Reed bee (*Exoneura* spp.) thermal physiology, body pollen composition, and species diversity across an elevational gradient in Kosciuszko National Park, NSW, Australia

by

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Candidate's Declaration

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university. To the best of the author's knowledge, it contains no material previously published or written by another person, except where due reference is made in the text.

Noel Takeshima Date: 06/11/2024

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Abstract

Plant-pollinator communities play an important role in the functioning of mountain ecosystems, which are notably threatened by rapid climate change impacts. I investigated trends in the thermal tolerance (CT_{min}), body pollen composition, and genetic diversity in these communities by sampling 96 reed bees (*Exoneura*) at 7 sites along an elevational gradient ranging from 920m to 1850m. Reed bees underwent genome sequencing to test for genetic diversity, which revealed two clades, *Exoneura* c.f. *robusta* and *Exoneura* c.f. *bicolor*. I found that *E.* c.f. *robusta*, mostly present up until 1600m, was the more abundant clade up until 1450m, whereas *E.* c.f. *bicolor*, present at 6 out of 7 elevations, became more abundant above 1450m. Considering all bees, high-elevation bees were more tolerant to low temperatures than low-elevation bees ($p = 0.002$). However, there was an interaction between the effect of elevation and clade on CT_{min}, such that while CT_{min} was greater at higher elevations in *E.* c.f. *bicolor*, the effect was weaker in *E.* c.f. *robusta*. Pollen composition analysis showed that, on any given bee, the most abundant pollen type accounted for 86.5% (population mean) of their total body pollen. Therefore, our analysis focused on the identity of the most abundant pollen type found on bees. At elevations 920m to 1600m, the pollen family that both reed bee clades foraged from the most was Myrtaceae. However, at the highest elevation (1850m), where only *E.* c.f. *bicolor* was present, Asteraceae was foraged from the most as shown by 7 of 13 bees. Overall, my findings indicate that the nature of reed bee distribution is complex and interwoven with climatic variables. Despite exhibiting a broader range of CT_{min} across elevations, reed bees in the *E.* c.f. *bicolor* clade are more likely to be vulnerable to climate change-induced temperature increases than those in the *E.* c.f. *robusta* clade. A process which may drive this change is the potential upward range expansion of *E.* c.f. *robusta* populations, introducing competitive pressures at higher elevations. Additionally, my findings indicate that reed bees consistently forage from a single plant family at a time, regardless of elevation, while also demonstrating a capacity to forage from a variety of plant species. This foraging behaviour highlights reed bees' important role as pollinators, supporting plant reproduction in mountain ecosystems.

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List of acronyms and abbreviations

CT_{min} – Cold Tolerance

CT_{max} – Heat Tolerance

TOH – Trade Off Hypothesis

Chapter 1: Introduction

Across the globe, mountain environments are among the habitats influenced most strongly by the effects of climate change. Climate-induced changes in phenology create the potential for altered and new interactions. As lower-elevation species move upwards with warming temperatures, new competitors are introduced into high-elevation environments, generating changes in plant–pollinator networks (Slatyer, 2010; Dahlhoff et al., 2019; McCabe et al., 2019; Inouye, 2020). Kosciuszko National Park, situated in New South Wales, Australia, is one of the few mountain regions in the country. Organisms require specialised adaptations to tolerate the harsh conditions that the environment experiences and exhibit evolutionary trade-offs as a result (Gunderson, 2023). Mountain environments are important in the context of the unique biodiversity they support and the environmental value they provide for people. Due to their nature, mountain environments are subject to more rapid climate change impacts than many other systems (Encinas-Viso et al., 2023, Inouye, 2020). Considering the lack of literature in this field, it is important to understand how this will affect mountain environments and the values we derive from them. This project investigates this concern through the specific context of pollinators and plants. By examining variation along elevation gradients, we can make inferences about the role of temperature as a determinant of pollinator distributions and project shifts based on climate change impacts. Coates et al. (2024) have documented a change in plant-pollinator networks using an elevation gradient from 930m to 2000m in Kosciuszko National Park. They also document that while other bees decline in frequency at higher and cooler sites, reed bees occur along the whole gradient.

Mountain regions frequently experience extreme climatic events and temperature fluctuations (Dillon et al., 2010) which affect insect activity (Dahlhoff et al., 2019; Goodwin et al., 2021). Understanding how insects respond to the varied temperatures in mountain landscapes is necessary for determining the extent of impacts that environmental shifts may have on plant-pollinator networks. This can be done by measuring the thermal tolerance of an organism, which often matches the temperature extremes they are likely to encounter. Insect physiological thermal tolerance is defined as the temperature range between the two critical thresholds of minimum (CTmin) and maximum (CTmax) temperatures at which an organism loses motor function or fails to right itself (Fry, 1967), thereby losing the ability to escape from conditions that could lead to its death (Hutchison, 1961; Tougeron et al., 2016). Extensive literature documents the relationship between thermal limits and latitude, revealing strong increases in CTmax and decreases in CTmin at higher latitudes (Addo-Bediako et al., 2000; Overgaard et al., 2011; Sunday et al., 2014). Fewer studies have examined variation in critical thermal limits across elevations despite similar gradients in environmental temperatures (Oyen et al., 2016). Since the critical thermal limits of an organism are often closely bound to their current geographic distributions (Dennis and Hellberg, 2010), they can serve as valuable predictors for future range shifts

driven by changing environmental temperatures (Oyen et al., 2016). Taxa unique to these locations are more specialised in tolerating extreme temperatures than organisms from lower latitudes and altitudes due to prolonged selection pressures (Dahlhoff et al., 2019). Traditionally, studies incorporate transect gradient study methods to explore the changing effects in these environments. Elevational gradients are characterised by strong temperature gradients across even shorter geographic distances, but comparatively few studies have measured elevational variation in thermal tolerance (Gaston & Chown, 1999; Malo and Baonza, 2002). Studying the CT_{min} of pollinators in a mountain landscape across an elevational gradient will offer valuable ecological insights into the factors driving their distribution and allow us to predict the potential impacts of climate change on these patterns (Johanson et al., 2019, Ostwald et al., 2023).

Many plant-pollinator network studies also provide ecological insights by examining the body pollen of pollinators and informing us about vegetation community structures and the timing of flowering periods (Vitasse et al., 2021). Little research has been conducted investigating plant-pollinator networks in mountain environments despite their heightened vulnerability to climate change (Sánchez-Bayo and Wyckhuys, 2019; Goodwin et al., 2021). Climate change-driven changes in temperature and precipitation affect snowpack and snowmelt, inducing changes across the landscape, which can vary significantly if there is notable topographic complexity (Inouye 2020). Pollinator activity is constrained to the snow-free season and shifts in the timing and duration of flowering periods have the potential to reduce pollen availability via cascading effects, adversely impacting bees' capability to meet the nutritional requirements critical for their reproduction (IPBES, 2016; Kankasemsuk et al., 2023), Mountain plants that are the focus of pollen collection and are most likely to benefit from pollination (IPBES 2016), are equally likely to suffer adverse effects to their pollen dispersal and reproduction without pollination services (Marzinzig et al., 2018). Plants and their pollinators are both important components in the foundation of mountain landscapes. Elevational shifts in dominant pollinators could have important effects on plant reproductive success (McCabe and Cobb, 2021), because pollinators vary in their effectiveness as pollen vectors (Bischoff et al., 2013).

Reed bees (*Exoneura*), a genus endemic to Australia (Houston, 2018), are known flower visitors in the montane and subalpine zones of Kosciuszko National Park (Inouye and Pyke, 1988; Encinas-Viso et al., 2023; Coates et al., 2024). Their nesting behaviour has been observed to shift with elevation in this habitat. Typically, reed bees nest in dead fern fronds or bramble (Coates 2020, 2022), Silberbauer and Schwarz, 1995); however, they have recently been sighted nesting in dead snow gum branches at elevations of 1850m (Coates et al., in prep). This change in behaviour may align with the shift in environmental conditions between montane and subalpine zones.

Aspects of Coates et al. (in prep) and Encinas-Viso et al. (2024)'s work examine the subalpine pollination networks and behaviour of reed bees in the region. In their research, snow gum nests were abundant at high elevations, and reed bees visited flowers within relatively small distances of their nest. These findings allude to the possibility that high-elevation reed bee populations may have diverged from lower-elevation populations.

My thesis examines the forces generating patterns in genetic diversity, CT_{min}, and foraging behaviour of reed bees based on their elevational distribution and speculates on their implications in the context of a rapidly changing climate. As such, it can be hypothesised that reed bees from higher elevations will be genetically distinct, have stronger CT_{min}, and forage from different plant assemblages compared to reed bees from lower elevations. I aim to determine this by sequencing genomes, conducting thermal assays, and analysing the body pollen of reed bees sampled from a variety of elevations. Disentangling these will expand knowledge of an understudied taxa in an understudied field of research and speculate how mountain pollination networks may react to a changing climate.

1.1 Research Scope

My thesis examines reed bees, a genus of stem nesting Australian bee, their genetic diversity, thermal physiology, and pollen composition in an elevational gradient in Kosciuszko National Park, New South Wales, Australia. Reed bees have recently been observed abundantly nesting in dead snow gum branches in the high elevations of the region. While reed bees have been studied extensively in the context of social evolution (Bull and Adams, 2000; Michener, 1962; Schwarz and Okeefe, 1991), little work exists examining these bees in the context of mountain pollination. My research seeks to fill some of the knowledge gaps surrounding these Australian native bees, their role as pollinators in the mountain landscapes of Australia, and their vulnerability to climate change. I sequenced reed bee genomes to detect genetic variation, used thermal tolerance assays to assess thermal physiology, and assessed body pollen composition across my elevational gradient via light microscopy. By investigating genetics, thermal tolerance, and pollen composition, my thesis seeks to understand not only the forces shaping reed bees' patterns of distribution and foraging behaviour, but also whether the genus is at risk from projected impacts of climate change effects as well as how they are likely to adapt.

The context for this thesis arose from observations of snow gum nesting behaviour made by Coates and Encinas-Viso in Kosciuszko National Park, New South Wales, Australia. Due to the novel nature of the behaviour and the notably high elevation at which it occurred, it was supposed that snow gum nesting reed bees may be genetically distinct from lower elevation populations and may play an important role in the pollination services of the region.

1.2 Thesis Outline

My thesis has 6 chapters. This chapter outlines the context and scope for my research. Chapter Two outlines the relevant literature to my thesis in the field of mountain ecology with an emphasis on thermal and pollination ecology in the context of reed bees and ends by highlighting key knowledge gaps that my research will address. Chapter Three describes research methods employed in my study, including a description of my study sites and the statistical analysis used. Chapter Four details the results of my research. Chapter Five then interprets my results and their implications and suggests avenues for further research. Finally, Chapter Six presents a synthesis of my thesis and concluding remarks.

Chapter 2: Literature Review

In this Chapter I will discuss the current literature relevant to my thesis topic. This will include an overview of reed bee biology, their distribution, foraging patterns, and mountain environments. I then explore literature discussing pollinators in mountain landscapes, with a focus on vegetation community structures. I also review current literature examining insect CT_{min} in the context of mountain landscapes or variable gradients. I will also discuss various methods used to assess CT_{min}. Lastly, this Chapter will lead to a discussion of the current knowledge gaps in the literature, which my research will aim to address.

2.1 Mountain Environments

My chosen study sites were located within Kosciuszko National Park, part of the relatively small Australian Alps bioregion (7,938 km²) in southeastern Australia, home to Mt Kosciuszko (2,228m), the nation's tallest mountain (NSW National Parks and Wildlife Service, 2003; Slatyer, 2010). This bioregion is characterised by broad forested valleys and rugged mountain peaks, marking it as one of Australia's few true alpine environments. With its deep loamy soils, a rarity among alpine areas, the region supports uniquely structured vegetation communities, flora, and fauna found nowhere else on the planet. Additionally, it is a vital water catchment for the country and a popular tourist destination, offering numerous recreational opportunities (Scherrer, 2004; Tierney, 2024).

The bioregion is defined by its harsh abiotic conditions such as extreme climatic events, high precipitation, persistent winter snow, low average annual temperatures, frequent frosts, strong winds, wildfires, and high UV exposure (Costin, 1962; Scherrer, 2004; Inouye, 2020; Goodwin et al., 2021; Verrall et al., 2021). Complex topography further enhances environmental heterogeneity and microhabitat differentiation, contributing to high biodiversity and endemism (Encinas-Viso et al., 2023; Slatyer, 2003, 2010; Verrall et al., 2021). These conditions create distinct microhabitats that support rare plant communities, some of which depend on pollination to reproduce during the mountain ecosystem's brief growing season.

