-linked species was captured by an interception trap.

Neomyzia oblongoguttata (Linnaeus, 1758)

It is the second most dominant species in the inventory, represented by five specimens obtained from four distinct localities between 1800m and 2200m of altitude. Most were caught by interception traps, with a further positive result from a Malaise tent and, unique to this study, one capture by an attraction trap. The capture period is wide, covering from the end of June to the end of September. All of these elements tend to show a marked presence of the species in the high Pyrenean mountains, or at least in pine forests of the Andorran sector.

Propylea quatuordecimpunctata (Linnaeus, 1758)

The fourteen-spotted ladybird completes the species set results with three individuals from two localities all caught in a Malaise tent, at around 2070m and 2190m. The captures were made during the second half of July.

Discussion

From the outset, the Coccidulini tribe is poorly represented. This is not, however, attributable to frequent omission of small species when sorting samples, as all beetles collected during the study were determined. It would therefore seem that members of this tribe are indeed less present at high altitudes than other ladybirds.

This hypothesis is confirmed by our own observations in the French massifs, with few mentions of *Scymnus* and *Coccidula* above 1500m altitude: *Scymnus suturalis* Westman *in* Thunberg, *S. rubromaculatus* (Goeze), *S. impexus* Mulsant, *Coccidula rufa* (Herbst)... The *Rhyzobius* members, in particular *R. chrysomeloides* (Herbst), regular on pines, and the Pyrenean endemic *R. bipartitus* Fuente seem to reach higher altitudes (1900-2000m) without being very frequent *a priori*.

This also seems to be the case for the representatives of the Epilachnini tribe, who are totally absent from this study. According to our knowledge, only *Subcoccinella vigintiquatuorpunctata* (Linnaeus) occasionally exceeds 1500m.

Neomyzia oblongoguttata (photo : V. Nicolas)   *Adalia decempunctata* (photo : V. Nicolas)
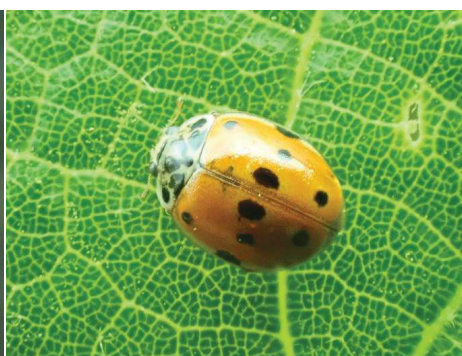
Systematic List of the Coccinellidae of Andorra

This list compiles the 23 coccinellids indicated in the literature including the five additional taxa highlighted by this study, for a provisional total of 28 species.

	Publications before 2011	Diéguez-Fernández & Pujade-Villar, 2011	Sesma, 2015	Nicolas *et al.*, 2021
Chilocorini				
Chilocorus bipustulatus (Linnaeus, 1758)		x		
Exochomus quadripustulatus (Linnaeus, 1758)		x		x
Platynaspis luteorubra (Goeze, 1777)		x		

	Publications before 2011	Diéguez-Fernández & Pujade-Villar, 2011	Sesma, 2015	Nicolas *et al.*, 2021
Coccidulini				
Rhyzobius chrysomeloides (Herbst, 1792)	x	x		
Clitostethus arcuatus (Rossi, 1794)		x		
Nephus bipunctatus (Kugelann, 1794)		x		
Nephus quadrimaculatus (Herbst, 1783)		x		
Scymnus interruptus (Goeze, 1777)		x		
Scymnus mimulus Capra & Fürsch, 1967				x
Scymnus suturalis Westman *in* Thunberg, 1795		x		
Stethorus pusillus (Herbst, 1797)	x	x		
Coccinellini				
Adalia bipunctata (Linnaeus, 1758)		x		
Adalia decempunctata (Linnaeus, 1758)		x		x
Calvia quatuordecimguttata (Linnaeus, 1758)	x	x		x
Coccinella hieroglyphica Linnaeus, 1758				x
Coccinella magnifica Redtenbacher, 1843				x
Coccinella septempunctata Linnaeus, 1758	x	x		x
Halyzia sedecimguttata (Linnaeus, 1758)		x		x
Harmonia axyridis (Pallas, 1773)			x	
Hippodamia variegata (Goeze, 1777)	x	x		
Myrrha octodecimguttata (Linnaeus, 1758)				x
Neomyzia oblongoguttata (Linnaeus, 1758)				x
Oenopia conglobata (Linnaeus, 1758)		x		
Oenopia lyncea (Olivier, 1808)		x		
Propylea quatuordecimpunctata (Linnaeus, 1758)	x	x		x
Psyllobora vigintiduopunctata (Linnaeus, 1758)		x		
Vibidia duodecimguttata (Poda, 1761)		x		
Epilachnini				
Henosepilachna argus (Geoffroy *in* Fourcroy, 1785)		x		
	6	**22**	**1**	**11**

Conclusion

The recent addition of relatively common ladybug species to the Andorran fauna reveals the knowledge gap present within this group in the Principality of Andorra. Indeed, it is difficult to provide even an approximation of the total number of species in this unique territory of less than 500km² yet whose average altitude is ~2000m with a low point culminating at 840m. Nevertheless, targeted prospecting using the traditional techniques of mowing and

threshing will undoubtedly unveil additional species. Among the most probable are *Aphidecta obliterata* (Linnaeus) and *Hippodamia notata* (Laicharting).

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CHAPTER 6

SIX INTERESTING SPECIES OF PTINIDAE (COLEOPTERA) FROM ANDORRA AND TARRAGONA, CATALONIA (IBERIAN PENINSULA)

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Six interesting species of Ptinidae (Coleoptera) from Andorra and Tarragona, Catalonia (Iberian Peninsula)

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Abstract

The Coleoptera belonging to the family Ptinidae have been separated from two projects carried out in Ordino (Andorra) and in Tarragona (Catalonia); the first study examined saproxylic Coleoptera and the latter created a methodology for the maintenance and conservation of *Pinus nigra* subsp. *salzmannii* (Dunal) Franco, 1943. Five of the studied species are the first records for Andorra: *Dryophilus anobioides* Chevrolat, 1832 (Dryophilinae), *Ernobius mollis mollis* (Linnaeus, 1758), *E. nigrinus* (Sturm, 1837) (Ernobiinae), *Ptinus (Pseudoptinus) auberti* Abeille de Perrin, 1869 and *P. (Ptinus) subpilosus* Sturm, 1837 (Ptininae). The sixth species, *Lapidoniptus hispanicus* (Pic, 1953) (Ptininae), was collected in Tarragona and is an interesting Iberian endemic. Of these six species, the habitus of the male and female (if necessary) are illustrated, as well as the aedeagus. The known distribution and biology are also discussed.

Key words: Coleoptera, Ptinidae, *Dryophilus, Ernobius, Lapidoniptus, Ptinus,* new records, Andorra, Catalonia, Iberian Peninsula.

Resumen

Seis interesantes especies de Ptinidae (Coleoptera) de Andorra y Tarragona, Cataluña (Península Ibérica)

De los coleópteros recolectados en sendos proyectos realizados en Ordino (Andorra) y en Tarragona (Cataluña), el primero para el estudio de saproxílicos y el segundo para crear una metodología para el mantenimiento y conservación de los bosques de *Pinus nigra* subsp. *salzmannii* (Dunal) Franco, 1943, se han separado los pertenecientes a la familia Ptinidae. Cinco de las especies estudiadas representan las primeras citas para Andorra: *Dryophilus anobioides* Chevrolat, 1832 (Dryophilinae), *Ernobius mollis mollis* (Linnaeus, 1758), *E. nigrinus* (Sturm, 1837) (Ernobiinae), *Ptinus (Pseudoptinus) auberti* Abeille de Perrin, 1869 y *P. (Ptinus) subpilosus* Sturm, 1837 (Ptininae). La sexta especie, recolectada en Tarragona, pertenece al interesante endemismo *Lapidoniptus hispanicus* (Pic, 1953). De todas ellas se representa el hábitus del macho y, si es necesario, el de la hembra, así como el edeago. También se comenta su distribución conocida y su biología.

Palabras clave: Coleoptera, Ptinidae, *Dryophilus, Ernobius, Lapidoniptus, Ptinus,* nuevas localizaciones, Andorra, Cataluña, Península Ibérica.

Laburpena

Sei Ptinidae espezie interesgarri (Coleoptera), Andorrakoak eta Kataluniako Tarragonakoak (Iberiar Penintsula)

Ordinon (Andorra) eta Tarragonan (Katalunian) garatutako proiektu banatan harrapatutako koleopteroetatik, Ptinidae familiakoak bereizi eta ikertu dira. Lehenengo proiektua saproxilikoen ikerketa bat da eta bigarrena *Pinus nigra* subsp. *salzmannii* (Dunal) Franco, 1943 basoen iraupena eta kontserbaziorako metodologia sortzeko egitasmoa. Aurkitutako espezieetatik bost Andorrarako lehenengo aipuak dira: *Dryophilus anobioides* Chevrolat, 1832

(Dryophilinae), *Ernobius mollis mollis* (Linnaeus, 1758), *E. nigrinus* (Sturm, 1837) (Ernobiinae), *Ptinus* (*Pseudoptinus*) *auberti* Abeille de Perrin, 1869 eta *P.* (*Ptinus*) *subpilosus* Sturm, 1837 (Ptininae). Seigarren espeziea, Tarragonan aurkitua, *Lapidoniptus hispanicus* (Pic, 1953) endemismo interesgarria da. Espezie horien guztien arren habitusa irudiztatzen da eta, behar izanez gero, emeena, bai eta edeagoa ere. Halaber, banaketa ezaguna eta biologia komentatzen dira.

Gako-hitzak: Coleoptera, Ptinidae, *Dryophilus, Ernobius, Lapidoniptus, Ptinus,* aipu berriak, Andorra, Katalunia, Iberiar Penintsula.

Introduction

From mid June 2017 to mid September 2017 a study examining elevational patterns of saproxylic Coleoptera was conducted in Vall d'Ordino, a valley located within the 90 km² parish of Ordino, Andorra. All our sites were located in *Pinus uncinata* Raymond ex A.DC. (Mountain Pine) forests or *P. uncinata* and *Pinus sylvestris* L. (Scots Pine) mixed forests. The study consisted of ten 1.77 ha sites: five higher elevation sites each paired with five lower elevation sites. The lower sites ranged from 1719.6 to 1997.9 m in elevation, and the higher sites were located at tree line and ranged from 2054.8 to 2187.1 m. Three types of traps (cross-vane window flight interception, attraction and Malaise traps) were deployed at each site.

In various *Pinus nigra* subsp. *salzmannii* (Dunal) Franco, 1943 (Black Pine) forests of Catalonia, the «Centre Tecnològic Forestal de Catalunya (CTFC)» conducted an invertebrate sampling using pit-fall traps during the months of July and August 2015 and July 2017. These collections were made in two forests in the province of Lleida and four in the province of Tarragona, located in the regions of Solsonès, Baix Ebre, Ribera d'Ebre and Baix Camp.

These collections were made in order to monitor the evolution of the invertebrate fauna in the plots in which silvicultural treatments had been carried out with the purpose of developing a suitable methodology for the conservation of the Black Pine forests.

The few so far known Andorran Ptinidae records belong to the subfamilies Dorcatominae (1 sp.), Dryophilinae (1 sp.), Mesocoelopodinae (1 sp.) and Ptininae (5 spp.). The five species studied in this paper (subfamilies Dryophilinae (1 sp.), Ernobiinae (2 spp.) and Ptininae (2 spp.)) are the first records for the Andorran territory. The Ptinidae collected in Tarragona is an interesting Iberian endemic of which very few specimens were known to date.

