# Role of Bogong moths (*Agrotis infusa*) in the nocturnal plant-pollinator network of Kosciuszko National Park

Andrea Groth*[1], Taia Artemov*[1]

* Corresponding authors: u7290923@anu.edu.au , u6867511@anu.edu.au

[1] Research School of Biology, ANU, Canberra, ACT, Australia

## Abstract

Nocturnal pollinators are essential for the maintenance of ecosystems because they allow for plant reproduction and play a crucial role in trophic interactions. Moths (Lepidoptera) are important nocturnal pollinators yet have been largely overlooked by researchers due to difficulties associated with field sampling at night. The endangered Bogong moth (*Agrotis infusa*) has recently been found to forage on local flowers during its summer aestivation period in the Australian Alps, but its role as a pollen vector is not yet well understood relative to other nocturnal moths. Understanding plant-pollinator interactions is of particular importance for species likely to be impacted by anthropogenic climate change and will help direct conservation efforts. This study examined how Bogong moths vary in their capacity as pollen vectors compared to other nocturnal moths in Kosciuszko National Park. This was achieved by comparing moth abundance, pollen grain abundance, and pollen richness between different taxa, as well as using network metrics to identify species of structural importance. Using UV light trapping, 132 individuals from 42 moth species were captured and swabbed for pollen, which was then identified and counted using light microscopy. Our findings indicated that Bogong moths were present in greater abundance and carried a more diverse range of pollen taxa than other moths. They had high among- and between-module connectivity in the network, indicating that they are playing an important pollen vector role. Bogong moths were also the only species to carry significant amounts of *Eucalyptus pauciflora* pollen, possibly suggesting the existence of a mutualistic relationship between two at-risk species.

## Introduction

Insects act as important vectors in the transfer of pollen between flowers of the same species. If successful, this results in fertilisation and seed development (Stevenson *et al.* 2022). As such, insects play an essential role in population recruitment of pollinator-dependent flowering plants. In turn, plants perform vital environmental functions, including carbon cycling, influencing water dynamics, and providing food and shelter for other organisms (Encinas-Viso *et al.* 2023; Fernando 2012). Thus, insect pollination is crucial for the maintenance of biodiversity and overall ecosystem function. Understanding the complex interactions between plants and their pollinators can help predict impacts of habitat disturbance and invasive species, and to direct conservation efforts (Devoto *et al.* 2011).

Insect pollination can be studied by building plant-pollinator networks – webs consisting of two trophic levels (plants and pollinators) with undirected links representing interactions between species (Devoto *et al.* 2011; Rivera-Hutinel *et al.* 2012). These networks can be constructed by observing pollinator visits (Gibson *et al.* 2011; Inouye & Pyke 1988) or by examining pollen on the bodies of insects, which is taken to represent interactions with certain plant species (Devoto *et al.* 2011; Encinas-Viso *et al.* 2023). The latter of these is termed a "pollen-transfer network" and is particularly useful when observational data is difficult to obtain (Banza *et al.* 2015). Pollen-transfer networks may provide a more accurate understanding of community structure because they are more likely to consider variation in pollinator quality and include infrequent pollinators that may be missed by visitation surveys (Barker & Arceo-Gomez 2021).

From plant-pollinator interaction data, a matrix dataset is produced, which can then be described and analysed using various quantitative indicators (Devoto *et al.* 2011; Rivera-Hutinel *et al.* 2012; Vanbergen *et al.* 2017). One such indicator is nestedness, which describes the extent to which specialists interact with a subset of the species that generalists interact with. Another common metric is modularity, where subgroups of species are highly internally linked but weakly connected to other modules (Devoto *et al.* 2011). A high level of nestedness is thought to indicate greater community resilience, and greater modularity may reduce community persistence by decreasing the functional redundancy of species (Dalsgaard *et al.* 2013; Thébault & Fontaine 2010). However, more work is needed to elucidate these patterns across different ecosystems (Brimacombe *et al.* 2023; Dormann *et al.* 2017). A modular structure results in the existence of "hubs" (species highly linked within their own module) and "connectors" (species that link together different modules). These species are particularly important for the structure and function of networks and should be prioritised by ecologists and conservationists (Olesen *et al.* 2007).

When constructing plant-pollinator networks, nocturnal data is often overlooked, partly due to the logistical difficulties of conducting plant visitation surveys at night (Banza *et al.* 2015; Coates *et al.* 2023; Devoto *et al.* 2011). However, nocturnal pollinators are crucial for plant herbivory and other trophic interactions, and diurnally pollinated plants may require additional nocturnal pollination to maximise their reproductive success (Banza *et al.* 2015; Devoto *et al.* 2011). Moths (Lepidoptera) play a particularly significant role in this process. They are some of the most common night-time flower visitors, as well as being indicators of environmental change and an important food source for predators (Banza *et al.* 2015; Devoto *et al.* 2011). Including nocturnal data in plant-pollinator networks provides a more comprehensive understanding of ecosystem function and improves our ability to predict responses to environmental change (Banza *et al.* 2015; Devoto *et al.* 2011).

Alpine and subalpine regions are particularly vulnerable to the effects of climate change. These regions are characterised by harsh, highly variable environmental conditions, which in turn influences pollinator behaviour (Lara-Romero *et al.* 2016; Goodwin *et al.* 2021). Plant-pollinator networks in these areas often include large numbers of generalist species, a high degree of nestedness, more insect species compared to plants, and a predominance of flies as pollinators (Encinas-Viso *et al.* 2023; Inouye & Pyke 1988).

In the Australian Alps, the major pollinators include flies (Diptera), bees (Hymenoptera), and butterflies/moths (Lepidoptera) (Inouye & Pyke 1988). A keystone species in this area is the Bogong moth (*Agrotis infusa*), which aestivates in high-elevation caves and rock crevices over the summer after undergoing its annual migration from Queensland, New South Wales, and Victoria (Warrant *et al.* 2016). The Bogong moth holds historical and cultural significance to Indigenous Australians, having acted as a major food source due to its high nutritional value and fat content. Aestivation sites also served as a place for intertribal meetings, corrobborees, marriages, initiation rights, and trade facilitation (Warrant *et al.* 2016).

Due to population declines, the Bogong moth has been listed as endangered by the IUCN (IUCN 2024). The population has experienced a steady decrease since the 1980s, but there has been a significant drop in numbers from 2017. The initial decline was attributed to improper farming practices around the Bogong moth's breeding ground, but the recent sharp decrease is the result of extreme drought conditions, which will only worsen with increasing climate change (Green *et al.* 2020). Warming temperatures may also impact Bogong moths' migration and aestivation patterns. The arrival and departure time of these moths from their breeding grounds has already changed significantly since the 1950s, and further changes could lead to a phenological mismatch between moths and flowering plants (Coates *et al.* 2023).

Whilst it was previously thought that Bogong moths do not forage on flowers while in the Alps (Common 1954), recent work has found evidence that they visit a wide range of local species during their aestivation period, including *Epacris, Grevillia,* and *Eucalyptus* (Coates *et al.* 2023). This suggests that Bogong moths may be important for plant reproductive success in the Australian Alps (Coates *et al.* 2023). However, their relative role as pollen vectors compared to other insect species is still poorly understood. To gauge how this species' ongoing population declines may impact alpine ecosystems, it is important to understand what pollination niche they fill and whether it differs from other nocturnal moths.