Mountain regions offer researchers unique opportunities to study the mechanisms shaping ecosystem structure within compact geographic areas along environmental gradients (Körner, 2000, 2003, 2007; Minachilis et al., 2020). They also provide valuable insights into the adaptations that enable flora and fauna to survive harsh environmental conditions (Hoiss et al., 2015). In Australia, researchers gained a rare chance to examine mountain fire regimes and plant community responses to large-scale fires when wildfires burned 70% of the alpine and subalpine zones in southeastern Australia in early 2003 (Slatyer 2010, Fairman et al., 2017).

2.2 Mountain Plant Communities and Pollinator Interactions

Rapid shifts in conditions across relatively small distances often result in the elevational partitioning of mountain vegetation communities, creating distinct zones (Coates et al., 2024; Costin et al., 1962, 2000; Slatyer, 2010). These zones are classified as alpine, subalpine, and montane, and are characterised by diverse ecosystem structures and plant communities. Consequently, mountain plant-pollinator networks exhibit specialised associations that typically correspond with zonal habitat distribution.

Zone position varies widely due to the landscape's complex topography and environmental heterogeneity. However, Australian montane environments typically begin at elevations above 1100m, and are characterised by tall, dense eucalypt forests dominated by mountain ash (*Eucalyptus regnans*) and alpine ash (*Eucalyptus delegatensis*) (Colloff et al., 2016). Montane habitats transition to subalpine environments approximately above 1400m, where vegetation includes smaller trees, shrubs, and herbaceous plants adapted to lower temperatures and seasonal snow cover, often featuring species like snow gums (Williams and Ashton, 1987; Verrall et al., 2023). In contrast, alpine plant communities, which occur above 1850m, are relatively simple in structure, and primarily consist of long-lived perennial herbaceous plants and exhibit low species richness (Bear et al., 2006; Inouye, 2020). Trees are generally absent in alpine zones due to the extreme abiotic conditions that hinder seedling recruitment and limit effective dispersal strategies (Slatyer, 2010; Inouye, 2020). Conservation of these environments is widely recognised for the ecosystem services they provide, including erosion control, carbon sequestration (Keith et al., 2009), water storage, and filtration (Colloff et al., 2016).

Although alpine environments cover roughly 15% of the Earth's surface and hold significant ecological importance, relatively little is known about the forces that shape their plant-pollinator networks and their responses to environmental change, especially in Australia (Bryant et al., 2023). This knowledge gap exists primarily because studies in these regions are often logistically challenging due to short growing seasons, extreme weather conditions, and limited accessibility (Inouye, 2020). The high environmental heterogeneity of mountain ecosystems also complicates efforts to generalise findings to wider habitats. While previous research has explored species richness, shifts in plant communities (Slatyer, 2000), and the effects of snowmelt timing on plant communities (Edmonds et al. 2006; Verrall et al., 2021), little attention has been given to how these changes may impact mountain pollinators or their adaptive potential to respond to them (Goodwin et al., 2021; Hodkinson, 2005).

2.3 Rapid Climate Change in Mountain Environments

Mountain forests are particularly sensitive to rising temperatures (Bryant et al., 2023), and in Australia, mountain ecosystems are projected to shrink at a faster rate relative to other environments as global warming intensifies (Edmonds et al., 2006). Faster warming rates at higher elevations make alpine ecosystems among the most vulnerable to climate change, with responses manifesting as increases in the frequency of extreme climatic events, including heatwaves, droughts, and fires (Goodwin et al., 2021; Hoiss et al., 2012, 2015; Inouye, 2020; Slatyer, 2010; Verrall et al., 2021, 2023). High-altitude areas are also already experiencing shifts in precipitation patterns, impacting snow cover, depth, and duration in alpine and subalpine zones (Edmonds et al. 2006; Slatyer, 2010).

Since the balance of zones in mountain ecosystems is reliant on climatic variables, environmental shifts may compromise the entire system. As an example, temperatures extremes are typical of mountain ecosystems and are important in relation to the precipitation and wildfire regimes of the region. Climate change induced temperature shifts are likely to advance snowmelt dates as well as increased frequency and severity of wildfires ((Doherty et al., 2017; Inouye, 2020). These effects are associated with irreversible consequences for slow growing mountain flora, especially in the case of eucalypt forests, such as snow gum, whose regenerative capabilities are significantly inhibited after several successive fires (Colloff et al., 2016, Verrall et al., 2021). Further ecological concern has grown in the context of subalpine snow gum forests dieback, likely driven by a combination of climate change-induced stress and increased activity of a native wood-boring beetle (Bryant et al., 2023).

Growing seasons of plants typically cannot begin until snow cover melts, and timing delays are likely to alter mountain flowering phenology (Vitasse et al., 2021). Shifts in these timings would subsequently have cascading implications for the availability of food resource for insect pollinators, which are confined to the timings of migration or emergence events (Coates et al., 2023). Temperature increases are also predicted to drive elevational positions of zones upwards (Matthews et al., 2010), resulting in subalpine and alpine habitat loss and disproportionate montane habitats (Slatyer, 2010; Dahlhoff et al., 2019). Temporal or spatial mismatches in these timings are likely to change species interactions in multiple trophic levels (Vitasse et al., 2021).

As a result, mountain plant-pollinator communities are especially vulnerable to accelerated climate impacts, since their effects are likely to compound on the highly interconnected components that sustain the ecosystem. As an example, collapse high-elevation forests is increasingly being driven by the effects of climate change on snow cover and more frequent fires, particularly impacting subalpine specialists and fire-sensitive species in south-eastern Australia (Milla and Encinas-Viso, 2020; Bryant et al., 2023;

Verrall et al., 2023). Considering this susceptibility, simultaneous reductions in pollinator abundances and pollination services they provide may occur as they struggle to adapt to a changing climate (Inouye, 2020), further lessening plant community reproduction and regeneration capabilities.

Overall, mountain environments are among the habitats most strongly at risk from climate change. Consequently, their unique plants and pollinators will likely be forced to adapt or avoid extinction (Inouye, 2020). As such, my research will focus on the ecological role of a mountain pollinator and speculate how they are likely to respond to climate change by examining reed bees across an elevation gradient.

2.4 Reed Bees (*Exoneura spp.*)

In my elevational gradient, reed bees are the study species which are the focus of my thesis. Reed bees have also been observed pollinating within the montane and subalpine regions of Kosciuszko National Park (Coates, 2024, in prep; Encinas-Viso, 2023; Inouye and Pyke, 1988; Johanson et al., 2019) including nesting within snow gums (*Eucalyptus pauciflora*) (Coates., in prep). This genus occurs primarily throughout temperature montane and coastal regions of southern Australia, with a smaller number of species occurring in arid inland habitats (Schwarz, 1986). Figure 1 shows indicative reed bee distribution, based on Atlas of Living Australia (ALA) recorded sightings.

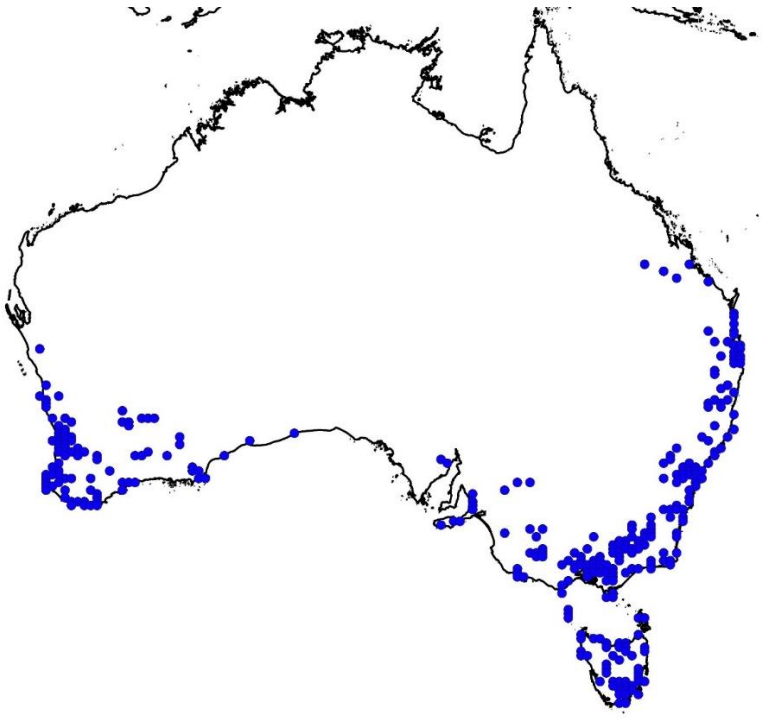

Figure 1: Reed bee distribution. Sightings are indicated by blue dots (Atlas of Living Australia, 2024)

Despite their extensive distribution, relatively little is known about the genus, particularly in relation to their role as pollinators and distribution within mountain landscapes. This is largely because previous research on reed bees has primarily focused on the genus' sociality and has not been studied in recent times (Batley and Hogendoorn, 2009).

Michael Schwarz of Flinders University has studied the genus extensively, focusing primarily on social evolution and evolutionary ecology (Schwarz, 1986; Schwarz and Okeefe, 1991). There is some research that explores nesting in reed bees, such as on nest translocation (Cronin, 2001) and nest predation, however it does not address the factors shaping their distribution, nor the pollination services they provide within mountain landscapes.

It should also be noted that while some studies on the genus focus on a specific species or group of species, the number of species present in my study sites is unclear. Due to difficulties in identifying reed bees to species level resolutions based solely on morphology, I will identify individuals through genome sequencing

Figure 2. A typical adult *Exoneura bicolor*. (Richter, R 2013, 'Bee on Blue Bells', Identified as *Exoneura* (*Exoneura*) *bicolor* (Bowerbird, 2024)

Exoneura is an endemic Australian genus of social bees belonging to the Allodapine tribe of the Apidae bee family (Hedtke et al., 2013; Houston, 2018). The genus is currently divided into two subgenera, *Exoneura sensu stricto*, *Brevineura*, which total approximately 67 described species (Hedtke

et al., 2013). However, due to a lack of recent taxonomic revisions, the number is likely not indicative of the true species diversity within the genus (Houston, 2018; Michener, 1965).

The genus typically has glossy black head and thorax with a black or red-orange abdomen. Individuals are small, reaching up to 8 mm in length, and often exhibit distinctive brown-orange colouring, and sometimes yellow facial markings (Cockerell, 1913; Rayment, 1951). Their abdomens are characteristically flattened at the rear (Figure 2), which they use to block the entrances to their nests (Michener, 1965). Within my study sites, past research suggests the presence of E. *bicolor* (Schwarz, 1986) and E. *robusta* (Bull and Adams, 2000).

Reed bees have been of interest to researchers due to the insights they provide into the evolution of social behaviour within Hymenoptera (Cronin and Schwarz, 1999). The genus exhibits a spectrum of social structures (Dew et al., 2014), ranging from complex eusocial behaviours seen in E. tridentata (Houston, 1977) to the primarily solitary behaviours exhibited by E. lawsoni (Michener, 1964). This diversity in social structure implies that species may vary significantly in their behavioural responses to environmental disturbances (Hall et al., 2019).

Members of the genus have been observed nesting within a variety of substrates, including *Xanthorrhoea minor*, *Rubus*, *Melaleuca squarrosa*, and recently *Eucalyptus pauciflora* (Encinas-Viso et al., 2024; Silberbauer and Schwarz, 1995; Cronin, 2001; Coates et al., 2024). While there is some flexibility in reed bees nest founding (Cronin, 2001; Silberbauer and Schwarz, 1995), they generally share the responsibility of rearing young in a single large cavity within a nesting substrate, uncommonly laying eggs in large numbers without partitioning the nest into separate cells. The entrance of each stem is typically narrowed to a pithy rim to allow for nest defence. Females will often guard nest entrances entrance either by blocking it with her flattened abdomen or using her mandibles to deter intruders (Michener, 1965).

While specific life cycles may vary between species and depend on habitat conditions (Silberbauer and Schwarz, 1995), the lifecycle of the genus is as follows (Michener, 1965). Foundresses (young, inseminated females) begin new colonies by occupying a previously used nest or by excavating a new cavity within a stem. This process can be undertaken alone or with other adult females, usually in the spring. Eggs are laid from spring through summer within a single brood cell. Colony members may be either reproductive or non-reproductive workers who gather pollen to feed the larvae and defend the nest. The larvae are fed continuously by adults until they mature and emerge from the nest. New colonies typically consist of new bee generations and some of the original foundresses. Some of the

new females will then disperse to establish new nests. Males seek mates near or within established nests, as well as on flowers, and generally have shorter lifespans than females (Michener, 1965).