Material and methods

The Ptinidae were separated from Coleoptera collections in the two projects (Andorra and Catalonia) to facilitate the determination by the authors. For the study of the aedeagus, specimens were extracted, cleaned, rinsed, and mounted for microscopic preparation on a sheet of transparent styrene (Evergreen® brand) with DMHF liquid. All specimens were mounted dry on entomological cards.

Photographs were taken with a Canon® model EOS 760D camera with the objective of microscopy using the layer method, with treatment of the images using the Zerene Stacker® program. The drawings were made with the Adobe® Illustrator CS5 program after obtaining PostScript® 3™ files.

Of these six species presented, the habitus of the male and female (if necessary) are illustrated, as well as the aedeagus. The known distribution and biology are also discussed.

Results

Family **PTINIDAE** Latreille, 1802

Subfamily **Dryophilinae** LeConte, 1861

Tribu **Dryophilini** LeConte, 1861

Dryophilus anobioides Chevrolat, 1832 (Fig. 1a)

Dryophilus anobioides Chevrolat, 1832. *Mag. Zool.* 2: 3
Anobium compressicorne Mulsant & Rey, 1853. *Ann. Soc. Linn. Lyon* 1[1852-53]: 15

Specimens studied:

1 ♂. Labelled as: «30-VII-2017, Besalí, el Serrat, Ordino,

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Andorra, S07IHFc2, CREAF leg.». Captured with Malaise trap. Deposited in the collection of A. Viñolas.

It is separated from the rest of the species of the genus by: the elytral intervals with short and uniform pubescence, the pronotum not transverse, the funiculus of the antennae with transverse antennomeres, the extremely projecting eyes, the particular conformation of the aedeagus (Fig. 1b) (Español and Bellés, 1981; Español, 1992; Español and Viñolas, 1995).

Distribution:

This species is known from central and southern Europe and North Africa. In the Iberian Peninsula it has been recorded from Cádiz, Castellón, Girona and Lleida (Español, 1992; Viñolas and Verdugo, 2009; Viñolas *et al.*, 2015b; Viñolas, 2016). The specimen of Ordino is the first record for Andorra.

Biology:

The species is found in different coniferous models (*Abies* Mill., *Pinus* L. and *Larix* Mill.) and also mentioned in hardwoods especially of the genus *Quercus* L. In Cádiz it has been captured in an area of *Quercus canariensis* Willd. (Viñolas and Verdugo, 2009) and in Lleida in a forest of *Pinus uncinata* (Viñolas *et al.*, 2015b).

It has also been mentioned in the shrub *Sarothamnus scoparius* (L.) Link, typical of the Mediterranean region, where it is predatory upon *Phloeotribus rhododactylus rhododactylus* (Marsham, 1802), a Scolytinae living in Cistaceae of the genus *Cistus* L. and in Fabaceae of the genera *Calycotome* Link, *Cytisus* Desf., *Genista* L., *Retama* Raf., *Spartium* L. and *Ulex* L. (Español, 1992).

Subfamily **Ernobiinae** Pic, 1912

Ernobius mollis mollis (Linnaeus, 1758) (Fig. 1c)

> *Dermestes mollis* Linnaeus, 1758. *Syst. Nat.*, ed. 10: 355
> *Anobium testaceus* Kugelann, 1792. *N. Mag. Liebh. Ent.* 1(4): 489
> *Anobium sybaris* Kugelann, 1792. *N. Mag. Liebh. Ent.* 1(4): 490
> *Anobium convexifrons* Melsheimer, 1846. *Proc. Acad. Nat. Sci. Philad.* 2(12): 309
> *Liozoum sulcatulum* Mulsant & Rey, 1863. *Opusc. Entom.* 13: 111
> *Liozoum consimile* Mulsant & Rey, 1863. *Opusc. Entom.* 13: 117
> *Liozoum consimile* var. *laetum* Mulsant & Rey, 1864. *Hist. Nat. Col. France. Térédiles*: 171

> *Ernobius tarsatus* Kraatz, 1881. *Deutsche Entom. Zeitschr.* 25(2): 302
> *Ernobius revercus* Sharp, 1916. *Ent. Mon. Mag.* 52: 222

Specimens studied:

1 ♂. Labelled as: «30-VII-2017, Canya de la Rabassa, el Serrat, Ordino, Andorra, S07ILFa2, CREAF leg.». Captured with flight interception trap. Deposited in the collection of A. Viñolas.

Species characterized by: the antennae with the sum of the antennomeres 6 to 8 longer than the 9th, the dorsal excavation of the 4th tarsomere not exceeding half of these, straight protibiae with the apex turned outward, and the aedeagus according to Fig. 1d.

Distribution:

A species of wide European distribution, present in North Africa and Palaearctic Asia, and introduced in the Afrotropical, Australian, Nearctic, Neotropical and Oriental regions. In the Iberian Peninsula it has been recorded from Spain (Alicante, Barcelona, Córdoba, La Rioja, Lleida, Murcia, Pontevedra and Tarragona) and Portugal (Vila Real); also known in the Balearic and Canary Islands (Español, 1992; Viñolas *et al.*, 2013, 2015a; Viñolas and Ghahari, 2017). The specimen of Ordino is the first record for Andorra.

Biology:

It develops in the bark and dead branches of resinous, also in their cones. The following tree species have been cited: *Larix decidua* Mill., *Picea abies* (L.) H. Karst., *Pinus heldreichii* Christ, *P. sylvestris*, *P. nigra*, *P. pinaster* Aiton, *P. strobus* L., *P. taeda* L. and *Pseudotsuga menziesii* (Mirb.) Franco. Some species of the genus *Ernobius* C.G. Thomson, 1859 can attack dead wood as long as it retains the bark (Español, 1992). In Spain it has been collected in *P. sylvestris* and in cones by means of a breeding box (Español, 1964; Pérez Moreno and Moreno Grijalba, 2009). In the Canary Islands it has been collected in cones of *P. canariensis* C. Sm. ex DC. (Español, 1964). The specimen from Andorra was collected in a mixed forest of *P. uncinata* and *P. sylvestris*.

Ernobius nigrinus (Sturm, 1837) (Fig. 1e)

> *Byrrhus mollis* Geoffroy, 1785. In: Fourcroy, *Entomol. Paris* 1: 26
> *Anobium nigrinum* Sturm, 1837. *Deutschlands Fauna (Insecten)* 11: 126

Anobium politum Redtenbacher, 1849. *Fauna Austriae*, ed. 1: 346 (non Duftschmid, 1825)

Liozoum fuscum Mulsant & Rey, 1863. *Opusc. Entom.* 13: 131

Ernobius canaliculatus C.G. Thomson, 1871. *Opusc. Entom.* 4: 380

Ernobius pueli Lavagne, 1914. *Bull. Soc. Ent. France*: 137

Ernibius pueli var. *berardi* Lavagne, 1914. *Bull. Soc. Ent. France*: 137

Ernobius pallidipennis var. *mayeti* Pic, 1914. *Echange* 32(377): 27

Ernobius nigrinus var. *rufescens* Pic, 1916. *Echange* 32(377): 3 (note)

Ernobius nigriclava Roubal, 1917. *Arch. Naturg., Ser. A* 82(3)[1916]: 51

Specimens studied:

2 ♂♂ and 2 ♀♀. Labelled as: 1 ♂ «12-VII-2017, Bordes de la Mollera, Llorts, Ordino, Andorra, S10ILMa1, CREAF leg.»; 1 ♂ «13-VII-2017, Pleta de Llom, El Serrat, Ordino, Andorra, S12IHFb1, CREAF leg.»; 1 ♀ «13-VII-2017, Canya de la Rabassa, El Serrat, Ordino, Andorra, S07ILMa1, CREAF leg.»; 1 ♀ «13-VII-2017, Besalí, El Serrat, Ordino, Andorra, S07IHFa1, CREAL leg.». Captured with flight interception trap and Malaise. Deposited in the collection of A. Viñolas.

This species is characterized by: the body of more or less blackish colour, the antennae with the sum of 6 to 8 antennomeres of the funiculus shorter than the length of the 9th, which is equal to the sum of the first 8 articles, the pronotum provided with a transverse basal depression, the elytra without or with only indications of basal depression in the humeral zone, the pronotum and elytra with fine and widely spaced granulation, the straight outlined protibiae, the dorsal surface of the 4 tarsomere excavated in the apical half, and the aedeagus according to Fig. 1f.

Distribution:

In the peninsular area, it is known to occur only in the Pyrenean region. It has been recorded from Huesca, La Rioja, Lleida and Navarra (Español, 1992; Pérez Moreno and Moreno Grijalba, 2009; Viñolas, 2017). The specimens of Ordino are the first record for Andorra.

Biology:

It develops in the thin branches of *Pinus cembra* L., *P. sylvestris*, *P. uncinata* and *Picea abies* after the primary attack by species of Curculionidae of the genera *Magdalis* Germar, 1817 and *Hylastes* Erichson, 1836 (Viñolas, 2017). The specimens of Ordino were captured in a forest of *P. uncinata*.

Subfamily **Ptininae** Latreille, 1802

Tribe **Ptinini** Latreille, 1802

Lapidoniptus hispanicus **(Pic, 1953)** (Figs. 2a-b)

Piarus hispanicus Pic, 1953. *Arch. Inst. Aclim. (Almería)* 1: 143

Lapidoniptus hispanicus (Pic): Bellés, 1981. *Spixiana* 4: 70

Specimens studied:

28 ♂♀. Labelled as: 1 spec. «13-VII-2015, Llaberia, Ribera d'Ebre, Tarragona, Z3_T1_V1_P1, D. Guixer *et al.* leg.»; 1 spec. «13-VII-2015, Llaberia, Ribera d'Ebre, Tarragona, Z3_T1_V1_P5, D. Guixer *et al.* leg.»; 1 spec. «20-VII-2015, Llaberia, Ribera d'Ebre, Tarragona, Z1_T2_V2_P4, D. Guixer *et al.* leg.»; 2 spec. «27-VII-2015, Llaberia, Ribera d'Ebre, Tarragona, Z3_T2_V2_P5, D. Guixer *et al.* leg.»; 1 spec. «10-VII-2015, Montsagre, Paüls, Baix Ebre, Tarragona, Z6_T1_V2_P4, D. Guixer *et al.* leg.»; 1 spec. «10-VIII-2015, Montsagre, Paüls, Baix Ebre, Tarragona, Z6_T1_V2_R3, D. Guixer *et al.* leg.»; 1 spec. «10-VIII-2015, Paüls, Paüls, Baix Ebre, Tarragona, Z6_T4_V2_P4, D. Guixer *et al.* leg.»; 1 spec. «13-VII-2017, Llaberia, Ribera d'Ebre, Tarragona, Z3_T1_V2_P1, D. Guixer *et al.* leg.»; 2 spec. «13-VII-2017, Llaberia, Ribera d'Ebre, Tarragona, Z3_T2_V1_P3_C, D. Guixer *et al.* leg.»; 2 spec. «13-VII-2017, Llaberia, Ribera d'Ebre, Tarragona, Z3_T2_V1_R2_C, D. Guixer *et al.* leg.»; 1 spec. «27-VII-2017, Llaberia, Ribera d'Ebre, Tarragona, Z3_T1_V2_R2_C, D. Guixer *et al.* leg.»; 1 spec. «27-VII-2017, Llaberia, Ribera d'Ebre, Tarragona, Z3_T2_V2_P5_C, D. Guixer *et al.* leg.»; 1 spec. «11-VIII-2015, Cops, Rasquera, Ribera d'Ebre, Tarragona, Z5_T1_V2_P5, D. Guixer *et al.* leg.»; 1 spec. «11-VIII-2015, Cops, Rasquera, Ribera d'Ebre, Tarragona, Z5_T2_V2_P1, D. Guixer *et al.* leg.»; 3 spec. «13-VII-2017, Coll del Guix, Colldejou, Baix Camp, Tarragona, Z3_T4_V1_R1_C, D. Guixer *et al.* leg.»; 1 spec. «20-VII-2017, Coll del Guix, Colldejou, Baix Camp, Tarragona, Z3_T3_V2_P3_C, D. Guixer *et al.* leg.»; 7 spec. «20-VII-2017, Coll del Guix, Colldejou, Baix Camp, Tarragona, Z3_T4_V2_P2_C, D. Guixer *et al.* leg.». Captured with pit-fall traps. Deposited in the collections of A. Viñolas, J.I. Recalde, J. Muñoz and Museum of Natural Sciences of Barcelona (MZB 2018-0132, 2018-0133).

