School of Doctoral Studies in Biological Sciences

University of South Bohemia in České Budějovice

Faculty of Science

The role of ecological gradients and host-parasitoid interaction in tropical insect communities

Ph.D. Thesis

Sam Finnie MSc.

Supervisor: Mgr. Katerina Sam, Ph.D.

Department of Zoology, Faculty of Science, University of South
Bohemia, České Budějovice, CZ
Biology Centre of the Czech Academy of Sciences, Institute of Entomology,
České Budějovice, CZ

České Budějovice 2024

This thesis should be cited as:

Finnie, S., 2024: The role of ecological gradients and host-parasitoid interaction in tropical insect communities. University of South Bohemia, Faculty of Science, School of Doctoral Studies in Biological Sciences, České Budějovice, Czech Republic.

Annotation

The thesis explores the various biotic and abiotic factors that drive change in insect communities with particular emphasis on host-parasitoid interactions. These factors range from ecological gradients to defensive traits and host specialisation. The aim of the first chapter was to determine how the assemblage of an ecologically and anthropocentrically significant group of insects changed in response to altitude. This study highlighted the elevational limit of fruit flies and discovered that a new and promising attractant lure used in agriculture was particularly useful at higher altitudes, potentially opening new avenues for pest management in mountainous regions. The aim of the second chapter was to ascertain the physiological and phylogenetic traits of galling insects that alter the diversity and host specificity of their parasitioids. This research not only uncovered a remarkably specialist community of gall parasitoids, and found significant physiological drivers of parasitoid assemblage, it also broached the possibility of a latitudinal gradient in gall parasitoid host specificity, that warrants further exploration due to its potential implications for biodiversity patterns. The final chapter, aimed to uncover patterns in the vertical stratification of a caterpillar-parasitoid community, and whether any potential patterns and interactions are driven by the defensive traits of the caterpillar hosts. This study provided further evidence that the defensive traits of caterpillars influence their vulnerability to parasitism, and discovered distinct patterns in the vertical stratification of caterpillar communities. Overall, this holistic research expands upon the existing literature on tropical insect ecology and advances our understanding on the complex interactions between insects and their environment.

Declaration

I hereby declare that I am the author of this dissertation and that I have used only those sources and literature detailed in the list of references.

Sam Finnie

České Budějovice,

14th of May 2024

This thesis originated from a partnership of **Faculty of Science**, **University of South Bohemia**, and **Institute of Entomology, Biology Centre of the ASCR**, supporting doctoral studies in the **Entomology** study programme.

Financial support

Grant Agency of the University of South Bohemia (GAJU n. 014/2022/P and 136/20101/P), the European Research Council Starting (BABE, 805189; PI: Katerina Sam) and Standard Grant (669609; PI: V. Novotny), the Grant Agency of the Czech Republic: Junior 18-23794Y (PI: Katerina Sam), Standard 14-04258S, 20-10205S (PI: V. Novotny) and Expro (19-28126X), ‘ the US National Science Foundation (Division of Environmental Biology 841885), the Christensen Fund (US), and the UK Darwin Initiative for the Survival of Species (14/054).

Acknowledgements

I wish to acknowledge the following individuals, whose contributions, whether direct or indirect, were pivotal in the completion of this doctoral thesis. Your support has been invaluable, and this achievement would not have been possible without you all.

First and foremost, I would like to thank my supervisor Katerina Sam, her understanding and acceptance of my unconventional work habits, including my late starts and independent work style, demonstrated a level of trust and confidence that played a significant role in the successful completion of this thesis. Thanks for all the advice, feedback and for setting a great example of how insanely productive an individual can be!

I would also like to thank anyone who went through thick and thin with me during the various fieldwork expeditions over the course of my Ph.D., especially anyone opening bird and bat exclosures at the crack of dawn: Honza K. in Japan, Sara and Xue in China, Marketa and Kore in Australia, and Matthias and Honza L. in Leipzig, I am grateful to all of you making that draining experience more enjoyable.

I would like to thank Rob, Dragomir, Patrick, Mario, Joel, Bojan, and Carol for all the fun we've had over the years, it has been an absolute pleasure. A special thanks to Conor, for taking me under his wing when I first arrived, and to Phil and Jimmy for the many native-speaker-only beer nights and concerts of late. A huge thanks to all the Tenerife lads and Rian, for keeping me sane on discord over lockdown, and visiting me plenty of times over the years.