2.5 Pollen Composition Study Methods

There are a variety of methods used to generate plant-pollinator networks in an ecosystem. This is because disentangling plant-pollinator associations is crucial for understanding ecosystem connectivity and the nested structure of plant assemblages (Johanson et al., 2019), particularly considering global pollinator declines (IPBES, 2016). Additionally, conservationists and ecologists are interested in pollinators as they can inform our understandings of plant distribution, habitat type, and the spread of invasive species (Bell et al., 2024). This is achieved through using identified pollen grains collected by pollinators as environmental DNA (Bell et al., 2024; Encinas-Viso et al., 2023). Use of these methods is being used by managers of Kosciuszko National Park to improve the detection of invasive hawkweed species (Cousens and Williams, 2011; Encinas-Viso et al., in prep).

In my thesis, I will analyse reed bee pollen compositions by liberating pollen, identifying pollen morphospecies, tallying the counts of each pollen type and comparing compositions against other variables. I used this method because it provides a straightforward way of determining which plants bees were visiting without observing flower visitations. This method's simplicity, compared to other pollination measurement techniques, enabled me to collect a large sample size within the time constraints of my honours project.

The use of this pollen composition method also meant that I was able to collect focused pollination data on reed bees, which has not been closely examined before in mountain landscapes. Further, collecting body pollen is a widely used approach, providing results that can be compared to other studies relatively easily. The use of an elevational gradient methodology also meant that I was able to compare pollen compositions of bees from different elevations.

2.6 Knowledge Gaps and Research Aims

In Australia, there has been an overall lack of research on mountain pollination networks and their responses to climate change (Inouye, 2020). Previous studies have primarily focused on other aspects of mountain ecology, rather than on the details of pollinator species interactions (Slatyer et al., 2003).

Due to the small number of alpine weather stations, literature has also been further limited by a lack of consistent climate data, which has led to a poor understanding of recent climate changes above the tree line (Verrall et al., 2021). Additionally, the lack of recent taxonomic revisions in the reed bee genus suggests the possibility of undescribed species in my study sites. Combined with the morphologically cryptic nature of the genus, this raises the likelihood that populations exhibiting novel nesting behaviour in snow gums may represent an undescribed species. Limited information is available on the thermal tolerances and pollination services of native bees (Tierney et al., 2023) as well as the effects temperature have on their physiology and foraging behaviour (Jaboor et al., 2022). Mountain-dwelling reed bee populations provide a valuable case study to help address this knowledge gap, particularly given the steep temperature gradients typical of mountain environments. Moreover, my study is the first to closely examine the plants that reed bees pollinate in mountainous environments and their pollination role. Recent findings of reed bees nesting behaviour in snow gum branches further raise questions about the nature of the relationship, including speculations into the origin of the relationship, the pollination services that reed bees provide E. pauciflora, and its importance for their reproduction.

By studying the genetic diversity, foraging patterns, and thermal tolerances of cryptic reed bees, I hope to learn more about their role as mountain pollinators, understand how they have adapted to selection pressures across the region, and explain novel nesting behaviour at high elevations. Conducting this research will also explore how climate change may impact mountain pollination networks, which will assist in informing land-use planning, conservation status assessments, and park management decisions. To address these research gaps, my study examines distribution patterns in genetic diversity, thermal tolerance, and foraging behaviours of reed bee populations across an elevational gradient. Further, if shifts across elevation are present, I aim to explain whether variation in each aspect can be explained by differences in elevation. To address these aims, my thesis will answer the following research questions:

1. What is the distribution of reed bee species across an elevational gradient?
2. Can the distribution of reed bees along an elevational gradient be explained by their thermal physiology?
3. How do the foraging patterns of reed bees change across an elevation gradient?

The methods which my study will use to answer my research questions include:

- Sampling reed bees along an elevational gradient.
- Measuring reed bee thermal tolerance.
- Surveying reed bee body pollen using light microscopy.
- Identifying and comparing reed bee haplotypes.

Chapter 3: Methodology and Methods

3.1 Study Sites

To capture an elevational gradient, I selected study sites in Kosciuszko National Park, New South Wales, starting in the montane zone at 920m and finishing at the upper end of the subalpine zone tree line at 1850m (Totterdell et al., 2000). Roughly spanning 25km of horizontal extent, site locations occurred at 920m, 1210m, 1240m, 1470m, 1600m, 1640m, 1750m, and 1850m (Figure 3). I chose the site locations based on the recorded presence of reed bees from previous work in the area (Coates et al., 2024). I started the study site locations from Gaden Trout Hatchery (-36.374, 148.577) and followed Gaden Road and Kosciuszko Road, increasing elevation until the last location near Charlotte Pass (-36.436, 148.321).

I sampled pollinators for two weeks from the 11th to the 25th of January 2024, capturing the peak of reed bee activity (Coates et al., 2024) and flowering at montane and subalpine sites following snowmelt, along with the onset subalpine flowering period (Scherrer, 2004).

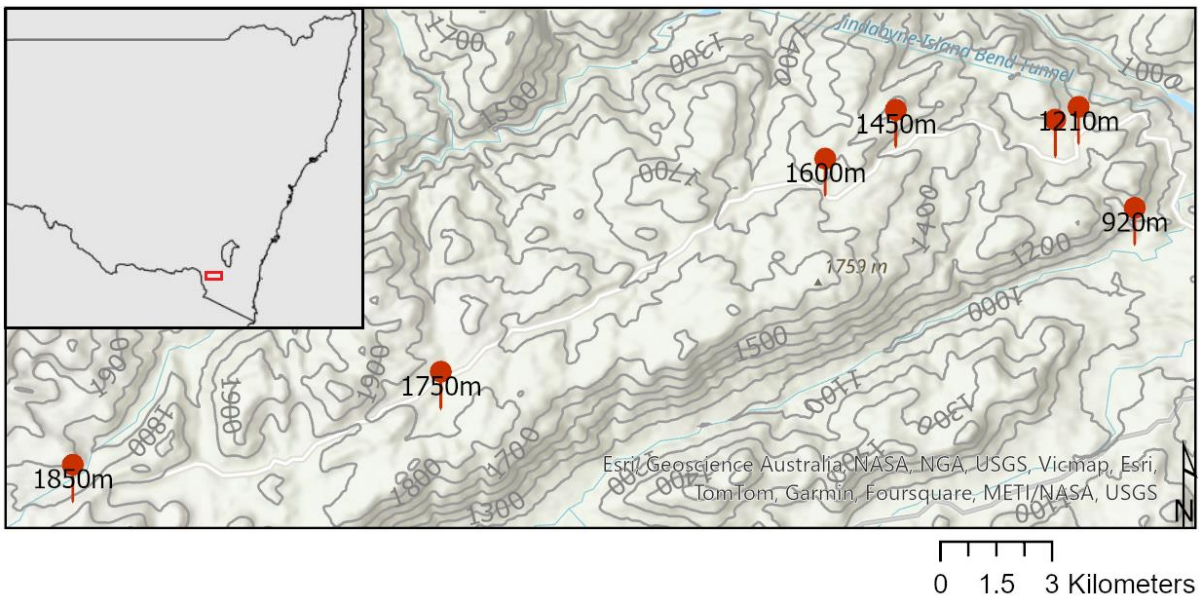

Figure 3. Location of 7 study sites in Kosciuszko National Park, Australia. Contour map labels shown in grey. Inset image shows New South Wales state border with study area indicated in red.

3.2 Sampling Protocol

Bee activity is influenced by weather and temperature (Coates et al., 2024), so more intensive sampling efforts occurred when weather conditions were forecasted to be warmer (around 20°C) with low wind speeds. However, I still sampled in cooler conditions to increase the likelihood for sampled bees to have varied CT_{min}. At each of the 7 study sites, I collected reed bees directly from flowering plants using either sweep netting or tubing. I stored them in the same tubes they were caught in and kept them in a cool bag for 4-5 hours before testing. Occasionally, I collected bees from leaves when

they took refuge from strong gusts or directly from nests at higher elevation sites. I recorded the plants the bees were caught from, and the time of collection. I identified plants in the field using field guides (Duncan, 1994), and iNaturalist (n.d.).

3.3 DNA Extraction and Haplotype Analysis

DNA extraction methods were conducted with the assistance of Dr Linda Neaves considering her extensive professional experience using genome sequencing techniques. DNA was extracted using the 'salting out' approach (Sunnucks and Hale, 1996). The cytochrome C oxidase subunit 1 (CO1) region was amplified using invertebrate specific primers (Lep_f1 and Lep_R1; Hebert et al., 2004). PCR reactions were performed in 12.5 µL reactions with 1x OneTaq (New England BioLabs, Australia), 0.5µM each forward and reverse primers and ~10 ng DNA. Thermocycling consisted of 10-minute denaturing step at 95oC, followed by 12 cycles of 30 seconds denaturing at 95oC, 30 seconds annealing at 56oC and extension of 72oC, followed by a further 35 cycles where the annealing temperature was changed from 56oC to 45oC, and a final extension step of 10 minutes at 72oC. Amplification was confirmed using UV visualisation of the project on a 1.5% agarose gel stained with SYBR Safe (Invitrogen, USA). Amplicons were then cleaned using 1.8 times Sera-Mag™ SpeedBeads (Cytiva, USA). Sequencing was performed on an AB 3730xl Sequencer by the Australian Genome Research Facility (AGRF). The result forward and reverse sequences were aligned, and chromatograms were inspected for errors, with the contig trimmed and adjusted accordingly in Uniprot UGENE (Okonechnikov et al., 2012). All sequences were then aligned using Muscle (Edgar, 2004) in AliView (Larsson, 2014), and haplotypes identified. The resulting sequence haplotypes were used for performing BLASTn searches against the National Center for Biotechnology Information Nucleotide nonredundant database (1 October 2024) to obtain a putative identification. All sequences included in the alignment shared at least 80% of their nucleotide positions with reference sequences to ensure that relevant and closely related sequences were compared. 3 sequences were excluded from the alignment due to errors associated with poor quality. Finally, an IQ-Tree, with 5000 bootstraps was built in UGENE to visualise the clustering of identified haplotypes.

3.4 Thermal Tolerance (CTmin) Assays:

I conducted thermal assays using thermoelectric plates (Model Number CP-121HT, TE Technology, INC.) and controller (Model Number TC-720, TE Technology, INC.) to assess the thermal tolerances of reed bees. I placed collected samples upright on the plate in glass or plastic tubes arranged in a 4x4 grid. I double-coated the tubes with fluon before sampling to prevent the bees from climbing tube insides and drilled small holes in the tops of the tubes to allow airflow. Although the temperature controller had its own measurements, I used thermocouple loggers (DT-847U, Instrument Choice) to record the surface temperature inside the tubes. Two thermocouple probes were placed this way in control tubes at opposite ends of the plate, and I averaged the recorded values for accuracy.

Since low temperatures are frequently experienced at higher elevations, I used thermal tolerance assays to find lower critical thermal limits of reed bee CT_{min}. Traditionally, CT_{min} refers to a single temperature, but there are a few options of behavioural responses at which the value can be recorded (Oyen et al., 2016; Tougeron et al., 2016; da da Silva et al., 2021) I chose loss of righting response as the behaviour to record CT_{min} since it is commonly used to assess insect CT_{min} (Gibert and Huey, 2001; Kellermann et al., 2012), and cold-adaptation is less related to chill-coma temperature (Goller and Esch 1990). This method involves observing when an insect can no longer right itself after being flipped onto its back, which indicates the temperature at which motor function is impaired.

Cold thermal assays started at 22°C, held for 10 minutes, and cooled at a rate of 0.25°C/min, commonly used in the literature (Oyen et al., 2016). I recorded the CT_{min} value when bees could no longer right themselves after being flipped. I removed bees from the plate once they exhibited chill-coma behaviour and allowed them to warm up. Hot thermal assays also began at 22°C, remaining consistent for 5 minutes and warmed at the same rate of 0.25°C/min. I monitored the tubes to ensure no bees climbed the tube walls, shaking them gently to dislodge them if they did. Post-testing, each sample was submerged in a 95% ethanol solution in the same tube and stored in a freezer to preserve DNA.

3.5 Data Analysis

I used the software program R x64 version 4.3.1 to conduct statistical analysis of my datasets (R Core Team, 2023). I used generalised linear mixed models using the glmmTMB function within the ‘glmmTMB’ package (Brooks et al., 2017) to determine the impact of elevation and clade on reed bees’ lower and upper thermal limits. I included reed bee clade and elevation as predictor variables, as well as their interaction, with reed bee thermal tolerance as the response variable. I checked statistical significance using the ‘summary’ command and performed model validation using the check_model function within the ‘performance’ package (Lüdecke et al. 2020).

3.6 Pollen Composition

3.6.1 Pollen Liberation

I examined the composition of the bees' body pollen to determine their foraging patterns. Since there are few methods to liberate pollen from insects stored in ethanol, I used a trial-and-error approach to establish a robust method. I based my methods on the work of Botti-Anderson (2019), who used de-ionized water to remove pollen from bees, and Riding (2021), who used detergent to deflocculate palynomorphs. Consolidating both, I integrated detergent into my pollen liberation methods.