1.96–2.46 mm in length.

Male (Fig. 2a). Very convex body, ovoid contour and apterous. Body of black colour, with antennae, palps and legs reddish. Pubescence double, one woolly and recumbent and another erect and pale yellowish colour. Large head, covered by the prothorax at rest and with very developed eyes. Thick antennae of 11 antennomeres slightly longer than wide from 2 to 10. Pronotum as wide as long, with rounded margins and with the surface covered by contiguous, flattened and thick granules. Very convex elytra, striae formed by series of deeply marked dots, slightly convex intervals.
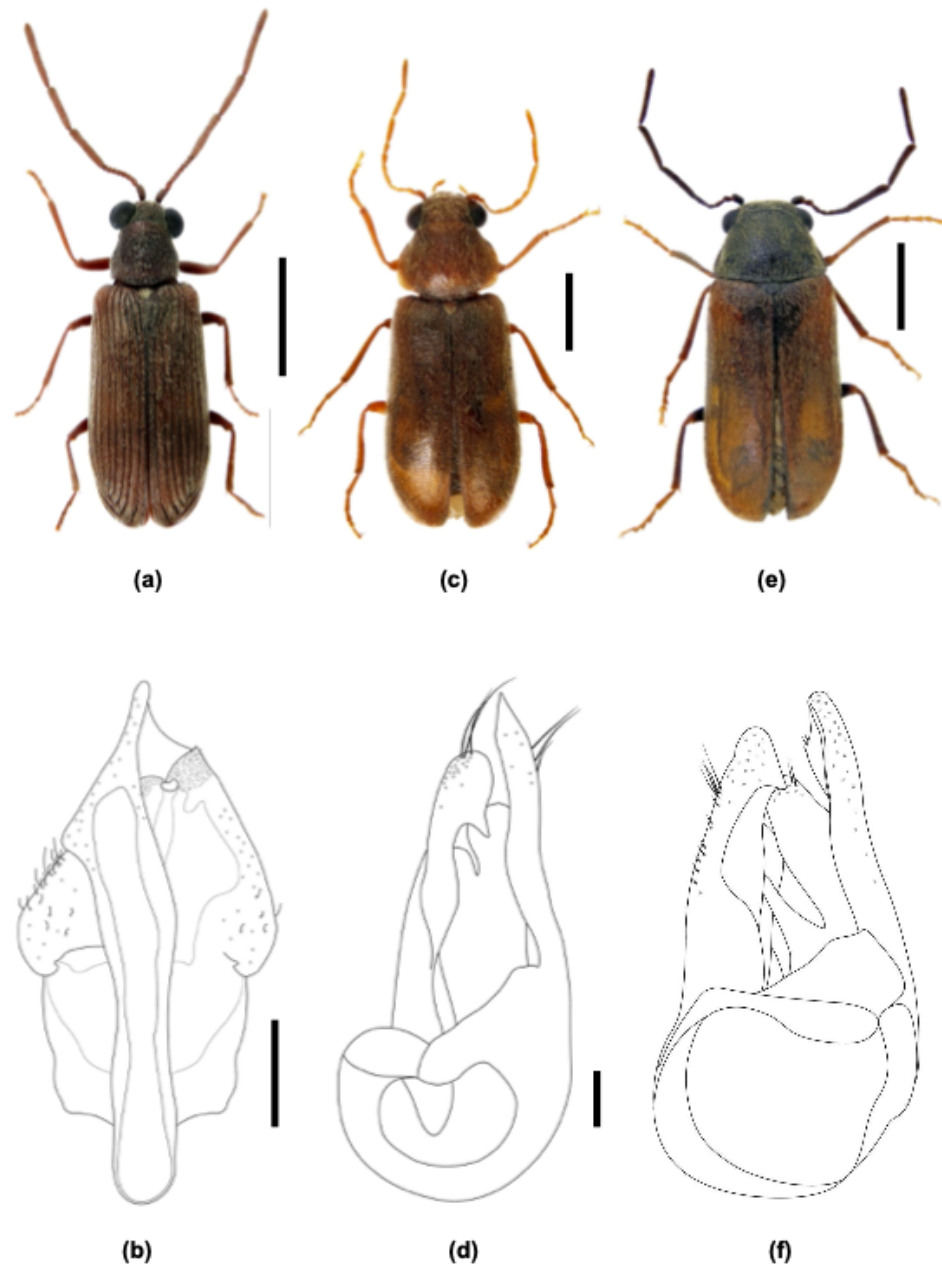
FIGURE 1. (a)-(b) *Dryophilus anobioides* Chevrolat, 1832: (a) Dorsal male habitus (collected in Ordino, Andorra); (b) Aedeagus in ventral view; (c)-(d) *Ernobius mollis mollis* (Linnaeus, 1758): (c) Dorsal male habitus (collected in Iriso, Navarra); (d) Aedeagus in ventral view; (e)-(f) *Ernobius nigrinus* (Sturm, 1837): (e) Dorsal male habitus (collected in Iriso, Navarra); (f) Aedeagus in ventral view (Scale bars: (a), (c), (e) = 1 mm; (b), (d), (f) = 0.1 mm).

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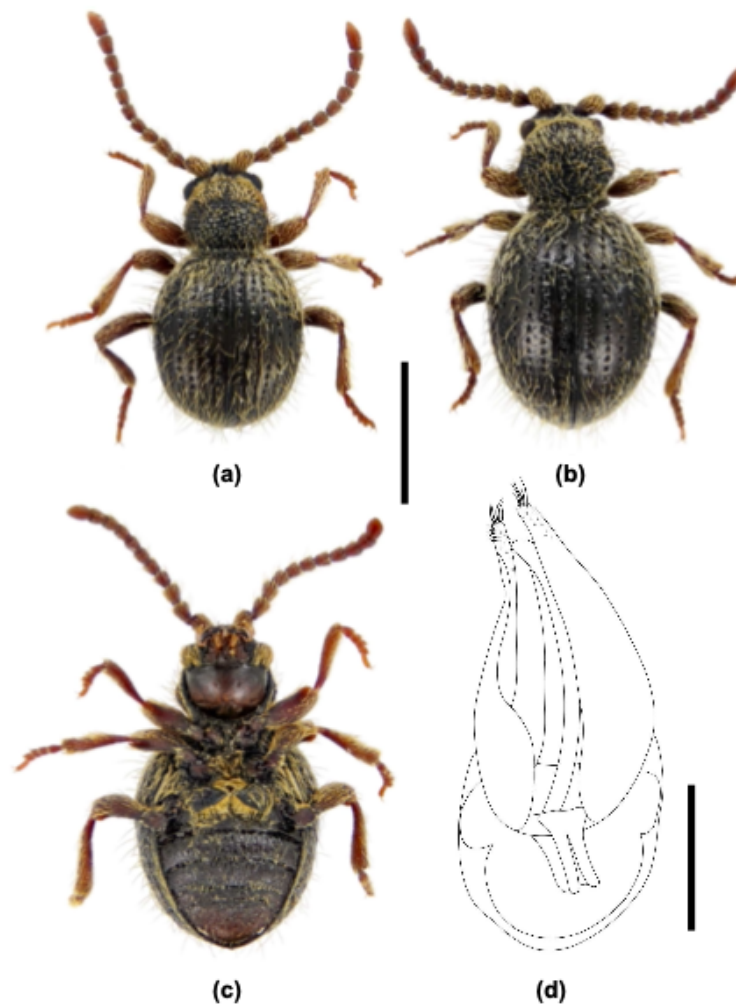
FIGURE 2. *Lapidoniptus hispanicus* (Pic, 1953): (a)-(b) Dorsal habitus: (a) Male; (b) Female (collected in Tarragona, Catalonia); (c) Ventral habitus (collected in Tarragona, Catalonia); (d) Aedeagus in ventral view (Scale bars: (a)-(c) = 1 mm; (d) = 0.2 mm).

Metasternum with a curious conformation (Fig. 2c). Metacoxae widely separated. Short and thick legs. Aedeagus very differentiated by its conformation of other species of the subfamily (Fig. 2d).

Female (Fig. 2b). Average size greater. Contour of body more elongated. Antennae shorter than those of the male.

Distribution:

Species endemic to Spain, described from two specimens collected in La Burrica (La Borrica [*sic*]), Mountains of Sierra María, municipal district of María, Almeria (Pic, 1953). Recorded from the provinces of Almeria (Andalusia) and Tarragona (Catalonia) (Bélles, 1981; Borowski, 2007). The locations where the spe-
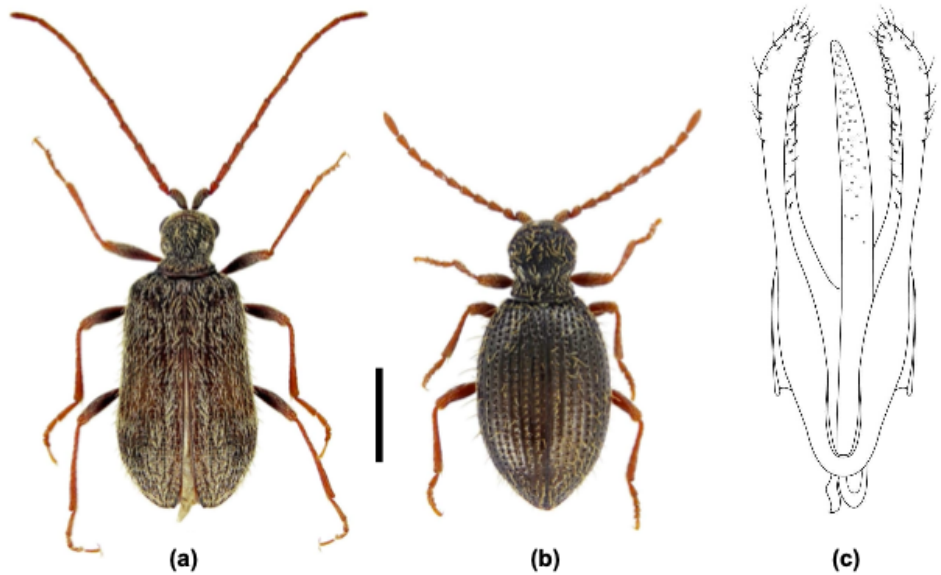
FIGURE 3. *Ptinus (Pseudoptinus) auberti* Abeille de Perrin, 1869: (a)-(b) Dorsal habitus: (a) Male; (b) Female (collected in Ordino, Andorra); (c) Aedeagus in ventral view (Scale bars: (a)-(b) = 1 mm; (c) = 0.2 mm).

cies has been collected are the following: La Burrica (Almeria); Colldejou, la Sénia, Llaberia, Paüls and Rasquera (Tarragona). The specimens studied expand the distribution area in Catalonia.

Bélles (1981) indicates the presence of four specimens collected in Catalonia (the Sénia and the Mola de Coll de Jou) in the collection of Ptinidae from the Museum of Natural Sciences of Barcelona. In the organization and documentation of the family collection carried out by Viñolas and Masò (2013) it was not possible to find these specimens.