Thank you to Romana, for tolerating me, supporting me and keeping me on my toes for past 3 years. To my parents and sister, for their unconditional support and enthusiasm in my career choices, I would not be here without you. To my late grandad, for instilling a love for the natural world in me from a young age, a love that never left and propelled to me where I am today, you are deeply missed.

For those whose names have not been listed, yet have contributed in numerous ways to the successful completion of this thesis, I express my deep gratitude.

A Ph.D. is one hell of an experience, I am extremely grateful to have overcome all the challenges presented over this five-year journey and want to thank all the people that made it possible.

List of papers and author’s contribution

The thesis is based on the following papers:

Chapter 1:

Finnie, S., Sam, K., Leponce, M., Basset, Y., Drew, D., Schutze, M. K., ... & Novotny, V. (2021). Assemblages of fruit flies (Diptera: Tephritidae) along an elevational gradient in the rainforests of Papua New Guinea. Insect Conservation and Diversity, 14(3), 348-355**.** (IF: 4.7)

ML, VN and YB designed the project. SF performed data analysis, and wrote the final draft of the manuscript. KS drafted the first version of manuscript, assembled data and prepared some analyses and assisted the field work. DD and MKS provided taxonomic expertise. All other authors participated in the field work and insect sorting of the project “Our planet reviewed Papua-New-Guinea 2012-2013” under the supervision of IBISCA expert network. All authors contributed to the writing of the final drafts and revisions.

Chapter 2:

Butterill, P., **Finnie, S.**, Sam, K., Fayle, T., Novotny, V. Gall apparency drives parasitoid richness in a highly specialised gall-parasitoid food web from a tropical rainforest. Manuscript.

VN established the project. VN designed the sampling protocols and the experimental approach. PB lead the fieldwork and processed and analysed the data. PB and SF wrote the first draft of the manuscript; all authors discussed the results and significantly contributed to writing the manuscript.

Chapter 3:

Finnie, S., Butterill, Novotny, V., Redmond, C., Jorge, L. R., Abe, T., Lamarre, G. P. A., Maicher, V., Sam, K. (2024). Vertical stratification and defensive traits of caterpillars against parasitoids in a lowland tropical forest in Cameroon. Oecologia, 1-16. (IF: 2.7)

VN and GPAL established the project. VN and GPAL designed the sampling protocols and the experimental approach. CR, TA and VM lead the field work. SF, PB, and CR processed the data. SF and LRJ analysed the data. SF wrote the first draft of the manuscript; all authors discussed the results and significantly contributed to writing the manuscript.

Co-author agreement

Katerina Sam, the supervisor of this thesis and co-author of chapters 1, 2, and 3 fully acknowledges the contribution of Sam Finnie as the first and co-author and his contributions as stated above.

Mgr. Katerina Sam, Ph.D.

Table of Contents

1.Introduction

1.1. Ecological gradients and insect communities

Ecological gradients represent a gradual change in biotic or abiotic environmental factors, which are essential determinants of the structure and functioning of ecological systems and their components (González et al., 2013). These gradients can be defined by a variety of factors, including but not limited to elevation, vertical stratification of vegetation, temperature, light exposure, and precipitation, all of which can have significant impacts on the distribution and diversity of insect communities within an ecosystem (Vetaas, 2021) and the interactions associated with them. Ecological gradients do not exist in isolation and are often interconnected, influencing each other in complex ways. As elevation increases, temperature typically decreases due to lapse rate (Anslow & Shawn, 2002) and precipitation often increases (McCain & Grytnes, 2010) resulting in distinct microclimates which greatly influences the assemblages of insect species occurring at different altitudes along elevational gradients (Hodkinson, 2005). Similarly, the vertical stratification within a forest is heavily influenced by the changing abiotic factors such as temperature, light, and humidity between the understory and upper canopy. These changes create a mosaic of microhabitats, which can dramatically alter the compositions of insect communities across localised spatial scales (Basset et al., 2003). In addition to changes in the composition, ecological gradients have also been shown to alter the total abundance (e.g., de Souza Amorim et al., 2022), density (e.g., Seifert et al., 2020), predation and parasitism rates (e.g., Libra et al., 2019), defensive strategies (e.g., Corff & Marquis, 1999), and the herbivory damage (e.g., Sam et al., 2015) by insects.