To develop my pollen liberation methods, I collected trial bees from ANU Campus. I euthanized these bees in a freezer and submerged them in ethanol to replicate field sample conditions. After removing them from ethanol and letting them dry, I inspected their body pollen count under a dissecting

microscope. I vortexed the test bees for either 15, 30, 45, 60, or 120 seconds in either a 0%, 1%, 5%, or 10% detergent solution. After, I let the bees dry and recounted their body pollen. Bees vortexed in a 0% detergent solution retained many pollen grains, regardless of vortex time, while bees vortexed in the 1% solution had visibly fewer grains remaining. Efficacy of the detergent continued to increase at higher concentrations which justified the use of a 10% detergent solution.

I emptied the tube contents and rinsed them with ethanol into 15ml Eppendorf tubes, added detergent to achieve a 10% concentration, and vortexed the samples for 60 seconds. I transferred paper ID tags and bees back into their original tubes using tweezers and re-submerged them in ethanol. I rinsed tags, bees, and utensils with ethanol into the tube, ensuring cross-contamination was minimized by washing and wiping utensils between sample handlings.

3.6.2 Slide Mounting:

Following Jones (2012) methods, I conducted two trials of centrifuging 15ml Eppendorf tubes at either 1000rpm or 3000rpm for 5 minutes. Since I noted little difference between the two settings, I used the prior option. I gradually removed the excess solution from the top of the Eppendorf using an autopipette, ensuring the pollen pellet at the bottom remained undisturbed, until around 1ml of solution remained. I then disturbed the pellet using the pump, removed 100µL of solution, and dispensed it onto a flat microscope slide. I continued mounting slides until no visible pollen pellet remained. I used fuschin dye to stain the pollen grains, making them easier to identify among plant matter. Unlike traditional methods that collect pollen grains from dry pollinator parts, my method used detergent and ethanol, which reduced surface tension. To circumvent this, I pre-heated the slides to 50°C, allowing the solution to evaporate faster. Once it evaporated, I applied a small amount of fuschin dye gel. After the dye melted, I mounted cover slips and sealed the edges with acrylic paint. Following the work of Jones 2012, I conducted two trials of centrifuging 15ml Eppendorf tubes. Centrifuge settings were set at 1000prm or 3000rpm for 5mins. Little difference was noted between them, so the prior option was used. I gradually removed the excess solution from the top of the Eppendorf using an autopipette, taking care not to disturb the pollen pellet at the bottom of the tube, until around 1ml remained. I then used the pump to gently disturb the pollen pellet and removed 100µL of solution, dispensing it onto a flat microscope slide. I continued mounting slides until the pollen pellet was no longer visible.

The use of fuchsine dye is common practice in staining pollen grains, making them clearer to identify amongst plant matter (Beattie 1971; Nicotra 2010). However, like pollen liberation methods, it is more common to collect pollen grains directly from dry pollinator parts with fuchsine dye as opposed to wet samples stored in ethanol (Coates et al. 2023). Detergent and ethanol being incorporated into the solution meant that there was little surface tension keeping the aliquot on the slide. To circumvent this, slides were pre-heated to 50°C which made the solution evaporate faster than it could spread. Once completely evaporated, a small amount of fuchsine dye gel was added. Cover slips were mounted once the dye melted, and acrylic paint was used to seal cover slip edges.

3.6.3 Pollen Grain Counting:

Pollen grains were counted using a ZEISS Axio Imager M1 microscope fitted with ZEN software Version 3.8. Using the 25x magnification setting, I started with the centre of the field of view at the top of the fuchsine dye blob, I panned right until no part of the dye blob was visible and panned left to do the same. Then panning down one field of view and repeated this process until all parts of the dye blob had been sighted. Pollen grain images were taken and labelled shorthand names for later identification and tallies of how many of each pollen label were taken. Pollen grain identification was accomplished in collaboration with Joshua Coates by cross referencing images I had taken with a variety of sources, including ANU Australasian Pollen and Spore Atlas, (Duncan, 1994; Macphail and Hope, 2006), and the pollen reference library collected from the study site, used in Coates et al. (2023).

Preliminary investigations revealed extreme variability in pollen load sizes and subsequent total count of pollen grains per bee. Because in some cases multiple slides were needed to mount the entire pollen pellet, samples were often split across multiple slides. Further, mounting 3 pollen solution aliquots per microslide across multiple slides meant that multiple pollen counts were required per insect sample. For an example, one sample's pollen pellet could potentially yield 6 microscope slides, with three aliquots mounted per slide, resulting in 18 counts for the one sample. These factors resulted in highly variable counts and due to time constraints, it became unrealistic to count pollen grains beyond the first mounted slide. This decision created an issue, requiring myself to determine how to account for the pollen grains that went uncounted beyond the first slide. Further, counts for pollen morphospecies were exact for relatively smaller counts (<1000), but higher counts were systematically estimated in larger increments based on visible grain abundance within the field of view at 25x magnification. Proportions of each pollen type were calculated, per bee sample, to overcome the issues created by not counting total pollen loads.

Preliminary analyses displayed that most pollen grains on a sample were predominantly from one plant family and morphospecies. Several of the most abundant pollen taxa within the Myrtaceae family were difficult to identify to genus level resolution, namely Baeckea, Leptospermum, and Kunzea (Thornhill, 2011). In most cases, unidentified taxa were assigned morphospecies identifiers (Coates et al., 2023) but were later grouped by family before undergoing analysis.

Chapter 4: Results

In total, I collected 96 reed bees distributed across an elevation gradient (920m: n=22, 1210m: n=20, 1240m: n= 10, 1450m: n=13, 1600m: n= 13, 1750m: n= 4, 1850m: n= 14). Despite being morphologically indistinguishable, the results of genome sequencing revealed two haplotype clades, *E.* c.f. *robusta* and *E.* c.f. *bicolor*. I found that *E.* c.f. *robusta* was the more abundant clade up until 1450m and bees in the clade were present up until 1600m. On the other hand, *E.* c.f. *bicolor*, which was present at 6 out of 7 elevations (absent from 1240m), became the more abundant clade above 1450m.

4.1 Haplotype Identification

Instead of forward and reverse sequences, which are more robust, received sequence data was only in one direction. As a result, some of the differences in sequences lacked sufficient evidence to dismiss, resulting in more haplotypes than may exist, as shown in Table 2. Each haplotype grouped into one of two clusters in the data, one which groups with *E. bicolor* sequences from Genbank (*Exoneura* c.f. *bicolor*), and second which groups with primarily *E. robusta (Exoneura* c.f. *robusta)*.

Haplotype	Count	Clade
1	42	*E. bicolor*
2	27	*E. robusta*
4	7	*E. robusta*
5	8	*E. robusta*
6	2	*E. bicolor*
J117	1	*E. bicolor*
J118	1	*E. bicolor*
J20	1	*E. robusta*
J23	1	*E. robusta*
J71	1	*E. robusta*
J8	1	*E. bicolor*
J96	1	*E. bicolor*
Failed	3	N/A
Total	96	

Table 1: Count of reed bee haplotypes, clustering of haplotypes, and number of failed or removed sequences. The number of successfully genotyped bees totalled 93.

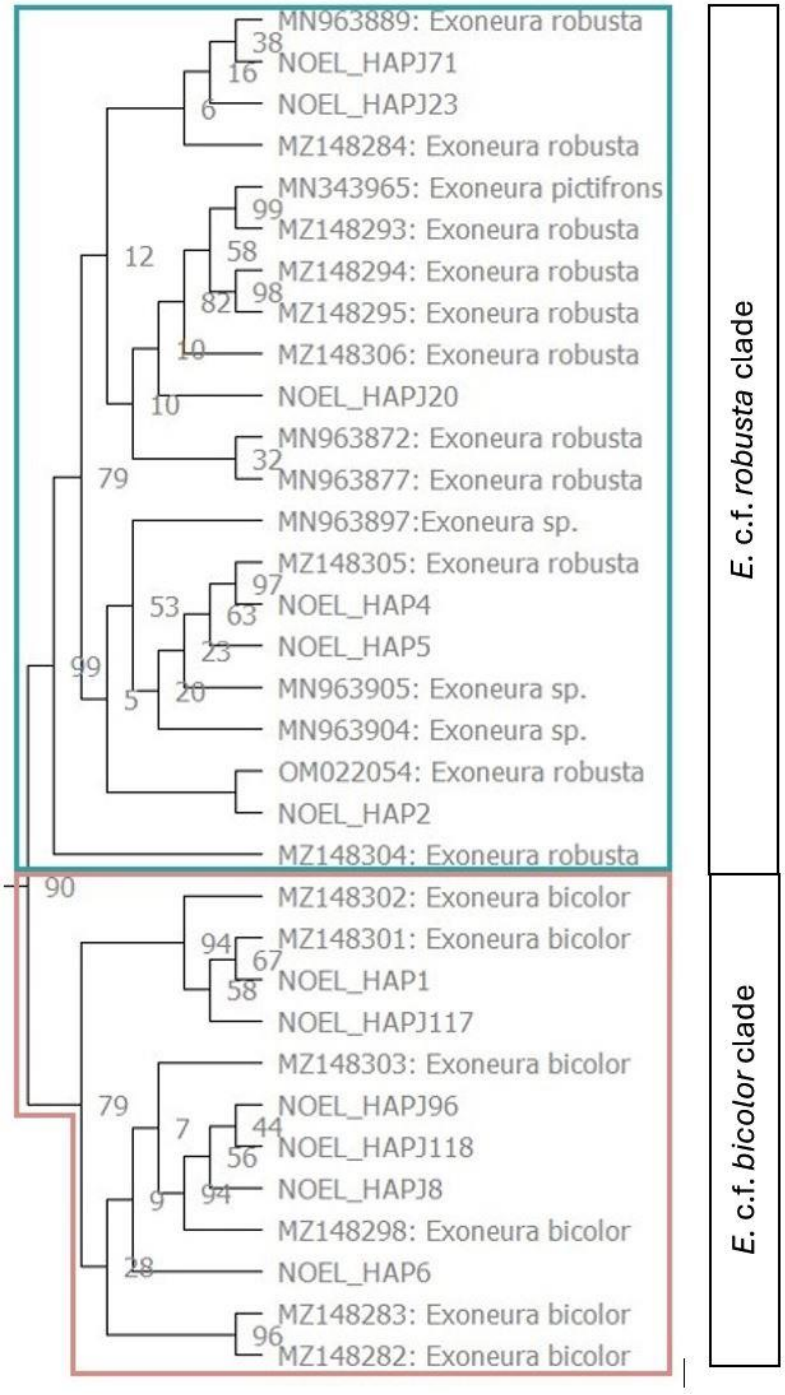

Figure 4: Genbank rapid IQ-TREE of *Exoneura* phylogeny, generated with default settings and 5000 bootstraps. The *E.* c.f. *robusta* clade is outlined in blue, and the *E.* c.f. *bicolor* clade is outlined in pink.

Although there are several low bootstrap values between GenBank archives and my sequenced reed bees within each clade, the structure of this IQ-TREE demonstrates confidence that the *E.* c.f. *bicolor and E.* c.f. *robusta* groups are genetically distinct, indicating they represent well-supported clades in the phylogenetic tree. High bootstrap values represent robust statistical support, and in this case, the bootstrap value of 90 at the first branch represents 90% support for the division at this node.

4.1.1 Clade Distribution

The *E.* c.f. *robusta* clade was most abundant at lower elevations. They were present at the 5 lowest elevations, and progressively decreased in abundance as elevation increased and did not occur above 1600m (920m: n=13, 1210m: n=13, 1240m: n=10, 1450m: n=9, and 1600m: n=1).

While more abundant at higher elevations, the *E.* c.f. *bicolor* clade was also more widely distributed across the elevational gradient, occurring at all elevations apart from the 1240m site (920m: n=8, 1210m: n=6, 1450m: n=2, 1600m: n=12, 1750m: n=4, and 1850m: n=13). This clade outnumbered *E.* c.f. *robusta* beginning at the 1600m site and remained more abundant along the rest of the elevation gradient

Distribution patterns of both clade are shown in Figure 7.

4.2 Thermal Tolerance

Reed bees sampled from higher elevations were more tolerant of lower temperatures compared to bees from lower elevation ($p = 0.002$). However, there was an interaction between the effect of elevation and clade on cold tolerance, such that while cold tolerance was stronger at higher elevations in *E.* c.f. *bicolor*, its effect was weaker in *E.* c.f. *robusta*.