Biology:

In the Sierra María the indigenous tree is *Pinus nigra* subsp. *salzmannii* with reforestation of *Pinus halepensis* Miller (Aleppo Pine) and in the term of María with *Pinus pinaster* Ait. (Maritime Pine). The material collected in Tarragona were obtained from pit-fall traps, located in forests of *P. nigra* subsp. *salzmannii*. Apparently the species is closely related to the resinous trees of the genus *Pinus* L.

Ptinus (Pseudoptinus) auberti Abeille de Perrin, 1869 (Figs. 3a-b)

Ptinus auberti Abeille de Perrin, 1869. *Pet. Nouv. Entomol.* 11: 42

Ptinus (Pseudoptinus) auberti Abeille de Perrin: Pic, 1895. *Échange* 11(129): 107

Ptinus (Pseudoptinus) auberti var. *dalmatinus* Pic, 1895. *Feuille Jeun. Natur.* 26[1895-1896]: 28

Ptinus (Pseudoptinus) auberti var. *rufipennis* Pic, 1929. *Échange* 45(438): 14

Specimens studied:

2 ♂♂ and 1 ♀. Labelleds as: 1 ♀ «02-IX-2017, Comis Vell, Vallnord-Arcalis, 2150 m, Ordino, Andorra, S02IHMa4, CREAF leg.»; 2 ♂♂ «19-IX-2017, Comis Vell, Vallnord-Arcalis, 2150 m, Ordino, Andorra, S02IHMa5, CREAF leg.». Captured with Malaise trap. Deposited in the collection of A. Viñolas.

2.8–3.2 mm in length.

Male (Fig. 3a). Elongated body, convex and with sub-parallel contour. Dark brown body, antennae, antennomeres 2-11, tibiae and tarsus reddish. Dense

and regular pubescence of slightly yellow colour. Head, eyes included, wider than the pronotum. Graceful antennae, no longer than body length. Pronotum longer than wide, strongly strangled in the basal quarter, surface covered with round granules. Long elytra and parallel contour. Striae formed by square points and flat intervals. Legs graceful. Aedeagus model of the subgenus according to Fig. 3c.

Female (Fig. 3b). Body more convex, ovoid contour and more blackish colour. Antennae and legs reddish. Shorter and thicker antennae. Pronotum as long as wide and less strangulated in the basal part. Elytra with an ovoid contour and very convex. Legs shorter than the male.

Distribution:

Described from a series of specimens collected in Toulon (France). Known from Crete, Slovenia, Spain, France, Italy and Portugal. From the Iberian Peninsula we are aware only of records originating in Spain (Madrid and Salamanca) and Portugal (São Martinho) (Fuente, 1932; Calmont, 2016). The specimens of Ordino are the first record for Andorra.

Biology:

Type specimens were collected by beating holm oak sheaves; also obtained by beating dead branches of *Pinus* sp. The larva develops in dry sheep droppings and the adults are located in the sheepfolds under excrement or stones (Calmont, 2016).

Ptinus (Ptinus) subpilosus Sturm, 1837 (Figs. 4a-b)

Ptinus subpilosus Sturm, 1837. *Deutschlands Fauna (Insecten)* 12: 82

Specimens studied:

1 ♂ and 1 ♀. Labelled as: 1 ♂ «28-VII-2017, Bordes de la Mollera, Llorts, Ordino, Andorra, S10ILMa2, CREAF leg.»; 1 ♀ «18-IX-2017, Bordes de la Mollera, Llorts, Ordino, Andorra, S10ILMa5, CREAF leg.». Captured with Malaise trap. Deposited in the collection of A. Viñolas.

2.1–3.0 mm in length.

Male (Fig. 4a). Elongated body, convex and with sub-parallel contour. Body, antennae and legs reddish brown. Pubescence not very dense, semi-erect and yellowish. Head, eyes included, wider than the pronotum. Very graceful antennae, its length equals that of the body. Pronotum longer than wide, little strangled in the basal quarter, with two small central crests and two small lateral projections. Long elytra and parallel contour. Striae formed by square points, smooth and convex intervals. Legs graceful. Aedeagus model of the subgenus according to Fig. 4c.

Female (Fig. 4b). Body more convex, ovoid contour. Head, antennae and legs densely pubescent. Wider pronotum with four small crests. Elytra with an ovoid contour and very convex. Legs shorter than the male.

Distribution:

Species with a broad European distribution (Calmont, 2016). In the Iberian Peninsula it seems to be circumscribed to the Spanish northeast, with records from: Barcelona, Girona, La Rioja, Lleida, Navarra and Zaragoza (Fuente, 1932; Bellés, 1978; Recalde Irurzun and San Martín Moreno, 2017). The specimens of Ordino are the first record for Andorra.

Biology:

It is considered strictly associated with forests, located in rotted wood and sometimes in anthills. Specimens from Lleida were captured with pit-fall traps located in a leafy area. Specimens from Andorra were captured with Malaise traps installed in a resinous trees area.

Acknowledgments

FIGURE 4. *Ptinus (Ptinus) subpilosus* Sturm, 1837: (a)-(b) Dorsal habitus: (a) Male; (b) Female (collected in Ordino, Andorra); (c) Aedeagus in ventral view (Scale bars (a)-(b) = 1 mm; (c) = 0.4 mm).

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CHAPTER 7

DISCUSSION

Our first study (Chapter 2), *Fecal matters: Implementing classical Coleoptera species lists with metabarcoding data from Passerine bird feces*, combined a metabarcoding approach and a traditional insect trap schema to determine if and how this new technology can influence results from conventional methods. The study was performed just below and at tree-line elevations. While neither the elevation of our plots nor the amount of open space showed influence upon biodiversity, Jaccard dissimilarity analysis did show high taxonomic variability between and within all collection types (3 traditional traps and feces collections). In addition, 39 species were found only in feces collections.

Feces collections not only added taxonomic coverage to the study, but functional feeding guild coverage was also broadened by the addition of metabarcoding; additional analyses of functional groups indicated that richness of respective functional groups varied according to collection type. For example, the richness of parasite-guild Coleoptera was higher in feces collections than the other three types of collections. However, a K-means analysis did indicate some collection types varied together. For example, attraction traps and feces tended to cluster together when species were organized into functional groups. The cluster analysis results were underlined by the lower levels of functional guild and taxonomic richness in feces and attraction collections relative to malaise and flight intercept. Feces collections also tended to contain a moderately higher species richness of phytophage-guild Coleoptera than flight intercept collections, suggesting that the supplementation of species lists produced from metabarocoding Passerine feces would add additional information to studies focused upon phytophage-guild Coleoptera diversity.

Unlike traditional traps, Passerine birds can actively search for prey, and feed upon insect species that are sessile. Thus, it's unsurprising that the Coleoptera found solely in the species lists created by metabarcoding Passerine feces included immobile Coleoptera such as *Rhamphus pulicarius* (Herbst, 1795), a leaf miner which has no abdominal legs in larval form.

These results indicate that metabarcoding Passerine feces can add both depth and breadth to a traditional survey of forest Coleoptera. However, biases are inherent in the metabarcoding pipeline. The process relies on correct morphological identification of voucher specimens whose DNA comprises DNA databases. Extracted DNA from samples of interest is compared to the DNA in these databases. The number of insect species both identified and DNA cataloged in DNA repository databases are still lower than the true

number of species. Also, raw abundance of specimens within samples of extracted DNA is thus far unattainable due to technological and biological biases.

Even with these limitations, however, advantages of the addition of a metabarcoding approach to a traditional trap schema still exist. Species-level identification of animals within samples using metabarcoding does not necessitate the use of taxonomic morphology group specialists, a type of specialization that is becoming less common and more expensive. Therefore, an experiment aiming to locate a specific species such a rare or invasive insect would particularly benefit from the use of metabarcoding. This focused approach would involve extracting DNA from voucher specimens of the species of interest and then including this DNA in a mock community, sequenced alongside the environmental or fecal samples. This technique would allow for a study plan with fewer biases inherent in the pipeline.

Chapter 2 in this dissertation examines Coleoptera community patterns through a methodology-based approach based on collection types and does not distinguish between bird species. Chapter 3, "*Metabarcoding Passerine bird feces at tree-line uncovers little intra- and inter-species dietary overlap*," does, and expands the focus to examining community patterns of both prey (insects) and predator (Passerines) at tree-line and involved a larger number of plots. This study also analyzed biodiversity of not only Coleoptera, but other insect orders as well.

Similar to Chapter 2, no difference in dietary overlap was found among Passerines species at different elevations, and links were found between richness of Passerines species and open space and elevation. Also similar to Chapter 2, extreme variation among all feces samples were found. When these data were separated along Passerine species lines, diets of Passerine species displayed high levels of both intra-and inter-species dissimilarity. Furthermore, even though high dietary overlap is generally more likely during times of high resource availability, no seasonal difference in dietary overlap was found among all Passerine species or the among the most common Passerine species. These data imply that Passerines are highly mobile feeders and have flexible diets. In turn, the flexible diets of these birds and lack of association to large scale landscape characteristics, including elevation, could indicate that future studies must collect smaller-scale or additional landscape-scale variables. Our results could also indicate that at least in the short- or mid-term, the upward shift of the tree-line in the Pyrenees, caused by land-use shifts and climate change, will not play a significant role in future community patterns of Passerines in this area.

Even though European Passerines are among the most studied birds in the world, conventional dietary studies have been limited to higher taxonomic level results or studies that that only involve nestlings. Our metabarcoding results recorded a different set of dietary components than those historically presented with conventional methods. Most notably, we found a very high affinity of European passerines to conifer aphids, especially *Cinara pini* (L., 1758) and *Eulachnus rileyi* (Williams, 1911). *Cinara pini* is a common European conifer pest, and the fact that it was found in over 50% of our samples indicates Passerines could play

important roles as top-down control in trophic cascades. *Eulachnus rileyi* is considered rare in Europe, yet it was the next most likely species to be found in our samples, suggesting the species may be more common than previously thought.

The taxonomic classification results of Chapter 2 and Chapter 3, however, should be tempered with the results of the mock community sequencing, which showed high levels of uncertainty in classifications of some prey insect groups. A complete mock community with extracted DNA of all species of interest, which is sequenced alongside the samples in question, is currently necessary for 100% taxonomic classification certainty. Even so, a majority of fecal metabarcoding studies have not included a mock community. However, despite the high levels of classification uncertainty in our metabarcoding studies, we still consider our results to be of interest as metabarcoding protocol and pipelines will continue to advance in efficacy and costs involved in the technique will undoubtedly continue to fall.

Our third study (Chapter 4), "*The Coleoptera community at tree-line is explained by divergent drivers: Taxonomic and functional guild approaches*" delved into Coleoptera species lists created by traditional traps (attraction, flight intercept, and malaise) in a set of plots at and below tree-line, some of which were utilized in the experimental design of Chapter 2. This study also expanded upon connections between biodiversity and large-scale (landscape) characteristics examined in the first two studies to include connections between biodiversity and a collection of smaller-scale (stand) characteristics. We found strong associations between abundance of saprophytes and large trees, an unsurprising finding as saproxylic Coleoptera biodiversity is often linked to old, veteran trees. However, the connections between saproxylic Coleoptera and dead wood, a link often found in other studies, was less clear in ours. Volume of dead wood did not affect the richness or abundance of saprophyte-guild or saproxylic Coleoptera, supporting previous studies that posit that either quality or connectivity of dead wood is a stronger predictor of saprophyte-guild or saproxylic biodiversity. In addition, we hypothesized that the low levels of dead wood in our study were insufficient to support many rare species. Interestingly, abundance and richness of phytophage-guild Coleoptera were positively correlated with dead wood. This finding lends support to other studies that found more positive responses to dead wood by non-saproxylic Coleoptera than saproxylic Coleoptera.