This study aimed to explore how Bogong moths compare to other moths in their capacity as nocturnal pollen vectors in the Kosciuszko alpine region of NSW. Specifically, we asked the following questions:

i) How does the abundance of different nocturnal moths vary?
ii) Do Bogong moths carry a different abundance of pollen to other nocturnal moths?
iii) Do Bogong moths visit a different number and range of plant species to other nocturnal moths?
iv) Which species are structurally important for the nocturnal plant-pollinator network in Kosciuszko National Park?

Since Bogong moths are present in large numbers after their mass migration to the Australian Alps, we hypothesised that they would be the most abundant moth present in the system. Additionally, due to recent findings that these moths appear to be floral generalists (Coates *et al.* 2023), we expected Bogongs to have higher pollen abundance and pollen species richness compared to other nocturnal moths, and to fill a structurally important role in the network.

# Methods

*Moth collection*

We collected moth specimens from a site near Charlotte Pass (1800m a.s.l., 36°25'54.1"S 148°19'43.9"E) for one hour between approximately 8.30-10.30pm on the 22$^{nd}$, and 26$^{th}$–29$^{th}$ of November 2023. This site was chosen for its high moth abundance and its proximity to a nearby Bogong moth aestivation site. To attract the moths, we used light traps which are commercially available under the name 'night collecting tents' (180 x 160cm). This was used in conjunction with a 12 W UV black light to allow for easy capture with specimen jars. We collected a variety of moth species; however, there was a particular focus on capturing Bogong moths. Once collected, we labelled the jars with the catcher's initials, moth name and the time/date of capture. We froze the samples overnight for pollen analysis the following day.

*Pollen collection*

To collect pollen from the moth specimens we viewed them under a dissecting microscope. If pollen grains could be seen, we swabbed them using fuchsin jelly on the end of a pinning needle (Coates *et al.* 2023). Only a very small amount of this gel was used because this reduces the surface area and makes counting the pollen easier. We swabbed areas which are most likely to interact with the reproductive structures of flowers, including the face, head, eyes, and the extended proboscis. This also helped us to avoid contaminating the gel with scales that would obscure the view when counting the pollen. Due to time constraints in the pollen-counting stage, we collected only up to ~100 grains from each moth.

After obtaining the pollen from the moth sample, we placed the gel onto a glass slide labelled with the sampler's initials and number of the sample. We heated the gel until it melted, at approximately 50°c, following the methods of Coates *et al.* (2023). We placed a cover slip over the melted gel and sealed it with black acrylic paint to prevent movement or desiccation of the sample.

During pollen collection, we identified the moths to the family-level using an ID guide by Johnson & Triplehorn (2004). To assign the moths to lower taxonomic groups or morphospecies we used iNaturalist and Canberra Nature Map (Canberra Nature Map 2023; iNaturalist 2023) as well as a moth reference collection from a previous study conducted by Coates *et al.* (2024). To determine the sex of the moths we examined their antennae; those with feathery antennae were classified as male and those with smooth antennae were classified as female.

*Pollen identification and counting*

We manually counted the pollen by viewing the specimen under a compound microscope and panning across the slide at 25x and 40x magnification. We identified the pollen using reference pollen photos (data unpublished) used by Coates *et al.* (2023) and the Natural Histories Handbook (Macphail & Hope 2018). We entered the number of pollen grains from each plant species into an Excel spreadsheet and those with a pollen count greater than 100 were noted as 100+.

*Data analysis*

We carried out all statistical analyses in R (R Core Team 2023). Following the methodology of Devoto *et al.* (2011), we ranked the moths by the number of pollen-carrying individuals of each species, the total number of pollen grains they carried, and the total number of plant taxa in their pollen load. These criteria assess the importance of each moth as a pollen vector in the plant-pollinator network by quantifying their pollen transport ability and the range of plants they visit (Devoto *et al.* 2011). We carried out an ANOVA of pollen load (log-transformed) to test for significant variation between species. We also used post-hoc analysis (Tukey's test) to test for differences in pollen load between species pairs.

To calculate network-level metrics we used the R bipartite package (Dormann *et al.* 2008). These metrics included modularity and weighted nestedness (values close to 1 indicating highly modular and nested networks) (Dormann 2023; Dormann *et al.* 2009). Weighted nestedness is a version of nestedness that takes into account the frequency of interactions (Galeano *et al.* 2009). We calculated the significance of these metrics by comparing them with null models (500 randomised networks with equivalent species richness and number of interactions) (Encinas-Viso *et al.* 2023).

We performed further network analysis to identify modules (groups that are linked together more tightly than they are to species in other modules) and to calculate c- and z- values for each moth species, which represent among- and within-module connectivity, respectively (Olesen *et al.* 2007). Species with a c-value above 0.625 or a z-value above 2.5 were assigned a structurally important role within the network. Those above the c-threshold were classified as "connectors", those above the z-threshold as "module hubs", and those that met both criteria as "network hubs". Species below both the c- and z-cutoffs were assigned a peripheral role (Olesen *et al.* 2007).

## Results

A total of 132 individuals from 45 moth species were collected in our study. Of these, 69 individuals from 21 species were carrying pollen and were included in the nocturnal plant-pollinator network, along with 19 plant species (Figure 1).