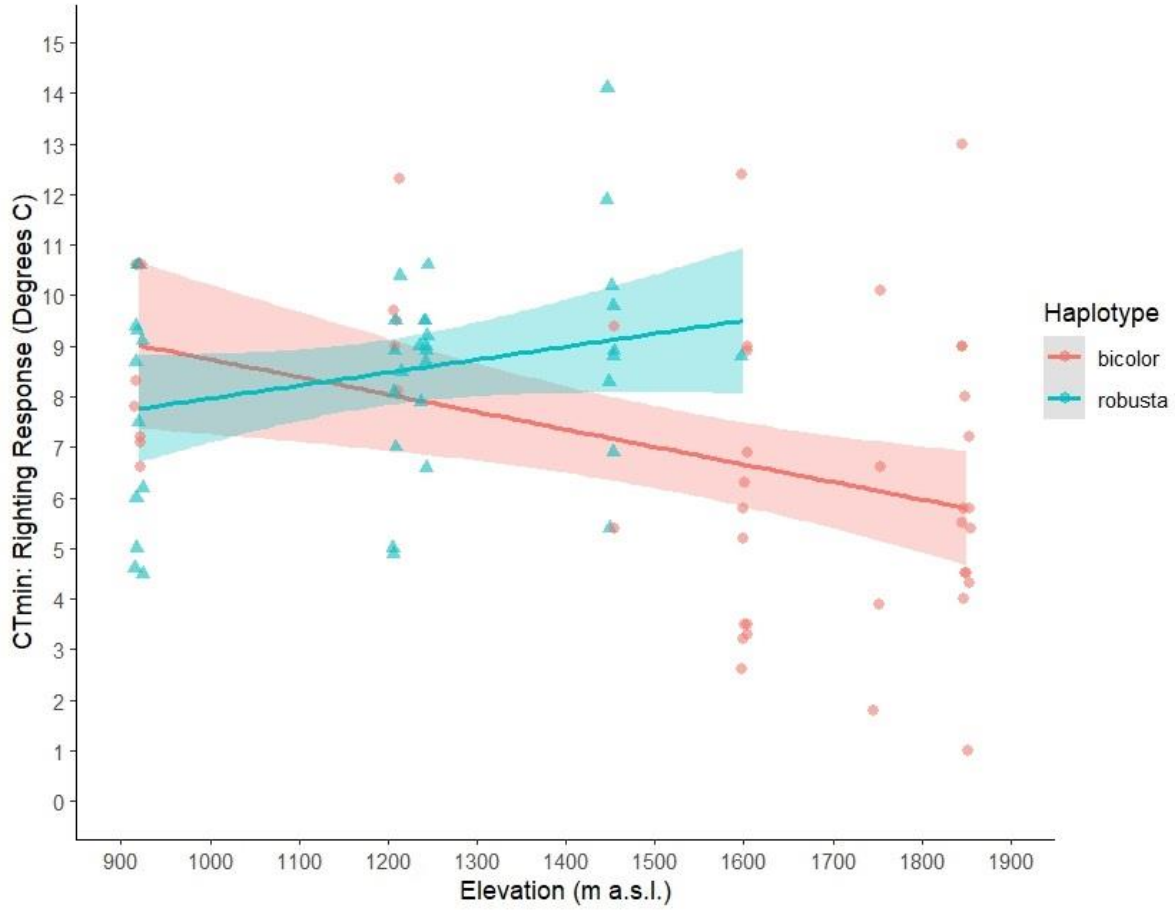

Figure 5: Multiple linear model visualisation of the righting response critical thermal limit (CT_{min}) of two reed bee clades (*E.* c.f. *bicolor* and *E.* c.f. *robusta*) collected at 7 different elevations.

Figure 5 represents the multiple linear model containing reed bee clade and elevation as independent variables, and reed bee righting response as the dependent variable. Only one reed bee in the *E.* c.f. *robusta* clade was observed at 1600m and none were observed at higher elevations.

Coefficient	Estimate	s.e.	*t*-value	Pr(>|t|)	*P*
Intercept	12.199	1.648	7.404	1.05e-10	≪0.001
Elevation	-0.003	0.001	-3.272	0.00156	<0.002
Clade	-6.798	2.743	-2.478	0.01527	<0.02
Elevation:Clade	0.006	0.002	2.864	0.00532	<0.006

Table 2: Multiple linear model summary of the effect of elevation, clade, and their interaction on the righting response of reed bees (CT_{min}).

The model had a residual standard error of 2.408 on 82 degrees of freedom, a multiple R2 of 0.205, and an adjusted R2 of 0.1759.

4.3 Pollen Composition Analysis

Pollen composition analysis showed that on average for any given bee, the most abundant pollen type accounted for 86.5% (population mean) of their total body pollen (Figure 6). This finding warranted the focus of my analysis on the identity of the most abundant pollen type found on reed bees. At elevations 920m to 1600m, the pollen type that both reed bee clades foraged from the most was Myrtaceae. However, at the highest elevation (1850m), where only *E.* c.f. *bicolor* was present, Asteraceae was foraged from the most as shown by 7 of 13 bees (Figure 7).

4.3.1 Pollen Type Cumulative Rank Abundance

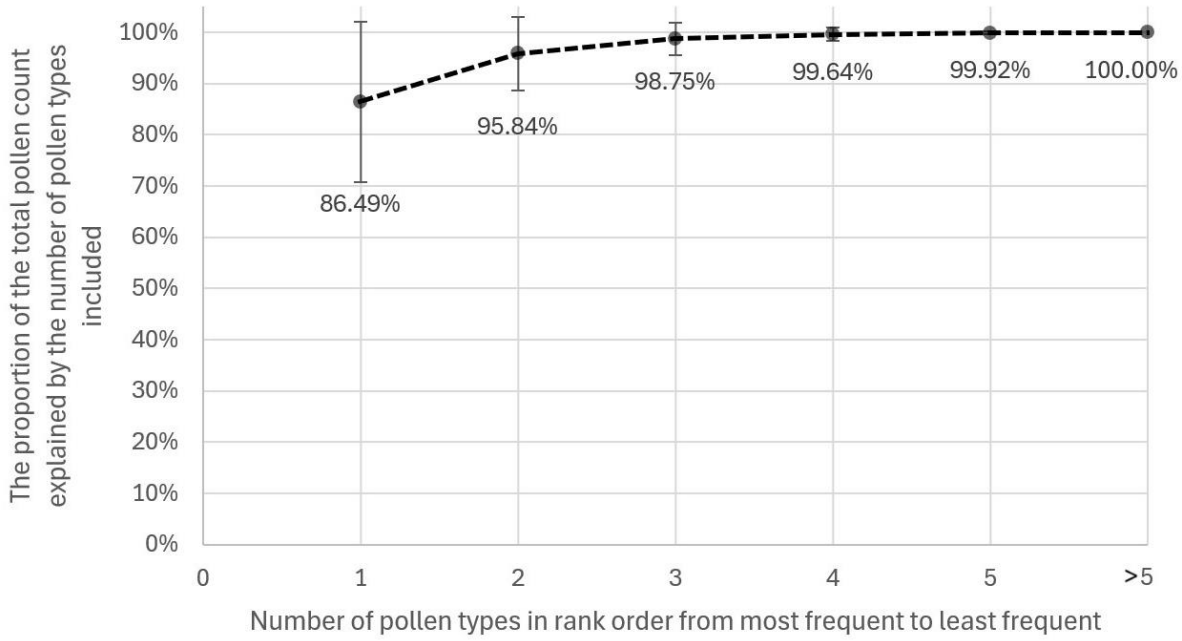

Figure 6. Rank abundance curve of the number of pollen types from most to least frequent by the mean cumulative proportion of total pollen grains per bee (explained by the number of pollen types included).

Figure 6 shows the accumulating mean percentage of the most abundant pollen morphospecies present on sampled bees. i.e. Of all pollen grains counted, the most abundant pollen type accounted for 86.49%. The sum of the 1st and 2nd most abundant pollen types accounted for 95.84% of pollen grains, the sum of the 1st, 2nd, and 3rd most abundant pollen types accounted for 98.75%, and so on. Error bars represent standard error.

While the largest number of pollen types on any one bee was 13, the number of pollen types is limited to 5 in the figure since it accounts for most of the pollen.

4.3.2 Elevational Patterns of Pollen Types Found on Bees

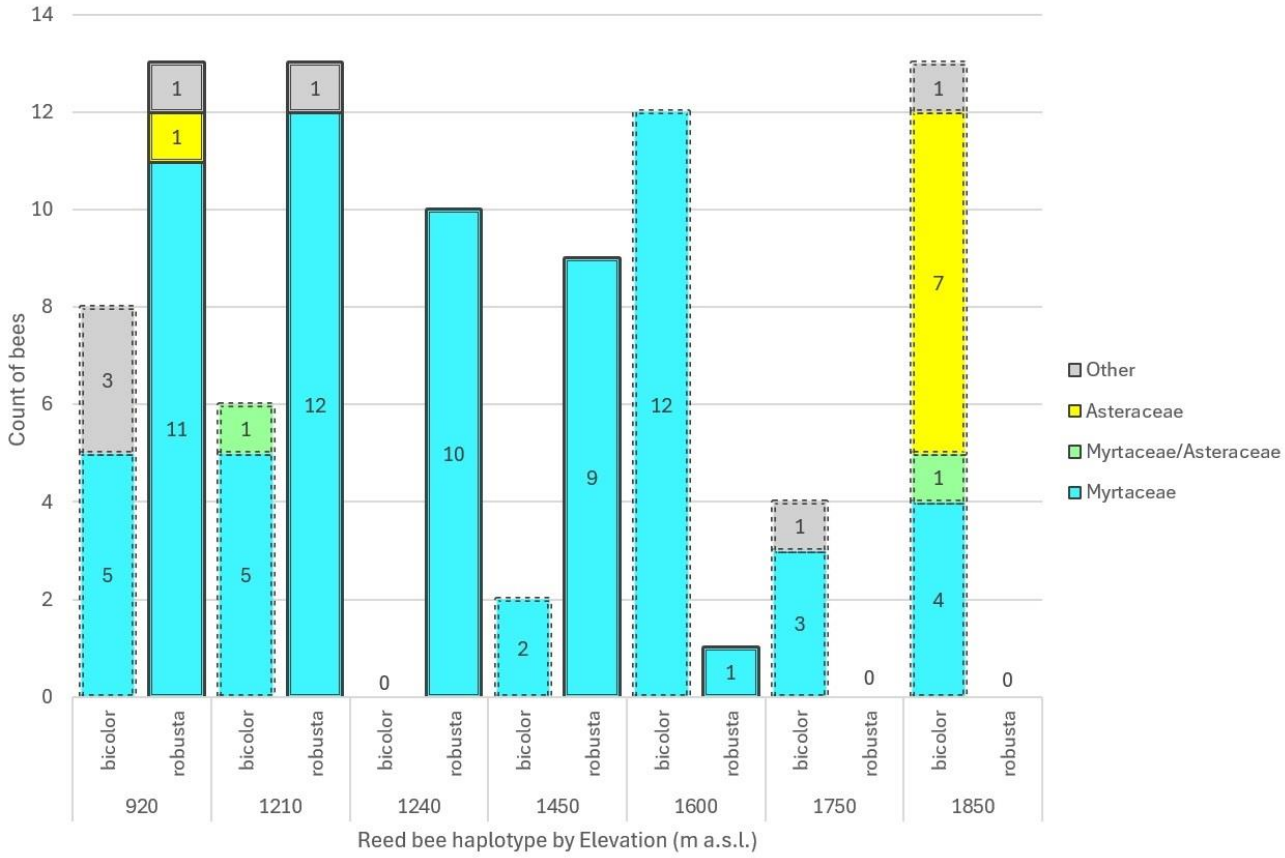

Figure 7. Count of reed bees per clade collected at different elevations, categorised by the most abundant plant family pollen type(s) they were carrying.

Figure 7 shows the number (n=91) of reed bees (*E.* c.f. *bicolor* or *E.* c.f. *robusta*) categorised by the predominant body pollen found on them by plant family. Colour fill within each column section represents pollen plant family label, while column outline distinguishes the clade (hatched: *E.* c.f. *bicolor*, solid: *E.* c.f. *robusta*). Issues that arose caused the exclusion of 5 samples in Figure 7 including failed genotyping (n = 3) or no pollen grains being present on the sample (n = 2). A count fell into a pollen type category if the proportion of a single pollen type accounted for more than 75% of that reed bee's total body pollen. Any pollen type below 75% was fitted into a blended category with the next

most prominent pollen type. Pollen types with either single values or considered less important were grouped together and categorised into “Other” for simplicity. These included one bee with >75% Lamiaceae and 6 bees with varying blends of Ericaceae, Fabaceae, Myrtaceae, Polygonaceae, Rosaceae, and an unidentified pollen type, no proportions of these blends accounted for >75%.