Edge effects were found to be important to biodiversity of Coleoptera, albeit through the action of different sets of forest characteristics. Coleoptera as well as saproxylic-only Coleoptera were more successful in areas with higher landscape-level (larger scale) tree coverage (i.e. lower percentage of openness) and lower stand-level (smaller scale) basal area. Coleoptera abundance was also positively correlated with higher elevation (at tree-line). As plots at tree-line tended to have higher openness, the data suggests sunlight and/or exposed substrate is important for Coleoptera success. Furthermore, higher open space is likely linked to a more complex architecture of forest with more sublevels.

Abundance of phytophage-guild Coleoptera was also linked to open space, but Coleoptera and saproxylic-only Coleoptera found positively correlated with larger areas of higher landscape-level tree coverage (low percent openness) interspersed with interior gaps in the stand-level tree cover (low basal area). Phytophage-guild Coleoptera was found positively correlated with both landscape-level open space and stand-level basal area and negatively correlated with medium tree density, i.e. positively correlated with edge habitat involving more open space and smaller copse of trees. Both taxonomic and some functional-guild groupings of Coleoptera were linked to edge effects through the interaction of different combinations of forest characteristics. These data emphasize the importance of utilizing both taxonomic and functional-guild approaches when analyzing Coleoptera community data.

Abundance of phytophage-guild Coleoptera was positively correlated to higher elevation, while the richness of phytophage-guild Coleoptera was negatively correlated to higher elevation. We hypothesize the higher abundance of phytophage-guild Coleoptera at higher elevation is due to the success of a few, less specialized species, while the higher richness at lower elevations is linked to warmer temperatures. Insects in general are strongly influenced by temperature, and even though the difference between the elevation of the highest plot and lowest plot in our study was only 498m, other studies have shown biodiversity differences along similar elevational gradients.

Climate change and land-use shifts seem to be driving the upward migration tree-line shift as well as tree-line densification in the Pyrenees. While the first two studies found no correlations between Passerine populations and elevation, the higher abundance of phytophage-guild Coleoptera at higher elevations does suggest that further tree-line densification without an upward migration tree-line shift could be detrimental to some members of this feeding guild. The further closure of tree cover in high elevation mountains could also be detrimental for many tree-associated Coleoptera population that are associated with edge effects.

The potential for bottom-up effects upon predators of both tree-associated and phytophage-guild Coleoptera could be acute. "Chapter 3: *Fecal matters: Implementing classical Coleoptera species lists with metabarcoding data from Passerine bird feces*" and "Chapter 4: *The Coleoptera community at tree-line is explained by divergent drivers: Taxonomic and functional guild approaches*" display complicated community patterns of insectivorous Passerines and their prey. We were not able to examine small-scale stand characteristics such as basal area and density of large trees as co-variables in our two studies examining Passerine diets. However, Passerines seem less affected by large-scale landscape characteristics, possibly due to their incredible diversity of diet. Passerine predators of phytophage-guild Coleoptera might shift to other prey in a future that includes a denser and higher tree-line. It's also possible the smaller-scale stand characteristics found linked to Coleoptera biodiversity are important drivers to Passerine populations as well. More studies are needed to examine stand characteristics such as edge effects at tree-line upon Passerines and other insectivorous predators. For example, the experiments

documented in this dissertation were conducted in a geographic area (Europe) and within a study system (European Passerines and Coleoptera) that is considered well-studied, yet we found 171 Coleoptera country records and recorded concentrations of Passerine dietary diversity previously undetected.

As climate change and land-use changes continue to shape an evolving and possibly alarming future for tree-line communities, it's imperative to use the best tools on hand to document the current state of biodiversity in these delicate landscapes. This dissertation demonstrates that using both morphological- and metabarcoding-based approaches to ecological challenges can be complementary. "Chapter 1: *Fecal matters: Implementing classical Coleoptera species lists with metabarcoding data from Passerine bird feces*" shows that metabarcoding Passerine feces can be combined with conventional collection types to create species lists with broader coverage. Therefore, while morphological-based taxonomic techniques are still critical due to wide knowledge gaps in DNA databases and other metabarcoding pipeline limitations, this modern technology should be a viable addition to ecologists' toolboxes.

CHAPTER 8

CONCLUSIONS

1. Insectivorous Passerines, as active predators, consume sessile forms of insects. Many sessile insects are undetectable by traditional insect traps. Metabarcoding Passerine feces can identify sessile and other functionally and taxonomically distinct insects. Combining this modern approach with a conventional trap schema brings breadth to non-saproxylic and saproxylic Coleoptera surveys.
2. European insectivorous Passerines are some of the most studied birds on earth, yet metabarcoding technology can uncover previously unknown Passerine dietary components, including an elevated affinity for aphids.
3. Overlap of dietary components within and among Passerine species was very high, open space and elevation did not affect dietary richness, indicating high dietary flexibility within these Passerine populations.
4. Connections between dead wood and saproxylic Coleoptera is complicated, and presence of veteran trees might be more important for Coleoptera success than volume of dead wood.
5. Different types of edge habitat (interior opening and perimeter borders) are strongly linked to Coleoptera biodiversity at tree-line.
6. Classifying Coleoptera into functional guild and taxonomic groupings broaden survey reach and expand our comprehension of the whole system.
7. Tree-line densification and upward shifts of the tree-line could lower future success of some groupings of Coleoptera.

APPENDIX 2

Appendix 2.1 List of all birds from which feces were collected

num. catches	Scientific name		Common name
53	*Periparus ater*	(L., 1758)	coal tit
20	*Lophophanes cristatus*	(L., 1758)	European crested tit
18	*Prunella modularis*	(L., 1758)	dunnock
9	*Regulus regulus*	(L., 1758)	goldcrest
7	*Cyanistes caeruleus*	(L., 1758)	blue tit
7	*Erithacus rubecula*	(L., 1758)	European robin
3	*Certhia brachydactyla*	Brehm, 1820	short-toed treecreeper
3	*Parus major*	L., 1758	great tit
3	*Sylvia atricapilla*	(L., 1758)	Eurasian blackcap
2	*Phoenicurus ochrurus*	(S. G. Gmelin, 1774)	black redstart
2	*Phylloscopus collybita*	(Vieillot, 1817)	common chiffchaff
2	*Phylloscopus trochillus*	(L., 1758)	willow warbler
2	*Regulus ignicapilla*	(Temminck, 1820)	common firecrest
1	*Anthus spinoletta*	(Linnaeus, 1758)	water pipit

Appendix 2

Appendix 2.2 Composition of mock community

Order	Family: Genus species	Ratio of amount of DNA within mock community
Mock 1		
Coleoptera	Melyridae: *Dastes aeratus*	0.25
Orthoptera	Acrididae: *Stauroderus scalaris*	0.13
Hymenoptera	Vespidae: *Vespula germanica*	0.13
Diptera	Calliphoridae: *Calliphora* vicina	0.25
Hemiptera	Miridae	0.25

Mock 2		
Hymenoptera	Formicidae: *Formica fusca*	0.22
Diptera	Tipulidae: *Tipula* pallidicosta	0.11
Hymenoptera	Vespidae: *Vespula vulgaris*	0.22
Diptera	Calliphoridae: *Calliphora* loewi	0.22
Hemiptera	Macropsinae	0.22

Mock 3		
Hymenoptera	Vespidae: *Vespula vulgaris*	0.14
Coleoptera	Busprestidae: *Anthaxia quadripunctata*	0.29
Hymenoptera	Vespidae: *Vespula germanica*	0.14
Diptera	Phoridae: *Phora* sp.	0.29
Lepidoptera	Zygaenidae: *Zygaena* filipendulae	0.14

Mock 4		
Hymenoptera	Vespidae: *Vespula rufa*	0.13
Orthoptera	Acrididae: *Stauroderus scalaris*	0.13
Lepidoptera	Zygaenidae	0.25
Diptera	Dilophus: *Dilophus femoratus*	0.25
Diptera	Calliphoridae: *Calliphora loewi*	0.25

Appendix 2

Order	Family: Genus species	Ratio of amount of DNA within mock community
	Mock 5	
Hymenoptera	Vespidae: *Vespula germanica*	0.25
Coleoptera	Melyridae: *Dastes aeratus*	0.25
Lepidoptera	Zygaenidae: *Zygaena filipendulae*	0.13
Coleoptera	Busprestidae: *Anthaxia quadripunctata*	0.13
Hemiptera	Miridae	0.25

Appendix 2

Appendix 2.3 QIIME2 pipeline

Import the fastq files as paired sequences.
```
qiime tools import \
 --type 'SampleData[PairedEndSequencesWithQuality]' \
 --input-path $PWD /paired \
 --input-format CasavaOneEightSingleLanePerSampleDirFmt \
 --output-path $PWD/demux-paired-end.qza
```

Visualize the imported file
```
qiime demux summarize \
 --i-data $PWD/ demux-paired-end.qza
 --o-visualization $PWD/demux-paired-end.qzv
```

Quality control and feature table construction with dada2
```
qiime dada2 denoise-paired \
 --i-demultiplexed-seqs $PWD/demux-paired-end.qza \
 --p-trim-left-f 10 \
 --p-trim-left-r 18 \
 --p-trunc-len-f 247 \
 --p-trunc-len-r 250 \
 --o-table $PWD/table_paired.qza \
 --o-representative-sequences $PWD/rep-seqs_paired.qza \
 --o-denoising-stats $PWD/denoising-stats_paired.qza
```

Visualize the files
```
qiime metadata tabulate \
 --m-input-file $PWD/denoising-stats_paired.qza \
 --o-visualization $PWD/denoising-stats_paired.qzv
```

Visualize FeatureTable and FeatureData
```
qiime feature-table summarize \
 --i-table $PWD/table.qza \
 --o-visualization $PWD/table.qzv \
 --m-sample-metadata-file $PWD/ mapping_file_samples.tsv
```

```
qiime feature-table tabulate-seqs \
 --i-data $PWD/rep-seqs.qza \
 --o-visualization $PWD/rep-seqs.qzv
```

Taxonomic assignment
```
qiime feature-classifier classify-sklearn \
    --i-classifier $PWD/bold_anml_classifier.qza \
    --i-reads $PWD/rep-seqs_paired.qza \
    --o-classification $PWD/sample_taxonomy2_paired_devon.qza
```

```
qiime taxa filter-table \
    --i-table $PWD/table_paired.qza \
```

```
    --i-taxonomy $PWD/sample_taxonomy2_paired_devon.qza \
    --p-exclude Protozoa,Fungi \
    --o-filtered-table $PWD/filtered_table2_bold_paired_devon.qza


Visulize in barplot
qiime taxa barplot \
    --i-table $PWD/filtered_table2_bold_paired_devon.qza \
    --i-taxonomy $PWD/sample_taxonomy2_paired_devon.qza \
    --m-metadata-file $PWD/mapping_file_samples.tsv \
    --o-visualization $PWD/barplot2_samples_bold_paired_devon.qzv
```

Appendix 2

Appendix 2.4 Available online at 10.5281/zenodo.7229509

APPENDIX 3

Appendix 3.1

Five mock communities of arthropods created with specimens caught in mist net plots. Ratio= Ratio of amount of DNA within mock community

Order	Family: Genus species	Ratio
	Mock 1	
Coleoptera	Melyridae: *Dastes aeratus*	0.25
Orthoptera	Acrididae: *Stauroderus scalaris*	0.13
Hymenoptera	Vespidae: *Vespula germanica*	0.13
Diptera	Calliphoridae: *Calliphora vicina*	0.25
Hemiptera	Miridae	0.25

	Mock 2	
Hymenoptera	Formicidae: *Formica fusca*	0.22
Diptera	Tipulidae: *Tipula pallidicosta*	0.11
Hymenoptera	Vespidae: *Vespula vulgaris*	0.22
Diptera	Calliphoridae: *Calliphora loewi*	0.22
Hemiptera	Macropsinae	0.22