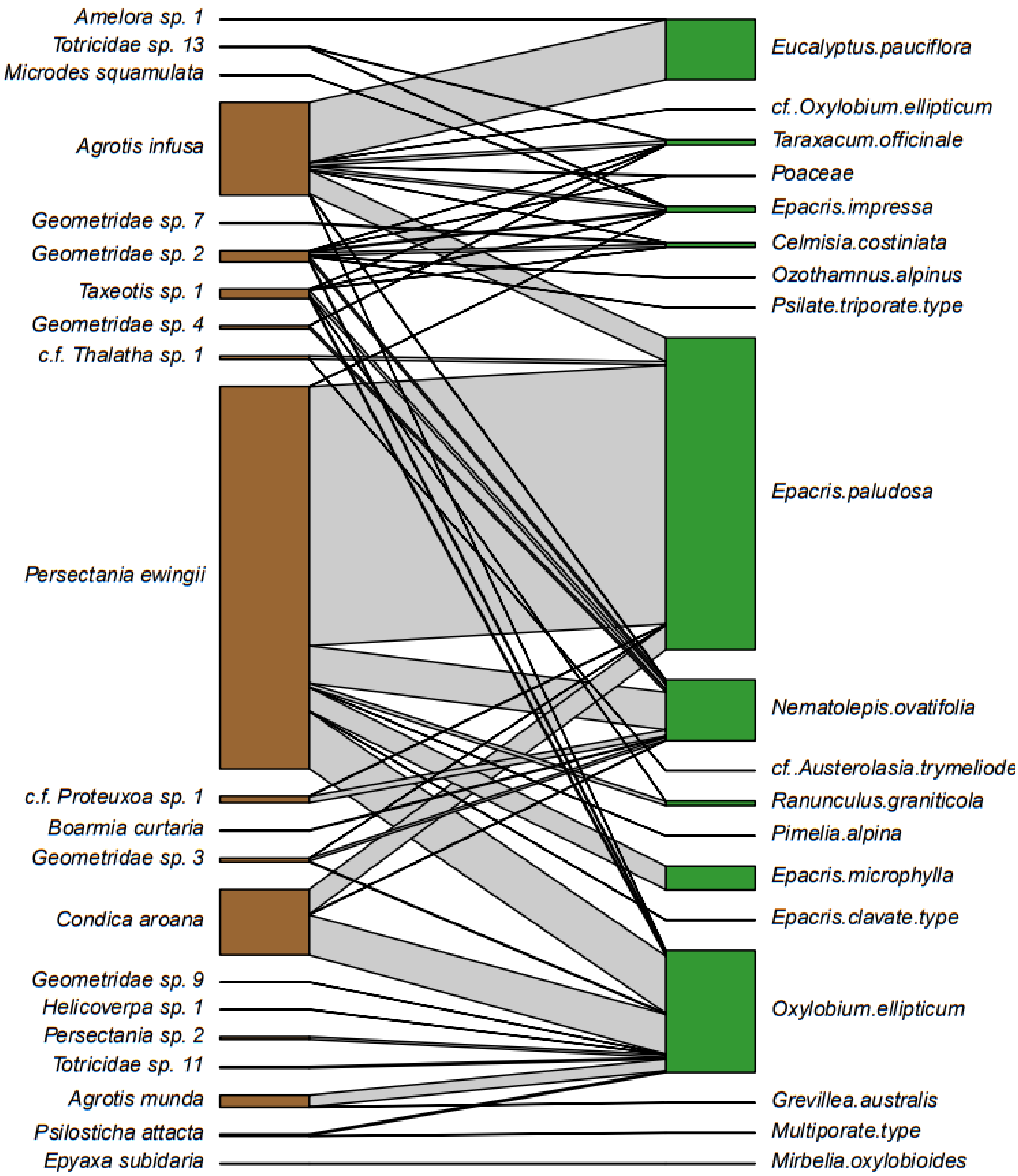


***Figure 1:*** *Plant-pollinator network of nocturnal moths at Kosciuszko National Park. Moth species are labelled along the left and plant species along the right. The width of the boxes on the left represents the total pollen carried by moths of each species. The width of the boxes on the right represents the number of pollen grains present from each plant species. The linking lines represent the proportion of pollen of each plant taxa carried by each moth species.*

*Abundance of moth species*

*A. infusa* was the most abundant moth species collected (18 individuals with pollen and 3 without), followed by *Persectania ewingii* (14 with pollen and 1 without), *Taxeotis* sp. 1 (6 with pollen and 7 without), and Geometridae sp. 2 (7 with pollen and 1 without). All other moth species had an abundance of less than 10 (Figure 2).

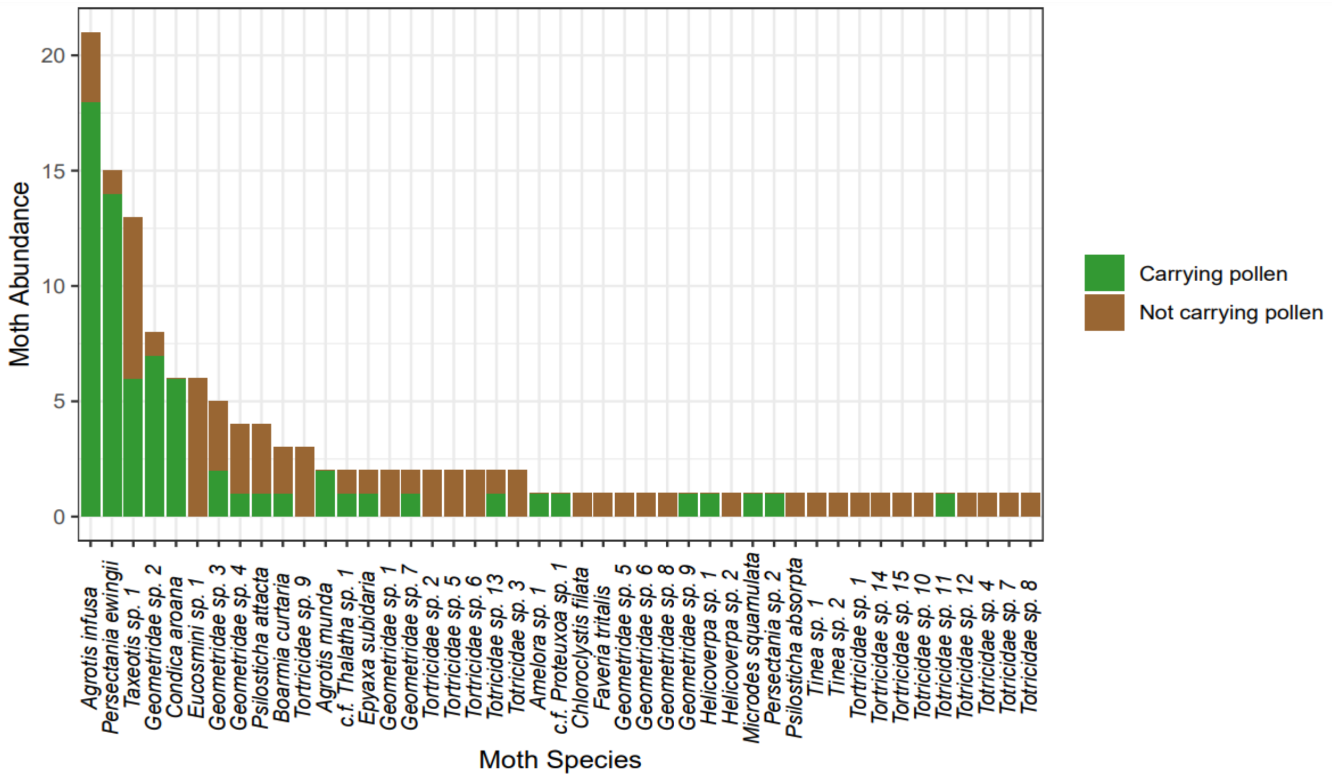


***Figure 2:*** *Abundance of pollen-carrying and non-pollen-carrying individuals of each moth species.*

*Abundance of pollen on moths*

Pollen load varied significantly between moth species that were carrying pollen ($F = 4.03$, $p < .001$). *P. ewingii* had the highest average pollen load per individual (108.6 grains), followed by *Condica aroana* (46.5 grains), and c.f. *Proteuxoa* sp. 1 (30.0 grains). *A. infusa* had the fifth-highest pollen load, with 21.9 grains per individual (Figure 3). *P. ewingii* carried significantly more pollen than 6 other species. No other species pairs differed significantly in pollen load.