4.3.3 Pollen Family Compositions

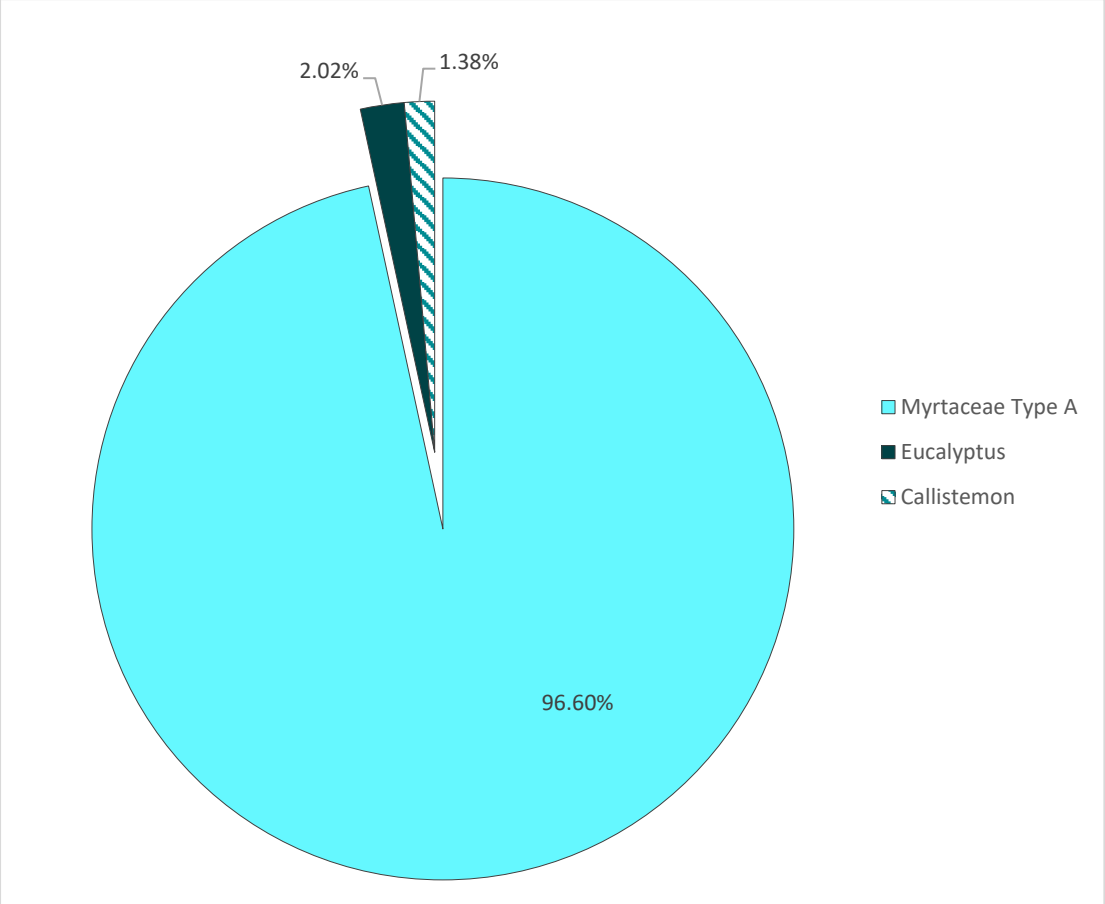

Figure 8. The proportions of Myrtaceae pollen accounted for by different morphospecies, collected across all elevations.

The high abundance of “Myrtaceae type A” should be interpreted cautiously since several pollen taxa within the pollen label were too morphologically cryptic to identify to genus level resolution. While identifiable as Myrtaceae pollen, the label likely consists of *Baeckea*, *Leptospermum*, and *Kunzea* genera based on anecdotally large numbers of big Myrtaceous shrubs in flower observed at multiple elevations during field work.

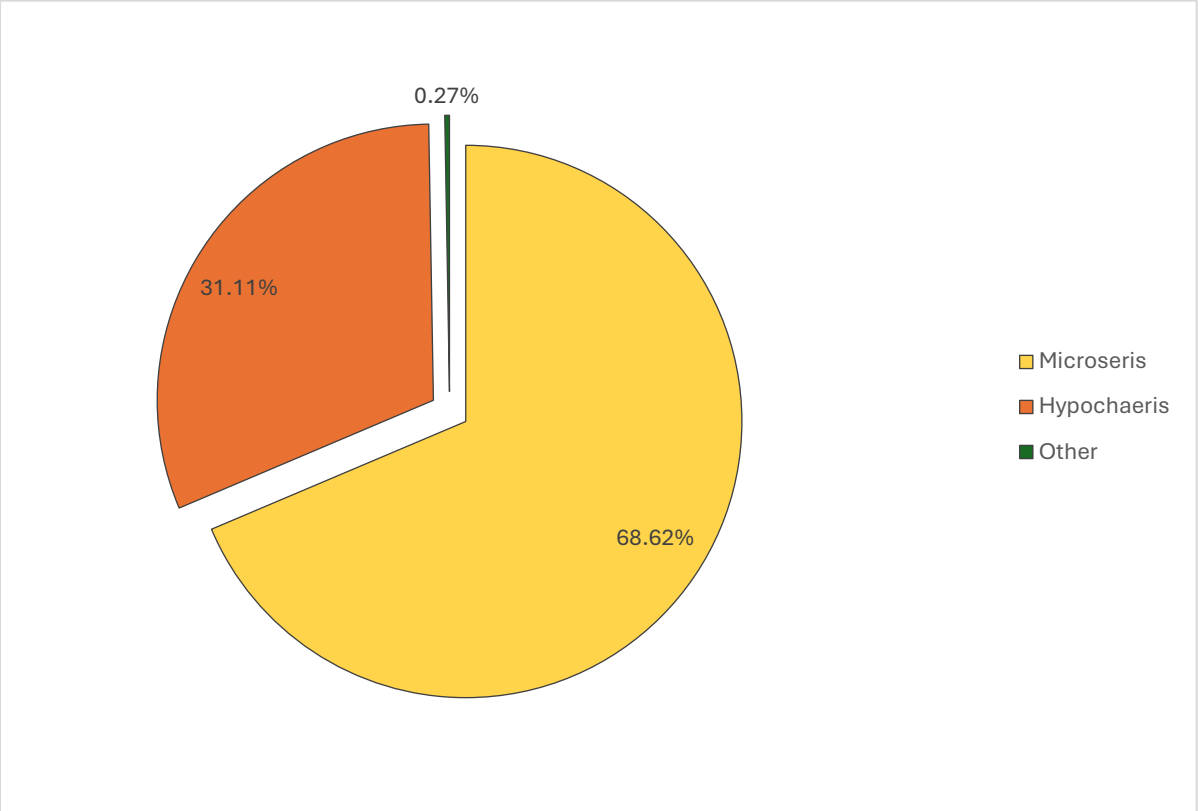

Figure 9. The proportions of Asteraceae pollen accounted for by different morphospecies, collected across all elevations.

5 pollen taxa (*Celmisia, Brachyscome*, Asteraceae spp: 1, 2, and 3) were joined under the "Other" label in this figure for clarity since their individual proportions were below 1%.

Chapter 5: Discussion

Given the temperature and floral community gradients in mountain ecosystems, I hypothesised that reed bees would be best adapted to the environmental conditions of the elevation they originated from, as reflected in their genetics, thermal physiology, and foraging patterns. DNA extraction results revealed two distinct reed bee clades with clear differences between their elevational distribution and thermal physiologies. Pollen composition results did not display a strong correlation to reed bee clade, instead indicating differences driven by elevation. My findings add further resolution into the factors influencing the species elevational distribution, which has previously been observed in nearby montane habitats (Cronin 2001; Bernauer et al. 2021). Of the two clades I identified, *E.* c.f. *bicolor* appears to be more specialised to higher elevations. The decreasing presence of *E.* c.f. *robusta* at higher elevations suggests a limited capacity to tolerate the lower temperatures characteristic of subalpine environments. However, they appear to thrive at lower elevations as indicated by their consistent abundance. Conversely, *E.* c.f. *bicolor* appears to be capable of persisting across the entire elevational gradient elevation, indicating a wider environmental flexibility. An organism's suitability to a specific niche is typically the result of a combination of morphological, behavioural, and physiological traits (Attiwilli et al. 2022). My study has identified two reed bee clades with overlapping ranges, which have the potential to be classified as distinct species. Considering this, establishing the features that define them may be valuable in understanding the abundance of each clade as explained by the differences in selection pressures in the elevation gradient. To do this, I will examine the differences in their distribution patterns, pollination role, and their environmental responses.

5.1 Genetic diversity

Haplotype identification is based on genetic differences in the genome (Sunnucks and Hales, 1996) between and within populations of a species. Reed bee species are notoriously difficult to distinguish visually due to their morphological similarities (Coates 2020; Silberbauer and Schwarz 1995). Reed bee haplotypes were identified using an 80% similarity threshold, which resulted in 12 identified haplotypes. While the use of an 80% threshold may have resulted in more haplotypes being identified than exist, the first IQ-TREE branch displayed a strong bootstrap value of 90, grouping haplotypes into either the *E.* c.f. *robusta* or *E.* c.f. *bicolor* clade (Figure 4). While the substantial bootstrap value indicates a species-level distinction between two haplotype clades, a more thorough investigation would be required to identify each clade as individual species. Previous studies conducted in the region have relied on morphology to identify reed bees, which has resulted in being limited to genus-level identifications (Coates. in prep, Encinas-Viso et al. 2024). The findings of my study are useful in clarifying the species-level identity of reed bees sampled in the studies since they occurred in the same region. As such, my results likely suggest that the reed bees collected in previous studies above 1850m a.s.l. (Inouye and Pyke, 1988; Encinas-Viso et al., 2023; Coates et al., 2024) are E. *bicolor*.

Cryptic species are defined as two or more species that have not been classified into separate taxa due to morphological similarities that make them visually indistinguishable (Hending 2024). A group of morphologically similar taxa can be considered cryptic if they have recently diverged, are reproductively isolated, can only be distinguished genetically or molecularly, or occur sympatrically in the wild (Hending 2024). The distribution patterns of each reed bee clade in this study suggest that they occupy different ecological niches. Additionally, flowering seasons commence later at higher elevations (Inouye and Wielgolaski 2003), suggesting that *E.* c.f. *bicolor* populations at elevations above 1600m are likely reproductively isolated spatially and temporally (Pyke et al. 2011). Moreover, reed bee clades co-occur at some elevations and DNA results reveal genetic distinctions between each clades. The combination of these findings highlights the strong likelihood that reed bees within my elevation gradient are a cryptic species.

The presence of two reed bee species along the elevation gradient raises questions about the factors that have shaped their distribution patterns. Within what we know of allodapine bees, several factors may provide an explanation. Findings from Chenoweth and Schwarz (2011) suggest that climate change during the Miocene had a significant impact in accelerating diversification rates, particularly in temperate exoneurine lineages. Despite the geographical proximity of sample sites, literature on speciation in mountain environments indicates that certain habitat characteristics can serve as key drivers of rapid speciation and promote ecological diversification along exposure gradients (McCulloch et al. 2019). One of these characteristics is the higher level of microclimate and habitat spatial heterogeneity at higher elevations compared to lower-elevation ecosystems (Encinas-Viso et al. 2023), which ultimately creates a variety of ecological niches for reed bees to exploit and adapt to (Verrall et al. 2021).

As shown by translocation experiments, reed bees demonstrate highly plastic behavioural responses to their environment (Cronin 2001), and it is likely that this ecological flexibility influences their distribution. Studies have suggested that behavioural variation is linked to differences in nesting substrates, floral resource availability, and thermal effects (Silberbauer and Schwarz 1995; Cronin 2001; Bull et al. 2003; Chenoweth and Schwarz 2011; Bernauer et al. 2021; Jaboor et al. 2022). It has also been established that temperature directly impacts brood development time in insects, with direct solar radiation potentially playing a role at higher elevations (Cronin 2001; Bryant et al. 2023; Ostwald et al. 2023; Stabentheiner et al. 2022, Silberbauer and Schwarz 1995). These observations are particularly relevant at higher elevation sites in my study, since *E.* c.f. *bicolor* nests in dead snow gum (Coates et al. in prep), where canopy covers can be sparse and more exposed to direct solar radiation.

It has also been suggested that durability, availability and accessibility of nesting substrates could be important for the development of multifemale reed bee colonies (Silberbauer and Schwarz 1995). Past comparisons between nesting substrates outlined in Silberbauer and Schwarz (1995), found that *Melaleuca squarrosa* supports larger and higher proportions of social colonies than *Xanthorrhoea minor*, likely due to differences in nesting substrate durability. Social living may also provide valuable benefits to *E.* c.f. *bicolor* populations, as they facilitate strategies for coping with abiotic pressures (Bernauer et al., 2021; Cronin, 2001; Michener, 1962; Ostwald et al., 2023). These factors overall suggest that the benefits associated with snow gum nesting habitat, may provide valuable benefits explaining the successful colonisation of *E.* c.f. *bicolor* at high elevations.