	Mock 3	
Hymenoptera	Vespidae: *Vespula vulgaris*	0.14
Coleoptera	Busprestidae: *Anthaxia quadripunctata*	0.29
Hymenoptera	Vespidae: *Vespula germanica*	0.14
Diptera	Phoridae: *Phora* sp.	0.29
Lepidoptera	Zygaenidae: *Zygaena filipendulae*	0.14

Appendix 3

Order	Family: Genus species	Ratio
	Mock 4	
Hymenoptera	Vespidae: *Vespula rufa*	0.13
Orthoptera	Acrididae: *Stauroderus scalaris*	0.13
Lepidoptera	Zygaenidae	0.25
Diptera	Dilophus: *Dilophus femoratus*	0.25
Diptera	Calliphoridae: *Calliphora loewi*	0.25

	Mock 5	
Hymenoptera	Vespidae: *Vespula germanica*	0.25
Coleoptera	Melyridae: *Dastes aeratus*	0.25
Lepidoptera	Zygaenidae: *Zygaena filipendulae*	0.13
Coleoptera	Busprestidae: *Anthaxia quadripunctata*	0.13
Hemiptera	Miridae	0.25

Appendix 3

Appendix 3.2

QIIME2 pipeline
Import the fastq files as paired sequences.
```
qiime tools import \
 --type 'SampleData[PairedEndSequencesWithQuality]' \
 --input-path $PWD /paired \
 --input-format CasavaOneEightSingleLanePerSampleDirFmt \
 --output-path $PWD/demux-paired-end.qza
```

Visualize the imported file
```
qiime demux summarize \
 --i-data $PWD/ demux-paired-end.qza
 --o-visualization $PWD/demux-paired-end.qzv
```

Quality control and feature table construction with dada2
```
qiime dada2 denoise-paired \
 --i-demultiplexed-seqs $PWD/demux-paired-end.qza \
 --p-trim-left-f 10 \
 --p-trim-left-r 18 \
 --p-trunc-len-f 247 \
 --p-trunc-len-r 250 \
 --o-table $PWD/table_paired.qza \
 --o-representative-sequences $PWD/rep-seqs_paired.qza \
 --o-denoising-stats $PWD/denoising-stats_paired.qza
```

Visualize the files
```
qiime metadata tabulate \
 --m-input-file $PWD/denoising-stats_paired.qza \
 --o-visualization $PWD/denoising-stats_paired.qzv
```

Visualize FeatureTable and FeatureData
```
qiime feature-table summarize \
 --i-table $PWD/table.qza \
 --o-visualization $PWD/table.qzv \
 --m-sample-metadata-file $PWD/ mapping_file_samples.tsv
```

```
qiime feature-table tabulate-seqs \
 --i-data $PWD/rep-seqs.qza \
 --o-visualization $PWD/rep-seqs.qzv
```

Taxonomic assignment
```
qiime feature-classifier classify-sklearn \
    --i-classifier $PWD/bold_anml_classifier.qza \
    --i-reads $PWD/rep-seqs_paired.qza \
    --o-classification $PWD/sample_taxonomy2_paired_devon.qza
```

```
qiime taxa filter-table \
```

```
    --i-table $PWD/table_paired.qza \
    --i-taxonomy $PWD/sample_taxonomy2_paired_devon.qza \
    --p-exclude Protozoa,Fungi \
    --o-filtered-table $PWD/filtered_table2_bold_paired_devon.qza
```

Visulize in barplot
```
qiime taxa barplot \
    --i-table $PWD/filtered_table2_bold_paired_devon.qza \
    --i-taxonomy $PWD/sample_taxonomy2_paired_devon.qza \
    --m-metadata-file $PWD/mapping_file_samples.tsv \
    --o-visualization $PWD/barplot2_samples_bold_paired_devon.qzv
```

Appendix 3

Appendix 3.4

GLM results (Beta estimates and P values): responses of prey richness of combinations of Passerines when examining open space, elevation, and season as independent variables. Post hoc Tukey tests are shown for the season variable.

		P. ater+L. cristatus	*L. cristatus*	*P. ater*	all birds
Elevation	Estimate	0	0.28	0	-0.19
	P value	0.63	0.23	0.16	0.07
Open space	Estimate	-0.09	-0.22	-0.11	0.12
	P value	0.45	0.37	0.32	0.3
Season: Spring:Summer	Estimate	0.15	0.02	0.34	0.17
	P value	0.42	0.94	0.16	0.36
Season: Spring:Fall	Estimate	0.32	0.38	0.59	0.11
	P value	0.09	0.11	0.01	0.52

Season: Summer:Fall		0.44	0.19	0.33	0.83
Season: Spring:Fall	Tukey tests	0.21	0.25	0.04	0.79
Season: Spring:Summer		0.7	1	0.33	0.62

Appendix 3

Appendix 3.5

a. Mean (±SD) mean overlap or dissimilarity between *Lophophanes cristatus/Periparus ater* (dark grey bar) and among all birds captured (light grey bar). b. Mean (±SD) mean overlap or dissimilarity within *L. cristatus*, within *P. ater*, between *L. cristatus/P. ater*, and among all birds captured in plots at tree-line and in plots below tree-line. Plot made with R version 1.3.1056 and MS Office
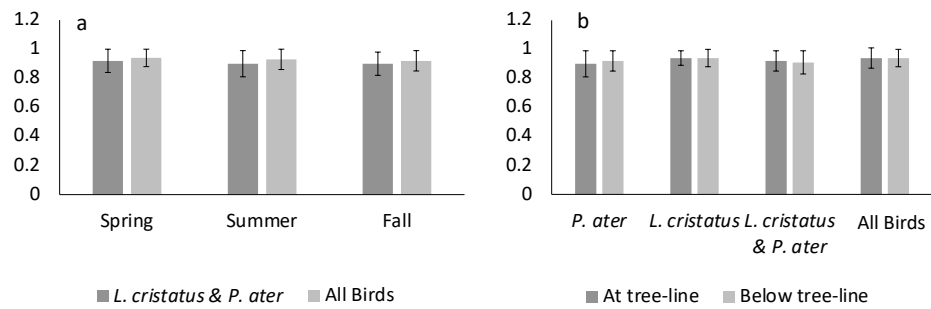
Appendix 3

Appendix 3.6

Eleven MOTUs present mostly likely to be present in birds' feces.

	MOTU	Biology and notes	Percentage of feces samples in which MOTU was present
Hemiptera	*Cinara pini*	aphid conifer pest	53.03
Hemiptera	*Eulachnus rileyi*	aphid conifer pest	37.88
Diptera	*Parasyrphus vittiger*	predatory fly upon aphids	29.55
Lepidoptera	*Panolis flammea*	moth conifer pest	28.03
Hymenoptera	*Pauesia* sp.	wasp parasitoid	25
Coleoptera	*Anthonomus phyllocola*	beetle conifer pest	19.7
Hemiptera	*Corylobium avellanae*	hazelnut pest	18.94
Hymenoptera	*Formica* sp.	wood ant	17.42
Lepidoptera	*Rhyacionia pinivorana*	moth conifer pest	17.42
Lepidoptera	*Hylaea* sp.	moth	16.67
Coleoptera	*Otiorhynchus singularis*	polyphagous weevil, including conifer and rhododendron	15.91

Appendix 3

Appendix 3.7

MOTU richness beta estimates and P values of prey families with open space and elevation as independent covariate variables. Season was included in the model as a fixed factor. Post hoc Tukey tests of seasonality are included. Orthoptera was unable to be analyzed due to low capture numbers. Numbers in bold indicate significant differences with P<0.05.

		Diptera	Coleoptera	Hemiptera	Hymenoptera	Lepidoptera
Elevation	Estimate	-0.34	-0.15	-0.10	-0.15	-0.07
	P value	0.11	0.37	0.46	0.33	0.58
Open space	Estimate	0.31	0.22	-0.01	0.06	0.00
	P value	0.49	0.22	0.93	0.70	1.00
Season: Spring:Summer	Estimate	-0.32	0.65	-0.22	-0.60	0.26
	P value	0.21	0.01	0.25	0.01	0.21
Season: Spring:Fall	Estimate	0.20	0.42	-0.60	-0.29	0.29
	P value	0.08	0.03	0.00	0.08	0.05
Season: Summer:Fall		0.05	0.57	0.49	0.42	0.42
Season: Fall:Spring	Tukey tests	0.38	0.07	0.00	0.18	0.13
Season: Spring:Summer		0.35	0.02	0.16	0.04	0.99

APPENDIX 4

Appendix 4.1. List of species captured. AR=Andorran Record. Obligate=Obligate saproxylic, facultative=Facultative saproxylic, NS=Not saproxylic, or ND=No data. "Multiple" is a designation given to a grouping with both non-saproxylic and saproxylic members. AR=Andorran Record. Phyt=Phytophagous. Wfee=Wood-feeding. Para=Parasitic. Pred=Predator. Fung=Fungivore. Detr=Detritivore.

	AR	Species	Family	Saproxylic	Phyt	Wfee	Para	Pred	Fung	Detr
1	yes	*Ernobius mollis*	Anobiidae	obligate	-	Yes	-	-	-	-
2	yes	*Ernobius nigrinus*	Anobiidae	obligate	-	Yes	-	-	-	-
3	no	*Ernobius pini*	Anobiidae	obligate	-	-	-	-	-	Yes
4	yes	*Protapion ruficroides*	Brentidae	NS	-	-	-	-	-	-
5	yes	*Anthaxia carmen*	Buprestidae	obligate	Yes	Yes	-	-	-	-
6	no	*Anthaxia quadripunctata*	Buprestidae	obligate	Yes	Yes	-	-	-	-
7	yes	*Anthaxia sepulchralis*	Buprestidae	obligate	Yes	Yes	-	-	-	-
8	no	*Buprestis rustica*	Buprestidae	obligate	Yes	Yes	-	-	-	-
9	no	*Cantharis obscura*	Cantharidae	NS	Yes	-	-	Yes	-	-
10	yes	*Malthodes atratus atratus*	Cantharidae	obligate	Yes	Yes	-	Yes	-	-
11	yes	*Malthodes chelifer*	Cantharidae	obligate	Yes	Yes	-	Yes	-	-
12	no	*Malthodes forcipifer*	Cantharidae	obligate	Yes	Yes	-	Yes	-	-
13	NA	*Malthodes group femoralis*	Cantharidae	obligate	Yes	Yes	-	Yes	-	-
14	yes	*Malthodes guttifer*	Cantharidae	obligate	Yes	Yes	-	Yes	-	-
15	NA	*Malthodes sp*	Cantharidae	obligate	Yes	Yes	-	Yes	-	-
16	NA	*Malthodes sp B*	Cantharidae	obligate	Yes	Yes	-	Yes	-	-
17	yes	*Calodromius spilotus*	Carabidae	facultative	-	-	-	-	-	-
18	yes	*Dromius fenestratus*	Carabidae	facultative	-	-	-	Yes	-	-