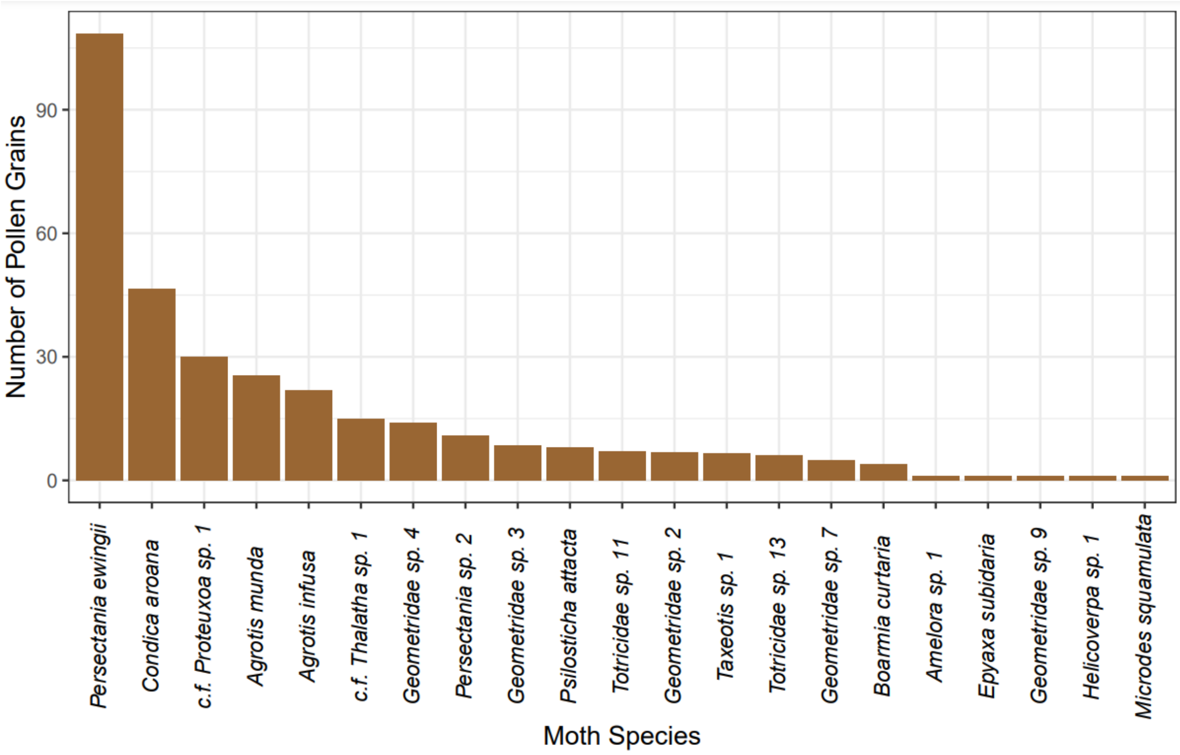


***Figure 3:*** *Average pollen load of pollen-carrying individuals of each moth species.*

*Richness and relative abundance of pollen*

*A. infusa* carried pollen from the greatest number of plant taxa (9), followed by Geometridae sp. 2 (8), *P. ewingii* (8) and *Taxeotis* sp. 1 (6). Nine moth species carried pollen from just one plant taxa (Figure 4).

Of the 9 pollen taxa carried by *A. infusa*, the greatest proportion of pollen was from *Eucalyptus pauciflora* (64.6%), followed by *Epacris paludosa* (25.3%), *Taraxacum officinale* (3.8%), and *Epacris impressa* (3.3%) (Figure 1). *A. infusa* also carried pollen from *Oxylobium ellipticum, Nematolepis ovatifolia, Celmisia constiniata, and cf. O. ellipticum,* as well as Poaceae pollen. *A. infusa* was the only moth species in our sample that carried pollen from *E. pauciflora,* apart from a singular grain found on *Amelora* sp. 1.

Of the 8 pollen taxa carried by *P. ewingii,* the majority was from *E. paludosa* (67.8%), followed by *O. ellipticum* (15.0%) and *N. ovatifolia* (9.7%). Geometridae sp. 2 carried the majority of its pollen from *N. ovatifolia* (29.8%), *C. costiniata* (27.7%) and *O. ellipticum* (21.3%).  *Taxeotis* sp. 1 carried the greatest proportion of pollen from *N. ovatifolia* (52.5%) and *O. ellipticum* (30%) (Figure 1).

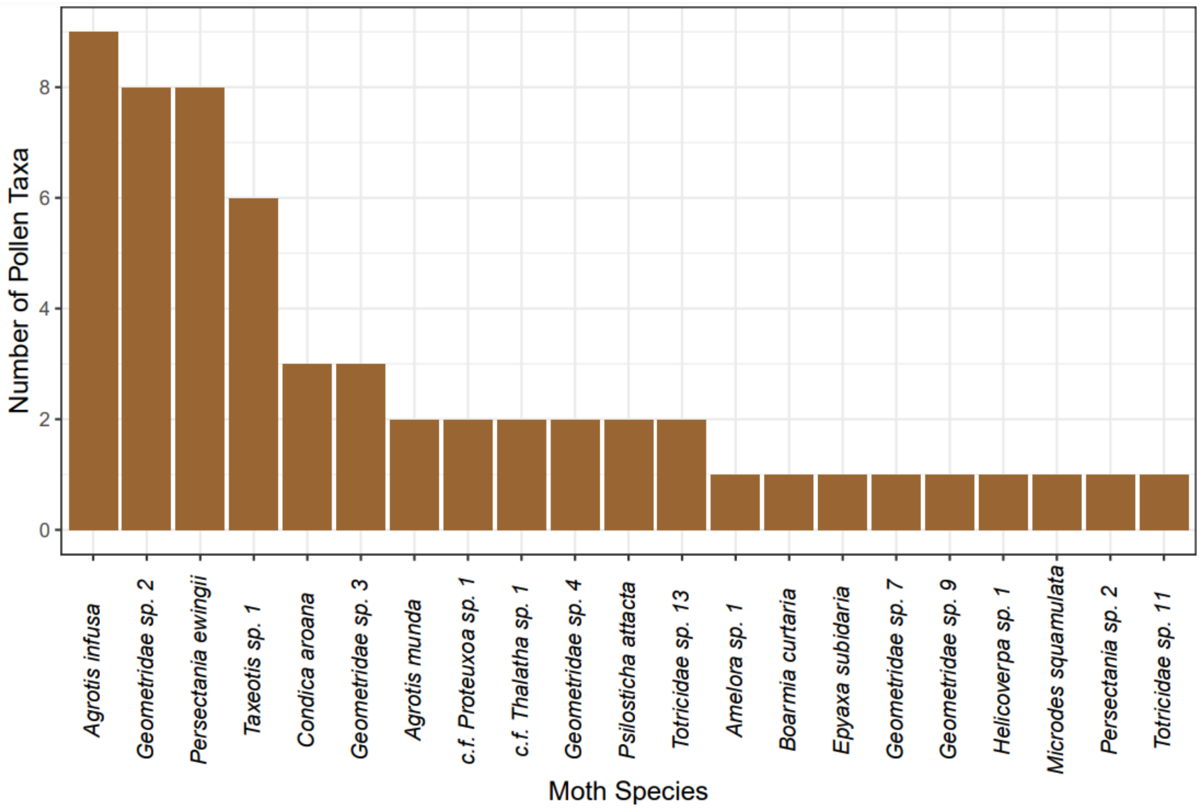


***Figure 4:*** *Total number of pollen taxa found on each moth species.*

*Family-level data*

Out of all the moths collected in our study, 17 species were from the family Geometridae, 16 from Totricidae, 9 from Noctuidae, two from Tineidae, and one from Pyralidae (Table 1). Moths in the family Noctuidae had the highest abundance of pollen-carrying individuals, greatest average pollen load, and highest average pollen taxa richness. Geometridae had the second highest moth abundance and pollen taxa richness, but the third highest pollen load, following Totricidae (although this family had only two pollen-carrying moths) (Table 2). No moths in Pyralidae or Tineidae carried any pollen.