E. c.f. *bicolor* abundance declines in the intermediate and lower elevations of the study gradient, where both clades occur, and a possible explanation for this decline could be explained by biotic competition pressures exerted by *E.* c.f. *robusta*. However, my results suggest that environmental temperature is a more defining influence that directly acts as a limiting factor on clade distribution in mountain areas (Hoiss et al. 2012).

5.2 Thermal physiology.

Analysing the differences in reed bee thermal physiology will assist in differentiating the two clades, explain their distribution (Oyen et al. 2016; McCabe et al., 2019), and allow us to forecast how each is likely to respond to a warming climate. Results from the CT_{min} assays of *E.* c.f. *bicolor* support my initial hypothesis that high elevations bees were more tolerant to colder temperatures than low elevation bees ($p = 0.002$). An interaction was also present between the effects of elevation and clade on cold tolerance, such that while cold tolerance was stronger at higher elevations in *E.* c.f. *bicolor*, the effect was weaker in *E.* c.f. *robusta*. These findings suggest that *E.* c.f. *robusta* is unable to tolerate the environmental conditions of higher elevations, as shown in Figure 7 through the decline of their distribution with elevational gain.

The differences between the elevational effects of CT_{min} on each clade's thermal physiology further supports the notion that each clade occupies distinct ecological niches (Tougeron et al., 2016). *E.* c.f. *bicolor*'s more adaptable thermal tolerance suggests that the clade is more specialised to operate in the lower temperatures of the subalpine zone. Conversely, cold tolerance appears to be less selected for in the *E.* c.f. *robusta* clade, as shown by their narrower cold tolerance. These notions are reflected in the distribution patterns of each clade (Figure 7). The elevation range of *E.* c.f. *robusta* extends well into the subalpine zone, but not beyond, which is consistent with the notion that temperature is a limiting factor. The findings of my thermal assays strongly suggest that low temperatures are a significant filter

shaping the distribution patterns of reed bee clades in mountain systems (Hoiss et al., 2012). Many other climatic factors unique to mountain habitats may also contribute to limiting reed bee distribution (Hodkinson, 2005). Understanding how climate shapes the trait relationships of reed bees are valuable in disentangling the mechanisms underpinning their distribution and survival (Kellermann et al., 2012) within mountains rapidly changing environments (Oyen et al., 2016).

Some processes by which organisms generally adapt to an environment include either adaptive plasticity or local adaptation. Adaptive plasticity allows individuals to cater their behaviour, physiology, or development in response environmental stimuli within their lifespan (Dewitt and Scheiner, 2004; West-Eberhard, 2003). Conversely, local adaptation occurs over generations and is defined by gradual genetic changes within a population selected by the environmental pressures that improve fitness (Kawecki and Ebert, 2004; Hereford, 2009). There is evidence that reed bee ancestors speciated within temperate conditions (Chenoweth and Schwarz, 2011), and likely further adapted through these processes to colonise higher elevation habitats. Adaptive hypotheses predict how abiotic variables affect physiological function, potentially explaining the elevational variations observed in clade thermal physiologies (Nicotra et al., 2015; Münzbergová et al., 2017; Ullah et al., 2024). According to the adaptive plasticity hypothesis, reed bees in the *E.* c.f. *bicolor* clade may have a wider thermal plasticity, explaining their wide elevational distribution (Nicotra et al., 2015; Drummond et al., 2020). Local adaptation would also explain distribution patterns, with stronger CT_{min} being selected for in the clade gradually over multiple generations. However, this differs in *E.* c.f. *robusta* as they exhibit thermal physiologies less adaptive to elevation. This suggests a divergence in each clades niche specialisations, with *E.* c.f. *bicolor* adapting to higher elevations, and *E.* c.f. *robusta* specialising in lower elevation environments.

5.3 Body Pollen Composition

Analysing the pollen collected by reed bees reveals more about the role they play as pollinators in Australia's mountain landscapes. The main findings from my study are centred around the plant families Asteraceae and Myrtaceae. The Myrtaceae family was overall more frequently foraged from across all elevations as reflected by 74 of the 91 genotyped reed bees. The high frequency of large Myrtaceae proportion in body pollen compositions suggests that Myrtaceae species are widespread and abundant in the region (Totterdell et al., 2000) and have resource rich flowers that are likely ecologically valuable considering their accessibility to a broad range of visitors (Coates et al., 2023). Myrtaceae was also the plant family most foraged from at each elevation apart from the highest elevation, where it shifted to Asteraceae. This is surprising because Asteraceae is phylogenetically distant from Myrtaceae, and

pollinators often display family-level floral associations (Grant and Grant, 1965, Tremblay, 1992, Coates et al., 2024). However, this can be explained by significant shifts in mountain vegetation compositions that occur at higher elevations (Verrall et al., 2021). While reed bee pollen was dominated by two plant families, a broad diversity was foraged from as reflected in a small number of reed bees. These plant families included Ericaceae, Fabaceae, Lamiaceae, Polygonaceae, and Rosaceae. Overall, these patterns may be explained by several factors, however there were no clear differences in pollen composition patterns between reed bee clades, which suggest that reed bee clades exhibit flexible or broad foraging preferences.

While extensive research has focused on how elevation shapes the distribution of pollinators directly (McCabe et al., 2019), there are still many factors unaccounted for in the context of Australian alpine pollinators (Encinas-Viso et al., 2024) which my research aims to address. I predicted reed bees to forage from multiple distinct plant assemblages at different elevations since mountain vegetation communities change with elevation (Edmonds et al., 2006; Slatyer, 2010) and heterogeneity of the landscape is typically high (Encinas-Viso et al., 2023).

Reed bees predominantly foraged from Myrtaceae across most of the elevation gradient and shifted to Asteraceae at higher elevations as initially hypothesised. However, only finding one shift is surprising because the strong environmental heterogeneity and elevational partitioning of mountain floral communities should create differing abundances of pollen resources. Furthermore, frequent Myrtaceae foraging occurred in both clades despite varying in their elevational distributions. Reed bee's tendency to forage primarily from two phylogenetically distant plant families despite their capability to forage from a diversity of plants suggests that they employ generalist flower-visiting habits. Low degrees of specialism are common in mountain plant-pollinator communities (Johanson et al., 2019; Verrall et al., 2023). In combination with the fact that generalists visit wider varieties of flowers (Coates et al. 2023) it is further unexpected that reed bees do not seem to exploit the diversity of pollen resources available across the mountain gradient (Brown et al., 2024). This pattern may be explained by a lacking diversity of pollen resources associated with an anecdotally early flowering period.

My findings on the abundance of Myrtaceae pollen are contrary to Coates' (2024) who found that bees respectively foraged more from Proteaceae and Fabaceae at the low and mid elevations. However, this disparity is likely due to differences between observation methods. Flower visitation observations are restricted by short distances since they need to be sighted, which pollen composition analyses are not limited by. In contrast to other plants, I observed anecdotally large numbers of big Myrtaceous shrubs in flower at multiple elevations. This suggests that reed bees foraged from myrtaceous plants frequently due to their larger floral availability. This logic may also apply to the higher elevations of

my gradient and explain the shift in reed bee body pollen from Myrtaceae to Asteraceae. Of the Asteraceae pollen liberated from reed bees, the majority belonged to two main taxa, Microseris lanceolata (69%) and Hypochaeris radicata (31%), the latter of which) has been shown to have significantly broad and sustained coverage (Johanson et al., 2019; Ng, 2019). Coates (2024) also found that Asteraceae abundance increased with elevation and that despite being rarely visited by bees, the family is more visited by pollinators overall at higher elevations.

While sampling reed bee nests at high elevations (~1900m a.s.l.) Coates (in prep) found that reed bees foraged close to their nests at high elevations, restricting them to forage from plants nearby. While the mechanisms of this finding are yet to be fully understood, it may be explained by rapid and frequent shifts of environmental conditions common at higher elevations (Goodwin et al., 2021). This suggests that *E.* c.f. *bicolor* foraging strategies may be opportunistic like many other subalpine organisms that rely on short growing and flowering periods (Inouye and Wielgolaski, 2003). Further investigation would be required to confirm this; however, the abundance of Asteraceae pollen on reed bees from 1850m could be greater due to a combination of greater abundances of Asteraceae, and *E.* c.f. *bicolor*'s limited foraging range.

While *E.* c.f. *bicolor* forages more from Asteraceae at 1850m, there appears to be no correlation between Asteraceae abundance and the clade's distribution given that it becomes more abundant than *E.* c.f. *robusta* at 1600m. This is unexpected since the floral association hypotheses states that elevation influences the distribution of plant species and is displayed in alpine plant-pollinator communities through elevational partitioning (Coates et al., 2024). Coates (2024) found that bees displayed elevational partitioning in the floral association of common plant families, however clade distribution did not align with this trend.

While the high frequencies of Myrtaceae in both clade's pollen composition point to a strong association between snow gum and reed bees, the mechanisms underpinning it remain unclear. Overall, my study suggests that the pollination role and services of *E.* c.f. *bicolor* and *E.* c.f. *robusta* overlap in the elevation gradient.

5.4 Study Limitations:

My study was limited by a variety of factors, including a limited fieldwork duration of period of two weeks. Sampling reed bees over more of the flowering period would provide a more robust sample representative of the populations. Sampling reed bees from multiple elevational gradients would also help to establish the distribution of reed bee clades in different locations. This would also test the consistency of clade distribution patterns and potentially affirm the hypothesis that the factors influencing distribution patterns are characteristic of the region. Further, high elevation reed bees are known to limit the distance of their foraging journeys and only sampling from one location may have caused body pollen to be limited to the floral resources in proximity of reed bee nests. It may also have narrowed the genetic diversity results of reed bees for the same reasons.

I also had to develop my own pollen liberation methods because past research primarily uses dry samples and reed bee samples were wet due to their ethanol submersion after undergoing thermal assays. Achieving a replicable and supported method involved a lengthy trial and error process which took a significant amount of time.

5.5 Ecological Implications and Further Research:

5.5.1 Genetic Diversity

Steep and compact environmental gradients (Coates et al., 2024; Verrall et al., 2023) create a mosaic of environmental conditions which suggest further species diversity within reed bee populations (Verrall et al., 2021). Several conservation implications arise from establishing reed bees in my elevation gradient as a cryptic species given our limited understanding of their role as mountain pollinators, especially in a landscape vulnerable to climate change (Hending, 2024). As demonstrated in Figure 5, variations between clades were apparent in their elevational distributions and thermal physiology, suggesting they may respond differently to exacerbated climate shifts in mountain landscapes. This forecasts alterations in plant-pollinator networks (Slatyer, 2010; Dahlhoff et al., 2019; Inouye, 2020; Jaboor et al., 2022), especially in subalpine *E.* c.f. *bicolor* populations considering their habitat ranges are likely to shrink whilst competition pressures increase.

These trait associations raise concern about reed bees' capability to adapt to rapid climate change, particularly in the case of *E.* c.f. *bicolor*, given its high frequency in the subalpine zone. To address these concerns, future genomic comparisons of reed bee populations would help to further determine the delineation of cryptic reed bee species (Hebert et al., 2004). Examining the association between reed bee population distribution and their trait relationships in other mountain environments would also help us better understand their relative ecological importance as pollinators (Herrera et al., 2023). Investigating genetic variability within clades may further reveal whether reed bee populations serve different pollination roles based on their microhabitat. These findings may shape our expectations of how different mountain microhabitats may respond to rapid climate change, particularly through expected shifts in pollinator-plant interactions that could impact the broader mountain ecosystem. Overlooking the ecological value reed bees provide in pollination services within the landscape could result in local extinctions, particularly in the case of *E.* c.f. *bicolor*, which may lead to serious consequences for mountain ecosystem function and management (Cardoso et al., 2020). Therefore, it is crucial to identify, protect, and conserve these species to ensure their ecological and economic value is not lost (Das, 2012; IPBES, 2016; Sánchez-Bayo & Wyckhuys, 2019).

If in the case that *E.* c.f. *bicolor* is described as a new species or subspecies, these findings point to the possibility of the clade being threatened according to IUCN Red List Criteria. The implications of projected range shifts also strongly align with IUCN Red List Criteria A and B, the first of which being expected population reductions. Given the limited area (7,938 km²) of the Australian Alps bioregion (NSW National Parks and Wildlife Service, 2003; Slatyer, 2010), it is likely that the second criterion of expected reductions in an already fragmented and restricted distribution range will also be

met. These possibilities warrant a more focused research effort into describing cryptic reed bee species in order to foster a more accurate assessment of mountain reed bee populations conservation status. The credibility of these concerns is further supported by the case of Bogong moths, a threatened pollinator that is also present in the landscape (Coates et al., 2023) and was recently added to the IUCN red list for similar reasons. Lastly, exploring the abundance and reproductive success of *E.* c.f. *bicolor* populations should be conducted to determine whether they meet remaining IUCN criteria requirements (C, D, E).