Appendix 4

	AR	Species	Family	Saproxylic	Phyt	Wfee	Para	Pred	Fung	Detr
19	yes	*Lebia cruxminor var*	Carabidae	ND	-	-	Yes	Yes	-	-
20	no	*Microlestes luctuosus*	Carabidae	ND	-	-	-	Yes	-	-
21	yes	*Alosterna tabacicolor*	Cerambycidae	obligate	Yes	Yes	-	-	-	-
22	no	*Anastrangalia dubia*	Cerambycidae	obligate	Yes	Yes	-	-	-	-
23	no	*Anastrangalia sanguinolenta*	Cerambycidae	obligate	Yes	Yes	-	-	-	-
24	yes	*Certallum ebulinum*	Cerambycidae	obligate	Yes	Yes	-	-	-	-
25	no	*Judolia sexmaculata*	Cerambycidae	obligate	Yes	Yes	-	-	-	-
26	yes	*Lepturobosca virens*	Cerambycidae	obligate	Yes	Yes	-	-	-	-
27	yes	*Pachytodes cerambyciformis*	Cerambycidae	obligate	Yes	Yes	-	-	-	-
28	yes	*Pogonocherus fasciculatus*	Cerambycidae	obligate	Yes	Yes	-	-	-	-
29	yes	*Stenurella melanura*	Cerambycidae	obligate	Yes	Yes	-	-	-	-
30	yes	*Stictoleptura maculicornis*	Cerambycidae	obligate	Yes	Yes	-	-	-	-
31	yes	*Stictoleptura rubra*	Cerambycidae	obligate	Yes	Yes	-	-	-	-
32	yes	*Stictoleptura stragulata*	Cerambycidae	obligate	Yes	Yes	-	-	-	-
33	yes	*Aphthona herbigrada*	Chrysomelidae	NS	Yes	-	-	-	-	-
34	yes	*Aphthona stussineri*	Chrysomelidae	NS	Yes	-	-	-	-	-
35	yes	*Calomicrus circumfusus*	Chrysomelidae	NS	Yes	-	-	-	-	-
36	yes	*Chaetocnema aerosa*	Chrysomelidae	NS	Yes	-	-	-	-	-
37	no	*Chaetocnema angustula*	Chrysomelidae	NS	Yes	-	-	-	-	-
38	no	*Clytra quadripunctata*	Chrysomelidae	NS	Yes	-	-	-	-	-
39	no	*Cryptocephalus labiatus*	Chrysomelidae	NS	Yes	-	-	-	-	-
40	yes	*Cryptocephalus pini*	Chrysomelidae	NS	Yes	-	-	-	-	Yes
41	yes	*Labidostomis humeralis*	Chrysomelidae	NS	Yes	-	-	-	-	-
42	no	*Longitarsus ochroleucus*	Chrysomelidae	NS	Yes	-	-	-	-	-

Appendix 4

	AR	Species	Family	Saproxylic	Phyt	Wfee	Para	Pred	Fung	Detr
43	no	*Longitarsus succineus*	Chrysomelidae	NS	Yes	-	-	-	-	-
44	yes	*Luperus pyrenaeus*	Chrysomelidae	NS	Yes	-	-	-	-	-
45	no	*Neocrepidodera melanopus*	Chrysomelidae	NS	Yes	-	-	-	-	-
46	yes	*Phyllotreta undulata*	Chrysomelidae	NS	Yes	-	-	-	-	-
47	yes	*Smaragdina concolor*	Chrysomelidae	NS	Yes	-	-	-	-	-
48	yes	*Thanasimus formicarius*	Cleridae	obligate	-	-	-	Yes	-	-
49	yes	*Trichodes apiarius*	Cleridae	facultative	Yes	-	Yes	Yes	-	-
50	no	*Adalia decempunctata*	Coccinellidae	NS	-	-	-	Yes	-	-
51	no	*Calvia quatuordecimguttata*	Coccinellidae	NS	-	-	-	Yes	-	-
52	yes	*Coccinella hieroglyphica*	Coccinellidae	NS	-	-	-	Yes	-	-
53	yes	*Coccinella magnifica*	Coccinellidae	NS	-	-	-	Yes	-	-
54	no	*Coccinella septempunctata*	Coccinellidae	NS	-	-	-	Yes	-	-
55	yes	*Exochomus quadripustulatus*	Coccinellidae	NS	-	-	-	Yes	-	-
56	no	*Halyzia sedecimguttata*	Coccinellidae	NS	-	-	-	Yes	-	-
57	yes	*Myrrha octodecimguttata*	Coccinellidae	NS	-	-	-	Yes	-	-
58	yes	*Myzia oblongoguttata*	Coccinellidae	NS	-	-	-	Yes	-	-
59	no	*Propylea quatuordecimpunctata*	Coccinellidae	NS	-	-	-	Yes	-	-
60	yes	*Scymnus mimulus*	Coccinellidae	NS	-	-	-	Yes	-	-
61	yes	*Arthrolips picea*	Corylophidae	facultative	-	-	-	-	Yes	-
62	yes	*Antherophagus similis*	Cryptophagidae	facultative	-	-	-	-	Yes	-
63	yes	*Cryptophagus cylindrellus*	Cryptophagidae	obligate	-	-	-	-	Yes	-
64	yes	*Cryptophagus denticulatus*	Cryptophagidae	facultative	-	-	-	-	Yes	-
65	yes	*Cryptophagus jakowlewi*	Cryptophagidae	obligate	-	-	-	-	Yes	-

Appendix 4

	AR	Species	Family	Saproxylic	Phyt	Wfee	Para	Pred	Fung	Detr
66	yes	*Cryptophagus saginatus*	Cryptophagidae	facultative	-	-	-	-	Yes	-
67	yes	*Cryptophagus scanicus*	Cryptophagidae	facultative	-	-	-	-	Yes	-
68	NA	*Cryptophagus sp*	Cryptophagidae	facultative	-	-	-	-	Yes	-
69	yes	*Anthonomus phyllocola*	Curculionidae	NS	Yes	-	-	-	-	-
70	yes	*Anthonomus rubi*	Curculionidae	NS	Yes	-	-	-	-	-
71	yes	*Brachonyx pineti*	Curculionidae	NS	Yes	-	-	-	-	-
72	yes	*Brachyderes incanus*	Curculionidae	NS	Yes	-	-	-	-	-
73	yes	*Curculio venosus*	Curculionidae	NS	Yes	-	-	-	-	-
74	yes	*Homapterus subnudus*	Curculionidae	NS	-	-	-	-	-	-
75	yes	*Ips acuminatus*	Curculionidae	obligate	-	Yes	-	-	-	-
76	yes	*Magdalis duplicata*	Curculionidae	obligate	-	Yes	-	-	-	-
77	yes	*Magdalis frontalis*	Curculionidae	obligate	-	Yes	-	-	-	-
78	yes	*Magdalis memnonia*	Curculionidae	obligate	-	Yes	-	-	-	-
79	yes	*Magdalis rufa*	Curculionidae	obligate	-	Yes	-	-	-	-
80	yes	*Mecinus dorsalis*	Curculionidae	NS	Yes	-	-	-	-	-
81	yes	*Miarus campanulae*	Curculionidae	NS	Yes	-	-	-	-	-
82	yes	*Micrelus ericae*	Curculionidae	NS	Yes	-	-	-	-	-
83	yes	*Orthotomicus laricis*	Curculionidae	obligate	-	Yes	-	-	-	-
84	no	*Otiorhynchus singularis*	Curculionidae	NS	Yes	-	-	-	-	-
85	yes	*Phloeotribus rhododactylus*	Curculionidae	obligate	-	Yes	-	-	-	-
86	no	*Phyllobius alpinus*	Curculionidae	NS	Yes	-	-	-	-	-
87	yes	*Phyllobius pomaceus*	Curculionidae	NS	Yes	-	-	-	-	-
88	yes	*Pityogenes bistridentatus*	Curculionidae	obligate	-	Yes	-	-	-	-
89	yes	*Pityogenes chalcographus*	Curculionidae	obligate	-	Yes	-	-	-	-

Appendix 4

	AR	Species	Family	Saproxylic	Phyt	Wfee	Para	Pred	Fung	Detr
90	yes	*Pityogenes conjuntus*	Curculionidae	obligate	-	Yes	-	-	-	-
91	yes	*Pityogenes quadridens*	Curculionidae	obligate	-	Yes	-	-	-	-
92	yes	*Pityogenes trepanatus*	Curculionidae	obligate	-	Yes	-	-	-	-
93	yes	*Pityophthorus buyssoni*	Curculionidae	obligate	-	Yes	-	-	-	-
94	yes	*Pityophthorus glabratus*	Curculionidae	obligate	-	Yes	-	-	-	-
95	yes	*Pityophthorus pityographus*	Curculionidae	obligate	-	Yes	-	-	-	-
96	yes	*Rhamphus pulicarius*	Curculionidae	NS	Yes	-	-	-	-	-
97	yes	*Strophosoma melanogrammum*	Curculionidae	NS	Yes	-	-	-	-	-
98	yes	*Tomicus piniperda*	Curculionidae	obligate	-	Yes	-	-	-	-
99	NA	*Tychius sp*	Curculionidae	NS	Yes	-	-	-	-	-
100	no	*Anthrenus fuscus*	Dermestidae	facultative	Yes	-	-	-	-	Yes
101	no	*Anthrenus museorum*	Dermestidae	facultative	Yes	-	-	-	-	Yes
102	yes	*Ctesias serra*	Dermestidae	facultative	-	-	-	-	-	Yes
103	yes	*Idolus picipennis*	Elateridae	ND	-	-	-	-	-	-
104	yes	*Pheletes aeroniger*	Elateridae	NS	Yes	-	-	-	-	-
105	yes	*Triplax russica*	Erotylidae	obligate	-	-	-	-	Yes	-
106	yes	*Helophorus glacialis*	Helophoridae	NS	-	-	-	-	-	-
107	NA	*Hister sp*	Histeridae	obligate	-	-	-	Yes	-	-
108	NA	*Cercyon sp*	Hydrophilidae	facultative	-	-	-	Yes	-	Yes
109	yes	*Cryptopleurum crenatum*	Hydrophilidae	NS	-	-	-	-	-	Yes
110	yes	*Sphaeridium bipustulatum*	Hydrophilidae	NS	-	-	-	Yes	-	Yes
111	yes	*Sphaeridium lunatum*	Hydrophilidae	NS	-	-	-	Yes	-	Yes
112	yes	*Sphaeridium marginatum*	Hydrophilidae	NS	-	-	-	Yes	-	Yes

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	AR	Species	Family	Saproxylic	Phyt	Wfee	Para	Pred	Fung	Detr
113	yes	*Brachypterolus longulus*	Kateretidae	NS	Yes	-	-	-	-	-
114	no	*Lampyris noctiluca*	Lampyridae	NS	-	-	-	Yes	-	-
115	yes	*Corticarina curta*	Latridiidae	facultative	-	-	-	-	Yes	-
116	yes	*Enicmus testaceus*	Latridiidae	facultative	-	-	-	-	Yes	-
117	yes	*Stephostethus lardarius*	Latridiidae	facultative	-	-	-	-	Yes	-
118	NA	*Agathidium sp*	Leiodidae	facultative	-	-	-	-	Yes	-
119	yes	*Anisotoma castanea*	Leiodidae	obligate	-	-	-	-	Yes	-
120	yes	*Anisotoma humeralis*	Leiodidae	facultative	-	-	-	-	Yes	-
121	NA	*Catops sp*	Leiodidae	multiple	-	-	-	-	Yes	Yes
122	yes	*Catops tristis*	Leiodidae	NS	-	-	-	-	-	Yes
123	yes	*Hydnobius multistriatus*	Leiodidae	facultative	-	-	-	-	Yes	Yes
124	yes	*Leiodes dubia*	Leiodidae	facultative	-	-	-	-	Yes	Yes
125	yes	*Leiodes obscura*	Leiodidae	facultative	-	-	-	-	Yes	Yes
126	NA	*Leiodes sp*	Leiodidae	facultative	-	-	-	-	Yes	Yes
127	NA	*Sciodrepoides sp*	Leiodidae	multiple	-	-	-	-	Yes	Yes
128	yes	*Sciodrepoides watsoni*	Leiodidae	facultative	-	-	-	-	-	Yes
129	yes	*Attalus amictus*	Malachiidae	facultative	Yes	-	-	Yes	-	-
130	no	*Axinotarsus tripatriae*	Malachiidae	facultative	Yes	-	-	Yes	-	-
131	yes	*Micrinus dimorphus*	Malachiidae	facultative	Yes	-	-	Yes	-	-
132	yes	*Troglops cephalotes*	Malachiidae	facultative	Yes	-	-	Yes	-	-
133	no	*Mylabris flexuosa*	Meloidae	NS	Yes	-	Yes	-	-	-
134	yes	*Aplocnemus alpestris*	Melyridae	obligate	-	-	-	Yes	-	-
135	no	*Danacea pallipes*	Melyridae	obligate	-	-	-	Yes	-	-
136	yes	*Dasytes gonocerus*	Melyridae	obligate	-	-	-	Yes	-	-