***Table 1:*** *Pollen-carrying and non-pollen-carrying species grouped by family*

| Family | Species carrying pollen | Species not carrying pollen |
|---|---|---|
| Noctuidae | *Agrotis infusa, Agrotis munda, Condica aroana, Persectania ewingii, Persectania* sp. 2, *Helicoverpa* sp. 1, c.f. *Proteuxoa* sp. 1, c.f. *Thalatha* sp. 1 | *Helicoverpa* sp. 2 |
| Geometridae | *Amelora* sp. 1, *Boarmia curtaria, Epyxa subidaria, Microdes squamulata, Psilosticha attacta,* Geometridae sp. 2, 3, 4, 7, 9, *Taxeotis* sp. 1 | *Chloroclystis filata, Psilosticha absorpta, Geometridae* sp. 1, 5, 6, 8 |
| Totricidae | Totricidae sp. 11, 13 | *Eucosmini* sp. 1, Totricidae sp. 1-10, 12, 14-15 |
| Pyralidae | | *Faveria tritalis* |
| Tineidae | | *Tinea* sp. 1-2 |

***Table 2:*** *Abundance, pollen load, and pollen taxa richness of each moth family*

| Family | Total abundance | Abundance with pollen | Proportion with pollen | Average pollen load | Average pollen taxa richness |
|---|---|---|---|---|---|
| Noctuidae | 50 | 44 | 88.0 | 32.44 | 3.5 |
| Geometridae | 51 | 23 | 45.1 | 5.17 | 2.45 |
| Totricidae | 28 | 2 | 7.1 | 6.5 | 1.5 |
| Pyralidae | 1 | 0 | 0 | 0 | 0 |
| Tineidae | 2 | 0 | 0 | 0 | 0 |

## *Network metrics*

Bipartite analysis of the plant-pollinator network showed a significant weighted nestedness value of 0.596 ($p < .001$) and a significant modularity value of 0.323 ($p < .001$). Further modularity analysis revealed the existence of 5 main modules within the network. Three species had c-values above 0.625, classifying them as “connectors” (T*axeotis* sp. 1, Geometridae sp. 3, and *C. aroana*) (Figure 5). No species had z-values above 2.5, meaning none were classified as “module hubs” or “network hubs”. Geometridae sp. 2 had the highest z-value (2.14), *Agrotis munda* and *Psilosticha attacta* the second highest (1.46), and *A. infusa* the third highest (1.45) (Figure 6).

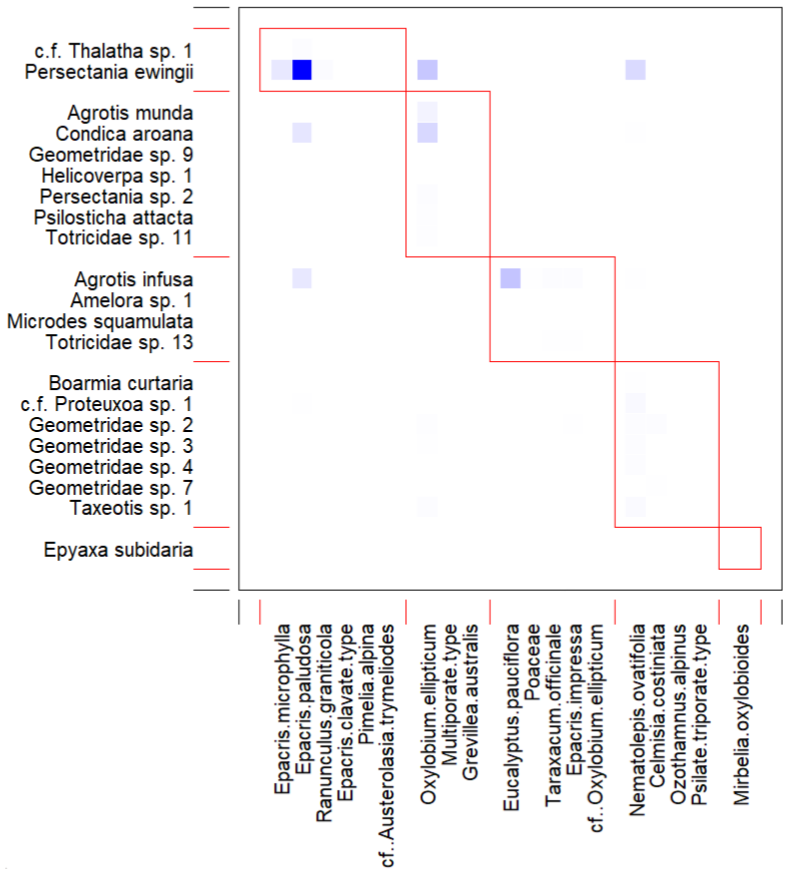


***Figure 5:*** *Module breakdown of the plant-pollinator network. Modules are shown in red, with moth species along the left and plant species along the bottom. Darker blue squares represent higher frequency of interactions.*

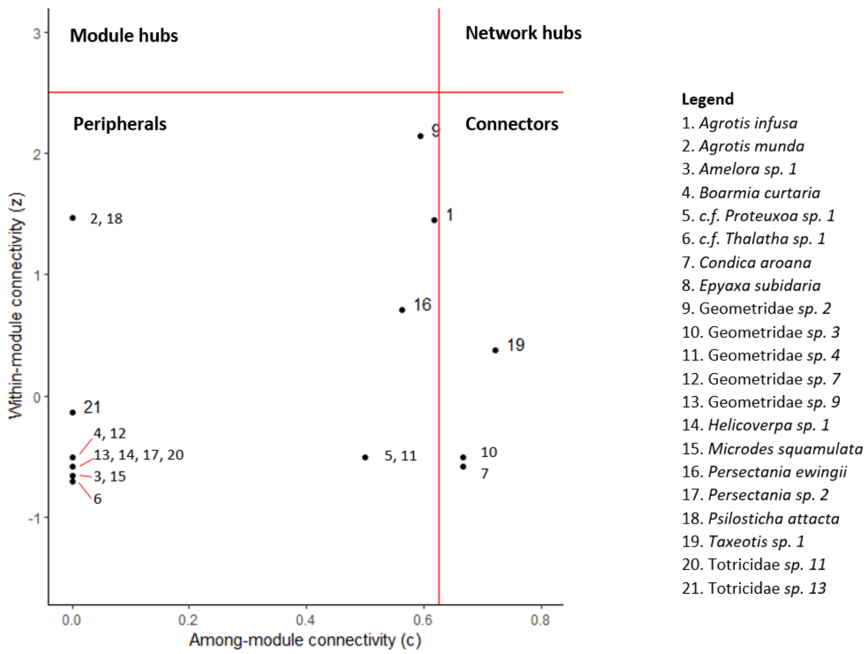


***Figure 6:*** *Among- and within-module connectivity scores of moth species in the network. Species with c > 0.625 are classified as "connectors", species with z > 2.5 as "module hubs", and species with both as "network hubs". Species with low c and z scores are "peripherals".*

# Discussion

The aim of this study was to understand the role of Bogong moths as pollen vectors compared to other nocturnal moths in Kosciuszko National Park. We assessed the abundance of pollen carrying individuals, total pollen loads, pollen richness, and relative pollen abundance of various moth species. High pollen carrying frequency may indicate the importance of a species within a plant-pollinator community. Pollen load can demonstrate pollinator importance for specific plant species, since moths that carry more conspecific pollen are likely to be more efficacious pollinators. Pollen richness is important for characterising a species' role in the plant-pollinator network. We also calculated key network metrics and assigned structural roles to moth species within the network. Since previous research has suggested the Bogong moth may be an important pollen vector in Kosciuszko National Park (Coates *et al.* 2023), we expected it to be higher in abundance, pollen count, and pollen richness, and to occupy a structurally important role in the network.