While The IUCN Red List guidelines are comprehensive for assessing vertebrate species, this effectiveness does not extend to insects (Cardoso et al., 2011) and other cryptic taxa like reed bees (Gaston and Fuller 2008). Many cryptic taxa are grouped together (Funk et al., 2011) resulting in the species comprising them failing to meet IUCN criteria, ultimately leaving them overlooked. Given the genus' distribution range is quite broad, cryptic mountain reed bee populations highlight this issue considering they operate in specific mountain ecosystems niches that are particularly vulnerable to climate change. Failure to assess conservation status under the IUCN Red List means species potentially vital for ecosystem function, similar to reed bees, may decline unnoticed. This gap underscores the need for IUCN guidelines to be revised, implementing accommodations of rare, cryptic, and understudied species into their criteria (Betts et al., 2020). Expanding IUCN guidelines in this direction would help to achieve a more accurate representation of insect biodiversity, allowing for targeted research and conservation efforts to protect overlooked species.

5.5.2 Thermal Physiology

My study reveals that the relationship between thermal physiology and elevation varies by reed bee genetic clade along an elevation gradient and indicates that each clade's CT_{min} is likely to respond differently to changing abiotic conditions (Figure 5). Establishing elevational differences in thermal physiologies suggests possible differences in adaptive responses. These findings highlight the need to better understand the genetic trade-off relationship between thermal physiology and reed bees' environmental fitness (García-Robledo and Baer, 2021).

Mountain systems offer a unique opportunity to investigate the role of plasticity in responding to global climate change (Gonzalo-Turpin and Hazard, 2009; Nicotra et al., 2015), since organisms consist of numerous interrelated traits (da Silva et al., 2022). Within-species trait variation has been observed in several alpine species (Nicotra et al., 2015; McCulloch et al., 2019). As some genotypes are suggested to have a greater potential to respond to stressful conditions (Nicotra et al., 2015), a divergence in the adaptive responses of reed bee clades would explain their varying distributions. Adaptive hypotheses highlight the direct impacts of abiotic factors on clade distribution. It has been suggested that trade-off

interactions can occur between adaptive plasticity and local adaptation and can be limited by the costs associated with maintaining plastic responses (Hereford, 2009; Gunderson, 2023). Implications of the baseline tolerance/plasticity trade-off hypothesis (TOH) may be relevant to high elevation *E.* c.f. *bicolor* populations due to their strong CT_{min}. According to TOH, stronger CT_{min} corresponds with weaker thermal plasticity (Gunderson, 2023), which may apply to *E.* c.f. *bicolor* since they demonstrate strong CT_{min}, especially at high elevations (Gunderson and Stillman, 2015; Gunderson, 2023). Further research is needed to validate the hypothesised correlation, assess the adaptive capacity of CT_{min} in each clade, determine whether a trade-off association occurs between CT_{min} and CT_{min} plasticity, and assess differences between clades. Examining this is likely to be more important in relation to the subalpine populations, since varied adaptive responses of CT_{min} could have implications for the way in which clades adapt to climate change, due to the finite and declining nature of similar habitat in Australia (da Silva et al., 2022).

As climate change rapidly alters mountainous environments, reed bees can respond through the same processes of adaptation or migrate to follow the conditions they are adapted to (Marshall et al., 2020). Slatyer (2010) predicted that Mt. Kosciuszko's snow line would rise from its current elevation of 1460m to between 1490m and 1625m. Temperature gradient and distribution patterns of *E.* c.f. *robusta* and *E.* c.f. *bicolor* may align with this prediction (Stabentheiner et al., 2022) but may also be a result of competition effects (Temeles et al., 2016). The thermal physiology of *E.* c.f. *robusta*, which displays a weaker influence of elevation, likely has a reduced fitness at high elevations compared to *E.* c.f. *bicolor*. The trade-offs associated with a stronger CT_{min} may explain why *E.* c.f. *bicolor* is less abundant than *E.* c.f. *robusta* except at higher elevations (Marshall et al., 2020). However, to fully understand the drivers of clade distribution, we must also consider the broader influences that abiotic factors have on mountain communities. Several studies predict modifications to occur in the biotic interactions of mountain plant-pollinator communities caused by shifting abiotic conditions (Das, 2012; IPBES, 2016; Dahlhoff et al., 2019; Inouye, 2020).

Testing this could be achieved by conducting reed bee nest translocations following methods used in Cronin (2001). Replicating the conditions of high and low elevations and rearing a lineage of each clade in each treatment could help determine whether reed bees adapted high CT_{min} in response to harsh abiotic mountain conditions via local adaptation, adaptive plasticity, or a combination of both (García-Robledo and Baer, 2021). It may also detect whether a trade-off association is present between the two traits and the extent of the fitness costs associated with having high CT_{min}. Quantifying the reproductive success of test lineages would also gauge the extinction pressures high elevation *E.* c.f. *bicolor* populations are likely to face, whether by increases in temperature, competition factors, or a combination of both. If reed bees survive across multiple generations, this experiment could yield

valuable insights into their role as pollinators. By assessing the behavioural plasticity of each clade, comparisons could be drawn between patterns in sociality, nesting behaviour, substrate preferences, and floral foraging choices. Overall, these experiments would provide valuable insights into the mechanisms that have shaped the reed bee distributions in mountain landscapes, as well as how they are likely to respond to climate change. The latter of which may be important in its relevance to mountain insect pollinators globally.

Overall, it is essential that research examines how climate tolerance traits are interconnected and evolve together across mountain landscapes (da Silva et al., 2022), which is particularly important considering the accelerated effects of climate change in alpine ecosystems.

5.5.3 Pollen Composition

While reed bee clades seem to exhibit patterns in their elevational distributions that vary based on genetics and thermal physiology, there does not appear to be an association between distribution and the plants they forage pollen from. Thus, reed bees are more likely to be in competition with one another for pollen resources without distinctions in foraging patterns. This is particularly concerning considering high elevation *E.* c.f. *bicolor* populations could be outcompeted by *E.* c.f. *robusta* as their population ranges shift upwards.

It is unusual that the two plant families that reed bees primarily foraged from are phylogenetically distant since pollinators often display family-level floral associations (Grant and Grant, 1965, Tremblay, 1992, Coates et al., 2024). The discovery that *E.* c.f. *bicolor* reed bees are foraging primarily from Asteraceae and not Myrtaceae at high elevations highlights that the conservation of Asteraceae in the subalpine zone may be particularly important in sustaining *E.* c.f. *bicolor* populations. This is supported by past literature which states that reductions in pollinator abundance are often correlated with reductions in host plant populations, encouraging conservation efforts catered to host plants (Biesmeijer et al., 2006). While my study suggests that Myrtaceae and Asteraceae serve as important food resources for reed bees, my study does not sufficiently explore the associations between them enough to make solid conclusions.

As such, it cannot be determined whether reed bees foraging patterns shifted due elevational partitioning of floral communities or for alternative reasons, such as temporal changes to growing and flowering seasons, or general pollen resource availability. A combination of floral community surveys and sampling reed bee pollen loads at these higher elevation sites across a wider time scale would help to address these research gaps. It may also additionally help assess the importance of reed bee's role as pollinators in the Australian Alps, and the plant species which benefit from their pollination services the most. Frequent pollen compositions comprising mostly of Myrtaceae and Asteraceae in my samples further allude to reed bees' pollination role and foraging preferences but remain inconclusive without surveying floral resource availabilities. Although I found that reed bees can carry large quantities of single pollen types at a time in mountain ecosystems, additional research is required to quantify the value of the pollination services they provide to host plants. Achieving this would involve investigating whether reed bees effectively transfer conspecific pollen when visiting flowers, and if this is important in assisting the conservation of ecologically important yet vulnerable mountain plants, such as E. pauciflora.

Given that in many cases thousands of pollen grains were found on an individual reed bee's body, and that the proportion of pollen loads frequently consisted of Myrtaceous pollen across the elevation

gradient, there are strong indications that reed bees are central to facilitating the reproduction and gene exchange for Myrtaceae plant species. This is particularly important in the context of subalpine snow gum forests, which have recently been experiencing widespread dieback due to increased activity of a native wood-boring longicorn beetle (Bryant et al., 2023).

Further examinations should be untaken in nearby mountain environments to confirm whether similar patterns occur between reed bees and Myrtaceae species and deepen our understanding of the pollination services reed bees provide to plant assemblages in the Australian Alps. This could be achieved by pollinator exclusion experiments (Turner et al., 2011) lab-based pollen transfer experiments (Buxton et al., 2022), controlled choice assays (Allen-Perkins et al., 2024), or flower resource sampling (Szigeti et al., 2016). These experiments would provide insights into the reproductive benefits that plants receive from pollination services and reed bee foraging preferences.

Light microscopy enabled us to determine sufficient taxonomic resolution necessary to confirm that reed bees are feeding on local plants in the montane and sub-alpine zones. However, due to morphological similarities in pollen grains, particularly in Myrtaceae, species and genus-level identifications were not possible in most cases (Thornhill, 2011). While light microscopy is commonly used in pollination ecology studies (Ribas-Marques et al., 2022), DNA-metabarcoding help to achieve pollen identifications at a finer taxonomic resolution (Encinas-Viso et al., 2022). Future studies should implement a combination of light microscopy and DNA-metabarcoding techniques clarify pollen species present in my study and disentangle the foraging patterns of reed bees (Liu et al., 2017; Suchan et al., 2019, Arstingstall et al. 2023).

My findings on reed bees underscore the complex implications reed bee's response to climate change may have on mountain ecosystems, providing conservation efforts, park managers, and researchers with a clearer understanding of how shifts in reed bee populations could affect mountain ecosystem structure.

Chapter 6: Conclusion

In my elevational gradient, cryptic reed bee populations displayed genetic distinctions and associated links to thermal physiology, which differed depending on their elevation. As such, my thesis calls for further research to clarify species diversity within the genus to allow a more accurate assessment of each clade's climate vulnerability, particularly given the challenges of identifying reed bee species morphologically.

I found that *E.* c.f. *bicolor* reed bees displayed values of CT_{min} that were considerably more adaptive to elevational effects than *E.* c.f. *robusta* reed bees. However, if such predictions are based primarily on reed bee's responses to abiotic conditions, without considering biotic factors such as competition pressures and plant-pollinator mutualisms, they may not accurately predict reed bee's response to rapid warming. Past research indicates that climate change is predicted to shift plant communities upwards (Auld et al., 2022; Encinas-Viso et al., 2023), disrupting mountain pollination networks. As many pollinator species also shift upslope in response to warming temperatures, they are likely to further alter ecosystem functions by disrupting plant-pollinator mutualisms and creating novel species interactions (Matthews et al., 2010; Slatyer, 2010; Dahlhoff et al., 2019; McCabe et al., 2019; Inouye, 2020).

The current distribution patterns of reed bee clades and their separate thermal physiologies project that despite their capability to persist across a variety of environmental conditions, *E.* c.f. *bicolor* populations are likely to decline due to increases in competition pressures evoked by upslope range expansions of *E.* c.f. *robusta*. My pollen composition analysis displayed a shift in reed bee foraging patterns across elevations, as demonstrated by the transition of frequent abundance from Myrtaceae pollen to Asteraceae pollen on reed bees at the highest elevations. Explanations for this pattern are not fully clear but insights from literature suggest this could be addressed by follow up research. These patterns offer insights into how reed bees may respond differently to climate shifts. For example, *E.* c.f. *bicolor* populations are likely to experience range reductions (Larson et al., 2001), while *E.* c.f. *robusta* populations are likely to expand upslope. Both predictions have concerning implications for high elevation *E.* c.f. *bicolor* populations, raising concern in relation to their susceptibility to rapid changing climate by biotic and abiotic factors.

My findings underscore the importance of considering both factors in understanding how reed bees are likely to respond to a rapidly changing climate, where previous research on the elevational distribution of pollinators only considered abiotic factors (Hoiss et al., 2012, 2015). My thesis contributes to the broader understanding of biotic factors within mountain ecosystems, illustrating the

complex relationships between reed bees, plants, and environmental gradients. Future research should continue to explore these interactions, particularly in light of climate change and its enhanced impacts on the subalpine habitats of mountain ecosystems.

Further research in different reed bee populations, in different areas and across a larger temporal scale, would substantiate whether the reed bee clades I identified are genetically distinct enough to be considered separate species. It may also corroborate whether the patterns I found in the thermal physiology, pollen composition, and distributions of reed bees occur consistently amongst other reed bee populations in Australia's mountain ecosystems. Overall, my research helps to provide the basis for the expected responses of mountain reed bees to climate change and highlights avenues for future research.

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