Appendix 4

	AR	Species	Family	Saproxylic	Phyt	Wfee	Para	Pred	Fung	Detr
137	no	*Dasytes niger*	Melyridae	obligate	-	-	-	Yes	-	-
138	no	*Dasytes nigropilosus*	Melyridae	obligate	-	-	-	Yes	-	-
139	no	*Dasytes plumbeus*	Melyridae	obligate	-	-	-	Yes	-	-
140	no	*Dasytes subaeneus*	Melyridae	obligate	-	-	-	Yes	-	-
141	no	*Dasytes virens*	Melyridae	obligate	-	-	-	Yes	-	-
142	NA	*Enicopus sp*	Melyridae	obligate	-	-	-	Yes	-	-
143	yes	*Trichoceble memnonia*	Melyridae	obligate	-	-	-	Yes	-	-
144	yes	*Rhizophagus depressus*	Monotomidae	obligate	-	-	-	Yes	-	-
145	yes	*Curtimorda maculosa*	Mordellidae	obligate	Yes	-	-	Yes	-	-
146	yes	*Mordella aculeata*	Mordellidae	obligate	Yes	-	-	-	Yes	-
147	no	*Brassicogethes viridescens*	Nitidulidae	NS	Yes	-	-	-	-	-
148	no	*Epuraea marseuli*	Nitidulidae	facultative	Yes	-	-	Yes	-	-
149	NA	*Epuraea sp*	Nitidulidae	facultative	Yes	-	-	Yes	-	-
150	no	*Fabogethes nigrescens*	Nitidulidae	NS	Yes	-	-	-	-	-
151	yes	*Glischrochilus quadripunctatus*	Nitidulidae	facultative	-	-	-	-	-	Yes
152	NA	*Meligethes sp*	Nitidulidae	NS	Yes	-	-	-	-	-
153	NA	*Nitidulidae gn sp*	Nitidulidae	multiple	-	-	-	-	-	-
154	yes	*Sagittogethes obscurus*	Nitidulidae	NS	Yes	-	-	-	-	-
155	no	*Chrysanthia viridissima*	Oedemeridae	obligate	Yes	-	-	-	-	-
156	yes	*Acrotrichis grandicollis*	Ptiliidae	facultative	-	-	-	-	Yes	-
157	yes	*Acrotrichis parva*	Ptiliidae	facultative	-	-	-	-	Yes	-
158	yes	*Acrotrichis rugulosa*	Ptiliidae	facultative	-	-	-	-	Yes	-
159	yes	*Ptenidium nitidum*	Ptiliidae	facultative	-	-	-	-	Yes	-

	AR	Species	Family	Saproxylic	Phyt	Wfee	Para	Pred	Fung	Detr
160	NA	*Ptiliidae gn sp*	Ptiliidae	facultative	-	-	-	-	Yes	-
161	yes	*Ptiliola brevicollis*	Ptiliidae	facultative	-	-	-	-	Yes	-
162	yes	*Ptiliolum fuscum*	Ptiliidae	facultative	-	-	-	-	Yes	-
163	yes	*Dryophilus anobioides*	Ptinidae	facultative	-	Yes	-	-	Yes	-
164	no	*Ptinus auberti*	Ptinidae	facultative	-	Yes	-	-	Yes	Yes
165	no	*Ptinus dubius*	Ptinidae	facultative	-	Yes	-	-	Yes	Yes
166	no	*Ptinus subpilosus*	Ptinidae	facultative	-	-	-	-	-	Yes
167	yes	*Rabocerus foveolatus*	Salpingidae	obligate	-	-	-	Yes	-	-
168	yes	*Sphaeriestes castaneus*	Salpingidae	obligate	-	-	-	Yes	-	Yes
169	yes	*Acrossus rufipes*	Scarabaeidae	NS	-	-	-	-	-	Yes
170	yes	*Agoliinus satyrus*	Scarabaeidae	NS	-	-	-	-	-	Yes
171	no	*Amidorus obscurus*	Scarabaeidae	NS	-	-	-	-	-	Yes
172	yes	*Loraphodius suarius*	Scarabaeidae	NS	-	-	-	-	-	Yes
173	yes	*Nimbus contaminatus*	Scarabaeidae	NS	-	-	-	-	-	Yes
174	yes	*Omaloplia ruricola*	Scarabaeidae	NS	Yes	-	-	-	-	-
175	yes	*Onthophagus fracticornis*	Scarabaeidae	NS	-	-	-	-	-	Yes
176	yes	*Rhizotrogus marginipes*	Scarabaeidae	NS	Yes	-	-	-	-	-
177	yes	*Trichius fasciatus*	Scarabaeidae	obligate	Yes	-	-	-	-	-
178	yes	*Elodes elongatus*	Scirtidae	NS	-	Yes	-	-	Yes	Yes
179	yes	*Anaspis frontalis*	Scraptiidae	obligate	-	Yes	-	-	Yes	Yes
180	yes	*Anaspis pulicaria*	Scraptiidae	obligate	Yes	-	-	-	-	-
181	yes	*Anaspis pyrenaea*	Scraptiidae	obligate	Yes	-	-	-	-	-
182	yes	*Anaspis ruficollis*	Scraptiidae	obligate	Yes	-	-	-	-	-
183	yes	*Anaspis rufilabris*	Scraptiidae	obligate	Yes	-	-	-	-	-

	AR	Species	Family	Saproxylic	Phyt	Wfee	Para	Pred	Fung	Detr
184	NA	*Anaspis sp*	Scraptiidae	obligate	Yes	-	-	-	-	-
185	yes	*Anaspis thoracica*	Scraptiidae	obligate	Yes	-	-	-	-	-
186	yes	*Anaspis varians*	Scraptiidae	obligate	Yes	-	-	-	-	-
187	NA	*Scraptiidae gn sp*	Scraptiidae	obligate	Yes	Yes	-	-	-	-
188	NA	*Scydmaenidae gn sp*	Scydmaenidae	facultative	-	Yes	-	Yes	-	Yes
189	yes	*Aspidiphorus lareyiniei*	Sphindidae	facultative	-	-	-	-	Yes	-
190	NA	*Aspidiphorus sp*	Sphindidae	facultative	-	-	-	-	Yes	-
191	yes	*Aleochara bilineata*	Staphylinidae	NS	-	-	Yes	Yes	-	-
192	yes	*Aleochara discipennis*	Staphylinidae	facultative	-	-	-	Yes	-	Yes
193	yes	*Aleochara intricata*	Staphylinidae	facultative	-	-	-	Yes	-	Yes
194	yes	*Aleochara sparsa*	Staphylinidae	NS	-	-	Yes	Yes	-	-
195	yes	*Aleochara tristis*	Staphylinidae	NS	-	-	Yes	Yes	-	-
196	yes	*Anotylus nitidulus*	Staphylinidae	obligate	-	Yes	-	Yes	-	Yes
197	yes	*Anthophagus alpinus pyrenaeus*	Staphylinidae	NS	-	-	-	Yes	-	-
198	yes	*Atheta ischnocera*	Staphylinidae	ND	-	-	-	-	-	-
199	yes	*Atheta nigritula*	Staphylinidae	facultative	-	-	-	-	Yes	-
200	yes	*Atheta parapicipennis*	Staphylinidae	ND	-	-	-	-	-	-
201	yes	*Atheta setigera*	Staphylinidae	NS	-	-	-	Yes	-	-
202	NA	*Atheta sp*	Staphylinidae	multiple	-	-	-	-	-	-
203	yes	*Atheta vaga*	Staphylinidae	facultative	-	Yes	-	-	-	Yes
204	yes	*Autalia rivularis*	Staphylinidae	facultative	-	-	-	Yes	-	-
205	yes	*Eusphalerum umbellatarum*	Staphylinidae	NS	Yes	-	-	Yes	-	-
206	yes	*Leptusa fumida*	Staphylinidae	obligate	-	Yes	-	-	-	Yes

	AR	Species	Family	Saproxylic	Phyt	Wfee	Para	Pred	Fung	Detr
207	yes	*Leptusa pulchella*	Staphylinidae	obligate	-	Yes	-	-	-	Yes
208	yes	*Lomechusoides strumosus*	Staphylinidae	NS	-	-	-	Yes	-	-
209	yes	*Lordithon bimaculatus*	Staphylinidae	facultative	-	-	-	Yes	Yes	-
210	yes	*Lordithon lunulatus*	Staphylinidae	facultative	-	Yes	-	-	-	Yes
211	yes	*Lordithon thoracicus*	Staphylinidae	facultative	-	-	-	-	Yes	-
212	yes	*Mycetoporus piceolus*	Staphylinidae	ND	-	-	-	-	-	Yes
213	yes	*Mycetoporus punctus*	Staphylinidae	facultative	-	-	-	Yes	-	-
214	yes	*Notothecta flavipes*	Staphylinidae	NS	-	-	-	Yes	-	-
215	yes	*Omalium excavatum*	Staphylinidae	facultative	-	-	-	-	-	Yes
216	yes	*Philonthus cognatus*	Staphylinidae	NS	-	-	-	Yes	-	Yes
217	yes	*Philonthus cruentatus*	Staphylinidae	NS	-	-	-	-	-	Yes
218	yes	*Philonthus marginatus*	Staphylinidae	NS	-	-	-	-	-	Yes
219	yes	*Philonthus montivagus*	Staphylinidae	ND	-	-	-	-	-	-
220	yes	*Philonthus splendens*	Staphylinidae	facultative	-	-	-	-	-	Yes
221	yes	*Phyllodrepa floralis*	Staphylinidae	facultative	Yes	-	-	-	-	Yes
222	yes	*Placusa tachyporoides*	Staphylinidae	obligate	-	-	-	Yes	-	-
223	yes	*Platystethus cornutus*	Staphylinidae	NS	-	-	-	-	-	Yes
224	yes	*Platystethus nitens*	Staphylinidae	NS	-	-	-	-	-	Yes
225	NA	*Proteinus cf ovalis*	Staphylinidae	facultative	-	-	-	-	Yes	Yes
226	NA	*Pselaphinae gn sp*	Staphylinidae	facultative	-	-	-	Yes	-	-
227	yes	*Quedius anceps*	Staphylinidae	ND	-	-	-	-	-	Yes
228	yes	*Quedius boops*	Staphylinidae	ND	-	-	-	-	-	Yes
229	yes	*Tachinus fimetarius*	Staphylinidae	NS	-	-	-	-	-	Yes
230	yes	*Tachinus marginellus*	Staphylinidae	NS	-	-	-	-	-	Yes

Appendix 4

	AR	Species	Family	Saproxylic	Phyt	Wfee	Para	Pred	Fung	Detr
231	yes	*Tachyporus nitidulus*	Staphylinidae	NS	-	-	-	-	-	-
232	yes	*Xantholinus linearis*	Staphylinidae	facultative	-	-	-	-	-	Yes
233	yes	*Cteniopus sulphureus*	Tenebrionidae	obligate	Yes	Yes	-	-	-	-
234	NA	*Isomira sp*	Tenebrionidae	obligate	Yes	Yes	-	-	-	-
235	NA	*Hallomenus sp*	Tetratomidae	obligate	-	-	-	-	Yes	-
236	yes	*Trixagus carinifrons*	Throscidae	obligate	Yes	Yes	-	-	-	-
237	yes	*Trixagus leseigneuri*	Throscidae	obligate	Yes	Yes	-	-	-	-

Appendix 4

Appendix 4.2. Boxplot of abundance (log scale) of Coleoptera by aspect.