*Abundance of moth species*

Bogong moths had the greatest abundance out of all the study species, both in terms of pollen-carrying individuals and total abundance. This may indicate a special significance of this species as a pollen vector during their aestivation period in the Australian Alps, as frequent flower visitors have the greatest impact on plant reproductive success (Maldonado *et al.* 2013). Bogong moths migrate to the Kosciuszko area by the billions and have been found lining the walls of aestivation caves with up to 17,000 individuals per $m^2$ (Common 1954), explaining their observed abundance in our study, which was conducted near these aestivation sites.

The Noctuidae and Geometridae families had the highest abundance of pollen-carrying individuals in our study. This supports the findings of Macgregor *et al.* (2015) and Hahn and Brühl (2016), who found that these families played a significant role in nocturnal pollination globally. In addition, Goodwin *et al.* (2021) reported Geometridae as the second-most abundant moth family engaging in diurnal pollination in Kosciuszko National Park.

It is important to note that during light trapping sessions, Bogong moths were of particular interest and one of the largest moth species present. Since they are easy to see and capture, and more focus was placed on trapping this species, our abundance data may be biased. Additionally, moth numbers can vary due to weather conditions on a particular night and throughout the entire summer period. Our study considers plant-pollinator interactions during peak Bogong activity and near Bogong aestivation sites. Future studies should explore nocturnal pollination across a longer time span, as this may reveal new insights about important pollinators in the area.

Another potential limitation of our moth capture methodology is the use of the light trap itself. These traps measure activity rather than abundance, and attraction to light varies among species, meaning our sample may not be representative of the community (Devoto *et al.* 2011). However, studying pollinators through direct observation is more difficult at night and light trapping is often the only viable methodology for nocturnal pollination studies (Banza *et al.* 2015; Devoto *et al.* 2011).

*Abundance of pollen on moths*

Contrary to our hypothesis, Bogong moths did not carry the most pollen out of all the species in the study. This result could be explained by the limited number of *E. pauciflora* in flower during the study period, as this was the primary pollen species found on Bogong moths. Coates *et al.* (2023) found an increase in pollen load from alpine/subalpine plant species (such as *E. pauciflora*) during the later weeks of the Bogong moth's aestivation period, reflecting more local activity by the moths during this later stage (December-February) than in November, when our study was conducted.

Other nocturnal moths varied significantly in the amount of pollen they carried, with *P. ewingii* carrying significantly more pollen than 6 out of 20 other species. The high abundance and large pollen load of this species suggests that it may be of particular importance as a nocturnal pollen vector in the Australian Alps. However, as mentioned above, it is not known whether this species is as highly represented throughout the entire summer season.

Differences in the pollen load of moth species could be explained by a variety of factors, including body size, proboscis length, time spent on the flower, and time between foraging and capture. Murúa (2020) found a significant effect of both body size and foraging time on pollen load in butterflies. Additionally, Inouye (1980) found that bumblebees with shorter proboscis length spent longer on flowers, which can in turn affect pollen load. Lastly, Del Socorro and Gregg (2001) found that pollen retention on Noctuid moths declines significantly just after two days. Therefore, moths who foraged more recently before being caught may have greater pollen loads. In future, a combination of field observations and pollen data may help elucidate the underlying causes of pollen load differentiation between species.

It is important to note that due to the time constraints of the project, we only collected up to ~100 pollen grains from each moth sample. These samples may not be representative of the total pollen on the moth's body, and our study could be improved with more standardised pollen collection procedures.

Additionally, pollen can vary in its adhesive properties and the amount produced between plant species (Smith *et al.* 2022). Therefore, pollen load may not be truly reflective of visitation. For instance, the large amount of *E. paludosa* pollen on *P. ewingii* could be the result of particular pollen characteristics rather than a greater number of interactions between these species. Studies should investigate the pollen properties of plants in Kosciuszko National Park, as well as adherence of pollen to moths in this area, including Bogong moths. While there have been no previous studies on the pollen grain retention of Bogong moths, similar studies have been performed on other species, for example on the nocturnal moth *Spodoptera exigua* (Jia *et al.* 2023).

*Richness and relative abundance of pollen*

Bogong moths carried pollen from the greatest number of plant species compared to other moths in the study, aligning with our hypothesis that they are a generalist species. This result coincides with Coates *et al.* (2023), whose experiment was conducted at the same site as ours. This study found a large diversity of pollen on Bogong moths and concluded that they are likely a generalist species. Plant families found on Bogongs by Coates *et al.* (2023) included Myrtaceae, Ericaceae, Rutaceae, Fabaceae, and Asteraceae, all of which were also identified in our study. Other Noctuidae species have also been found to be generalist flower visitors (Ribas-Marquès *et al.* 2022), which aligns with our findings of this family having the greatest pollen taxa richness on average.

Generalist pollinator species are those that visit a large diversity of flowers, as opposed to specialists, which interact with fewer species (Maldonado *et al.* 2013). Although generalists may have lower per-visit effectiveness due to deposition of heterospecific pollen, they are usually more abundant and less variable in abundance than specialists, which can result in a greater overall impact on plant reproductive success (Maldonado *et al.* 2013). This suggests that Bogong moths, along with other species with relatively high pollen richness (Geometridae sp. 2, *P. ewingii*, and *Taxeotis* sp. 1), may be of high importance in the Kosciuszko area. The existence of several generalist species within the network is not surprising, given that alpine plant-pollinator communities tend to be dominated by generalists (Ribas-Marquès *et al.* 2022; Encinas-Viso *et al.* 2023).

Differences in degree of generalisation between species may be explained by functional traits such as proboscis length. Inouye and Pyke (1988) found associations between proboscis length of flies and corolla length of the flowers they visited, and Klumpers *et al.* (2019) found a similar relationship between flower nectar tube length and proboscis length of various pollinators. Additionally, McCanna (2004) found greater pollen species richness in butterflies with longer proboscises. Thus, functional differences between alpine moths may explain variation in their degree of generalisation and overall pollination niche. Future studies should investigate these factors, as well as possible life-history differences between the moth taxa.

It is important to consider that during their migration, Bogong moths travel long distances from low to high elevations of the Kosciuszko alpine region. As a result, they can visit a wider range of plant taxa than moths with a more restricted geographic range. This factor may have contributed to the higher pollen taxa richness found on this moth species in our study.

To improve the population validity of our pollen abundance and richness data, equal numbers of each moth species should be captured and swabbed. Furthermore, it would be beneficial to employ genetic techniques to refine taxonomic resolution of pollen identification. Some plant groups, such as *Eucalyptus* species, have very similar pollen grain morphology. DNA metabarcoding would allow for species-level identification and identity confirmation (Coates *et al.* 2023).

*Bogong moths and Eucalyptus pauciflora*

Our study found a high frequency of *E. pauciflora* pollen grains on Bogong moths (255 grains). One single *E. pauciflora* grain was also found on an *Amelora* sp. 1 individual, but this was likely the result of cross-contamination during moth capture or pollen swabbing. Two prior studies have also found an association between Bogong moths and *E. pauciflora* through observational studies (Common 1954) and pollen data (Coates *et al.* 2023). This supports our findings, suggesting that *E. pauciflora* flowers may be an important food source for Bogongs, and Bogongs may be serving as nocturnal pollen vectors for this plant species.

Previous studies in Kosciuszko National Park have observed various species foraging on *E. pauciflora* during the daytime, including honeybees, blow flies, and soldier beetles (Encinas-Viso *et al.* 2023; Goodwin *et al.* 2021). However, little is known about nocturnal pollination of this species, which may be an important component of its reproductive success. Additionally, generalist species often benefit most from a small number of highly efficacious pollinators (Rossi *et al.* 2014; Watts *et al.* 2012). Future studies should investigate the relationship between *E. pauciflora* and Bogong moths to determine the efficiency of this moth as a pollinator, as well as diurnal and nocturnal pollination of this plant species more generally. Whilst Bogongs are persistent throughout the entire summer period, other species may be present during shorter time frames and could have been missed by our sampling. Pollinator exclusion experiments should be conducted to fully understand the importance of various species to *E. pauciflora*.

If Bogong moths are contributing to the reproductive success of *E. pauciflora,* this mutualistic relationship could have future implications due to growing impacts of anthropogenic climate change on alpine ecosystems. Bogong moth populations have already significantly declined as a result of severe drought during their breeding season, and further decreases may impact *E. pauciflora* reproductive success (Green *et al.* 2020). Additionally, changing weather conditions may shift Bogong moths' migration patterns, which could lead to divergence from flowering times in the Australian Alps and have adverse effects on *E. pauciflora* gene flow, that is, the distribution of its pollen (Coates *et al.* 2023). Conversely, *E. pauciflora* has also been impacted by climate change, becoming more susceptible to attack by Phoracantha beetles, which results in dieback of affected stands (Chua *et al.* 2021; Ward-Jones 2020). This may impact Bogong moths by reducing food availability. Given the at-risk state of both Bogong moths and *E. pauciflora*, conservation of these two species may be mutually beneficial to the other.

*Network characteristics*

Our study found significant modularity and weighted nestedness in the plant-pollinator network of nocturnal moths. Modularity is highly correlated with specialisation, since modules exist because some species do not interact with some others, i.e. because they are specialised (Dormann 2023). A modularity value of 0.323 is relatively low, which can be explained by the high level of generalisation in several of the species in the network.

In contrast, the nestedness value of the network was comparatively high, greater than that reported by Encinas-Viso *et al.* (2023) for a diurnal plant-pollinator network in Charlotte Pass, and by Devoto *et al.* (2011) for nocturnal pollinators in Scotland. High nestedness and low modularity are associated with increased functional redundancy of species and thus greater community persistence and resilience (Dalsgaard *et al.* 2013; Thébault & Fontaine 2010). Therefore, the nestedness and modularity values observed in our study suggest a high level of robustness in the moth pollen-transfer community at Kosciuszko National Park.

Modularity analysis revealed the existence of 5 distinct modules within the network, including one containing Bogong moths and *E. pauciflora*, emphasising the strength of the association between these two species. Three species of particular structural importance in the network were *Taxeotis* sp. 1, Geometridae sp. 3, and *C. aroana*. These species are "connectors", meaning they play a significant role in the transfer of pollen within their modules. All three of these species were present in relatively high abundance, and *C. aroana* had the second-highest pollen load. These factors likely contributed to their importance as pollen vectors within their subgroups. *C. aroana* had particularly strong interactions with *O. elipticum* and *E. paludosa*.

Although Bogong moths did not surpass the threshold to be considered a structurally important species, they did have a c-value of 0.617, very close to the critical point, as well as the fourth-highest z-value in the study. The combination of high among- and within-module connectivity for this species emphasises its importance in the network.

## Conclusions

This study revealed that Bogong moths vary in their role as pollen vectors compared to other nocturnal moths in Kosciuszko National Park. During their summer aestivation period, Bogong moths are present in greater abundance and visit a more diverse set of plant taxa than other nocturnal moths. They have high among- and between-module connectivity in the network, suggesting they are playing a particularly important pollen vector role. Furthermore, Bogong moths may be the only nocturnal moth in the area to contribute to *E. pauciflora* pollen transfer. This highlights the importance of conservation of the Bogong moth, not only for *E. pauciflora* but for the variety of plants the Bogong visits. To conserve the moths' population, it is important to protect their breeding grounds and the plant species they feed from.

## Acknowledgements

We acknowledge that our research was conducted on Ngarigo and Walgalu Country. We recognise and pay respect to the First Nations peoples who have nurtured and managed their sovereign lands for generations and continue to do so today.

We would like to thank our group members who helped conduct this experiment: Garrett Ball, Lisa Cary, Esther Duve, Natasha Erb, Ariel Neidorf, Ellie Shuetrim, Jessica Treloar, and our supervisor Joshua Coates. We would also like to thank people who contributed to moth collection efforts, including Adrienne Nicotra, Ashleigh Eason, Harry Whittaker, Ishbel Burns, John Burley, Julia D'Angelo, Jesse Hill, Kate Farkas, Kelvin Meng, Leah Thewlis, Megan Head, and Sean Martin.

Thank you to Adrienne Nicotra and Megan Head for organising this field course and supporting us in our learning throughout. Thank you to Tony Vorreiter and the Kahane Ski Lodge for their hospitality and the use of their space for our lab work and analysis.

## References

Banza P, Belo ADF, Evans DM, Stewart A, Brady S (2015) Structure and robustness of nocturnal Lepidopteran pollen-transfer networks in a Biodiversity Hotspot. *Insect conservation and diversity* **8,** 538-546.

Barker DA, Arceo-Gomez G (2021) Pollen transport networks reveal highly diverse and temporally stable plant–pollinator interactions in an Appalachian floral community, A*oB plants* **13**.

Brimacombe C, Bodner K, Michalska-Smith M, Poisot T, Fortin MJ (2023) Shortcomings of reusing species interaction networks created by different sets of researchers. *PLoS Biology* **21**.

Canberra Nature Map (2023) https://canberra.naturemapr.org/. Accessed on [22nd Novemeber 2023].

Chua E, Nicoll A, Bloomfield T, Boehm M, Farkas K, Kearns M, Kiu K, Martin E, Thompson R (2021) The effects of Phoracantha-induced dieback on photosynthesis, respiration and estimated leaf nitrogen in *Eucalyptus pauciflora*. *Field Studies in Ecology* **4**.

Coates J, Keaney B, Scheele B, Cunningham S (2023) Endangered Bogong moths (*Agrotis infusa*) forage from local flowers after annual mass migration to alpine sites. *Global ecology and conservation* **44.**

Coates J, Maldwyn J, Scheele B, Encinas-Viso F, Florez Fernandez J, Lumbers J, Cunningham S (2024) Influence of Climate, Weather and Floral Associations on Pollinator Community Composition Across an Elevational Gradient. *Oikos.*

Common IFB (1954) A study of the ecology of the adult bogong moth, *Agrotis Infusa* (Boisd) (Lepidoptera: Noctuidae), with special reference to its behaviour during migration and aestivation. *Australian journal of zoology* **2,** 223-263.

Dalsgaard B, Trøjelsgaard K, Martín González AM, Nogués-Bravo D, Ollerton J, Petanidou T, Sandel B, Schleuning M, Wang Z, Rahbek C, Sutherland WJ, Svenning JC, Olesen JM (2013) Historical climate-change influences modularity and nestedness of pollination networks. *Ecography* **36,** 1331-1340.

Del Socorro AP, Gregg PC (2001) Sunflower (*Helianthus annuus* L.) pollen as a marker for studies of local movement in *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae). *Austral Entomology* **40,** 257–263.

Devoto M, Bailey S, Memmott J (2011) The ‘night shift’: nocturnal pollen-transport networks in a boreal pine forest. *Ecological Entomology* **36,** 25-35.

Dormann CF, Fruend J, Bluethgen N, Gruber B (2009) Indices, graphs and null models: analyzing bipartite ecological networks. *The Open Ecology Journal* **2,** 7-24.

Dormann CF, Gruber B, Fruend J (2008) Introducing the bipartite Package: Analysing Ecological Networks. *R news* **8/2,** 8-11.

Dormann CF, Fründ J, Schaefer HM (2017) Identifying Causes of Patterns in Ecological Networks: Opportunities and Limitations. *Annual Review of Ecology, Evolution, and Systematics* **48**, 559-584.

Dormann CF (2023) Using bipartite to describe and plot two-mode networks in R. Germany: University of Freiburg.

Encinas-Viso F, Bovill J, Albrecht DE, Florez-Fernandez J, Lessard B, Lumbers J, Rodriguez J, Schmidt-Lebuhn A, Zwick A, Milla L (2023) Pollen DNA metabarcoding reveals cryptic diversity and high spatial turnover in alpine plant–pollinator networks. *Molecular Ecology* **32,** 6377-6393.

Goodwin EK, Rader R, Encinas-Viso F, Saunders ME (2021) Weather Conditions Affect the Visitation Frequency, Richness and Detectability of Insect Flower Visitors in the Australian Alpine Zone. *Environmental Entomology* **50,** 348-358.

Fernando W (2012) Plants: An International Scientific Open Access Journal to Publish All Facets of Plants, Their Functions and Interactions with the Environment and Other Living Organisms. *Plants (Basel, Switzerland)* **1,** 1-5.

Galeano J, Pastor JM & Iriondo JM (2009) Weighted-Interaction Nestedness Estimator (WINE): A new estimator to calculate over frequency matrices. *Environmental Modelling & Software* **24**, 1342-1346.

Gibson RH, Knott B, Eberlein T, Memmott J (2011) Sampling method influences the structure of plant-pollinator networks. *Oikos* **120**, 822-831.

Green K, Caley P, Baker M, Dreyer D, Wallace J, Warrant E (2020) Australian Bogong moths *Agrotis infusa* (Lepidoptera: Noctuidae), 1951–2020: decline and crash. *Austral Entomology* **60**.

Hahn M, Brühl CA (2016) Secret pollinators: an overview of moth pollination with a focus on Europe and North America. *Arthropod-plant interactions* **10,** 21-28.

iNaturalist (2023) BIOL2203/3303 ANU Field Course 2023. Version 3.3.4. https://www.inaturalist.org. Accessed on [22nd November 2023].

Inouye DW (1980) The Effect of Proboscis and Corolla Tube Lengths on Patterns and Rates of Flower Visitation by Bumblebees. *Oecologia* **45,** 197-201.

IUCN (2024) The IUCN Red List of Threatened Species. Version 2024-1. https://www.iucnredlist.org. Accessed on [9th August 2024].

Inouye DW, Pyke GH (1988) Pollination biology in the Snowy Mountains of Australia: Comparisons with montane Colorado, USA. *Australian Journal of Ecology* **13,** 191-205.

Jia H, Wang T, Li X, Zhao S, Guo J, Liu D, Liu Y, Wu K (2023) Pollen Molecular Identification from a Long-Distance Migratory Insect, Spodoptera exigua, as Evidenced for Its Regional Pollination in Eastern Asia. *International journal of molecular sciences* **24**, 8, 7588.

Johnson N, Triplehorn C (2004) Borror and DeLong's Introduction to the Study of Insects, Brooks/Cole ISE, 7th Edition.

Klumpers SGT, Stang M, Klinkhamer PGL, Irwin R (2019) Foraging efficiency and size matching in a plant–pollinator community: the importance of sugar content and tongue length. *Ecology letters* **22,** 469-479.

Lara-Romero C, García C, Morente-López J, Iriondo JM (2016) Direct and indirect effects of shrub encroachment on alpine grasslands mediated by plant–flower visitor interactions. *Functional Ecology* **30,** 1521-1530.

Macgregor CJ, Pocock MJO, Fox R, Evans DM (2015) Pollination by nocturnal Lepidoptera, and the effects of light pollution: a review. *Ecological entomology* **40,** 187-198.

Macphail M, Hope G (2018) Natural Histories: An illustrated guide to fossil pollen and spores preserved in swamps and mires of the Southern Highlands, NSW. Canberra, ACT: PalaeoWorks, Department of Archaeology & Natural History, Research School of Pacific & Asian Studies, The Australian National University.

McCanna C (2004) Butterfly proboscis length and pollen load. *Tropical Ecology and Conservation* **121**.

Maldonado MB, Lomáscolo SB, Vázquez DP (2013) The importance of pollinator generalization and abundance for the reproductive success of a generalist plant. *PloS one* **8,** e75482-e75482.

Murúa M (2020) Different Pollinators' Functional Traits Can Explain Pollen Load in Two Solitary Oil-Collecting Bees. *Insects (Basel, Switzerland)* **11,** 685.

Olesen JM, Bascompte J, Dupont YL, Jordano P (2007) The modularity of pollination networks. *Proceedings of the National Academy of Sciences - PNAS* **104,** 19891-19896.

R Core Team (2023) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/

Ribas-Marquès E, Díaz-Calafat J, Boi M (2022) The role of adult noctuid moths (Lepidoptera: Noctuidae) and their food plants in a nocturnal pollen-transport network on a Mediterranean island. *Journal of insect conservation* **26,** 243-255.

Rivera-Hutinel A, Bustamante RO, Marín VH, Medel R (2012) Effects of sampling completeness on the structure of plant-pollinator networks. *Ecology (Durham)* **93**, 1593-1603.

Rossi M, Fisogni A, Nepi M, Quaranta M & Galloni M (2014) Bouncy versus idles: On the different role of pollinators in the generalist Gentiana lutea L. *Flora. Morphologie, Geobotanik, Oekophysiologie* **209**, 164-171.

Smith G, Raguso R, Kim C (2022) Pollen accumulation on hawkmoths varies substantially among moth-pollinated flowers. *Journal of Pollination Ecology* **32**, 201–211.

Stevenson P, Koch H, Nicolson S, Brown M (2022) Natural processes influencing pollinator health. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences* **377**.

Thébault E, Fontaine C (2010) Stability of Ecological Communities and the Architecture of Mutualistic and Trophic Networks. *Science (American Association for the Advancement of Science)* **329**, 853-856.

Vanbergen AJ, Woodcock BA, Heard MS, Chapman DS (2017) Network size, structure and mutualism dependence affect the propensity for plant–pollinator extinction cascades. *Functional Ecology* **31,** 1285-1293.

Ward-Jones J (2020) *Dieback of subalpine snow gums, Eucalyptus pauciflora subsp. niphophila in Perisher Valley, Kosciuszko National Park: a description of symptoms and landscape drivers.* Master's Thesis, The Australian National University, Canberra, Australia.

Warrant E, Frost B, Green K, Mouritsen H, Dreyer D, Adden A, Brauburger K, Heinze S (2016) The Australian Bogong Moth *Agrotis infusa*: A Long-Distance Nocturnal Navigator. *Frontiers in behavioral neuroscience* **10,** 77.

Watts S, Ovalle DH, Herrera MM & Ollerton J (2012) Pollinator effectiveness of native and non-native flower visitors to an apparently generalist Andean shrub, *Duranta mandonii* (Verbenaceae). *Plant species biology* **27**, 147-158.