Numerous ecological gradients exist in nature, each offering unique insights into the structure and dynamics of ecosystems. However, this thesis specifically focuses on two spatial, ecological gradients and their distinct impacts on insect communities. The first is an elevational gradient (**Chapter 1**), showing how insect communities can change across an elevational transect. The second is a vertical forest gradient (**Chapter 3**), which due to its localised nature, offers a detailed view of changes within a specific forest stratum. Thus, both these gradients, each in their own unique way, shed light on the spatial variations in insect communities across ecological gradients.

Elevational gradients

Elevational gradients are considered important for understanding causes behind gradients in insect species richness and abundance due to the large variation in climate and habitat within a small spatial extent (Körner, 2007). Elevational gradients can be used as analogues to predict how different organisms will be affected by fluctuations in temperature due to climate change (Tito et al., 2020). Typically, a mountain with a large elevational and thermal range is chosen to measure the effect of an elevational gradient on insect communities. Mountains also often have high biodiversity and high levels of endemism (Noroozi et al., 2018; Rahbek et al., 2019), and are typically relatively less disturbed by people compared to flat lowlands, making them ideal for studying species under more natural settings. As you ascend an elevational transect, the changes in the abiotic conditions are generally consistent and predictable. Temperature will decrease (Barry, 2008), precipitation as rain or snow will increase (McCain & Grytnes, 2010), as well as atmospheric turbulence, wind speed and radiation exposure (Iziomon & Mayer, 2002). These parameters interact strongly with each other. As elevation increases, they collectively result in a less structurally complex habitat. This, in turn, leads to a decrease in insect diversity, particularly at higher elevations (Dianzinga et al., 2020). However, this trend of decreasing diversity with increasing elevation is not strictly followed by all insect groups (Colwell et al., 2016). Interestingly, although rainfall can be highest at peak altitudes due to the formation of clouds, rapid runoff results in less water availability. In contrast, intermediate elevations, with their warm temperatures, moderate precipitation, and abundant vegetation, maintain higher water availability. These intermediate conditions favour many insect communities, leading to mid-elevational peaks in their diversity (McCain & Grytnes, 2010).

Among all the elevational gradients, the one discussed in this thesis (see **Chapter 1**), Mt. Wilhelm in Papua New Guinea (PNG), is one of the most extensively studied. Spanning from 200 - 4509 m a.s.l., (**Fig. 1a**) this mountain has been the site of numerous research studies assessing how various vertebrate communities, such as birds, bats, and frogs (Dahl et al., 2024; Sam et al., 2019; Sivault, Amick, et al., 2023) change in response to elevation. Additionally, the insect communities have been extensively studied including ants (Moses et al., 2021), wasps (Souto-Vilarós et al., 2020), beetles (Weiss et al., 2024), moths (Toko et al., 2023), and true bugs (Le Cesne et al., 2015)) as well as ecological processes such as pollination (Souto-Vilarós et al., 2018), predation, and parasitism rates (Libra et al., 2019, Sam et al. 2015). These studies have shed light on how the abundance and richness of different insect taxa change in response to elevation. The species richness and abundance of certain insect groups showed a linear decline with increasing elevation such as ambrosia bark beetles (Weiss et al., 2024) and Hemiptera (Le Cesne et al., 2015), whereas geometrid moths, ants, and fig wasps exhibited unimodal, mid-elevational peaks in their species richness (Beck et al., 2017; Colwell et al., 2016; Moses et al., 2021; Souto-Vilarós et al., 2020; Toko et al., 2023). Additionally, previous studies have demonstrated that caterpillars experienced higher predation at lower latitudes compared to higher ones (Sam et al., 2015), while the rate of parasitism remained consistent across both altitudes (Libra et al., 2019). These studies have significantly advanced our understanding of insect communities across elevational gradients. However, further research is crucial to explore the many understudied insect groups and deepen our knowledge of their responses to elevational changes (**Chapter 1**).

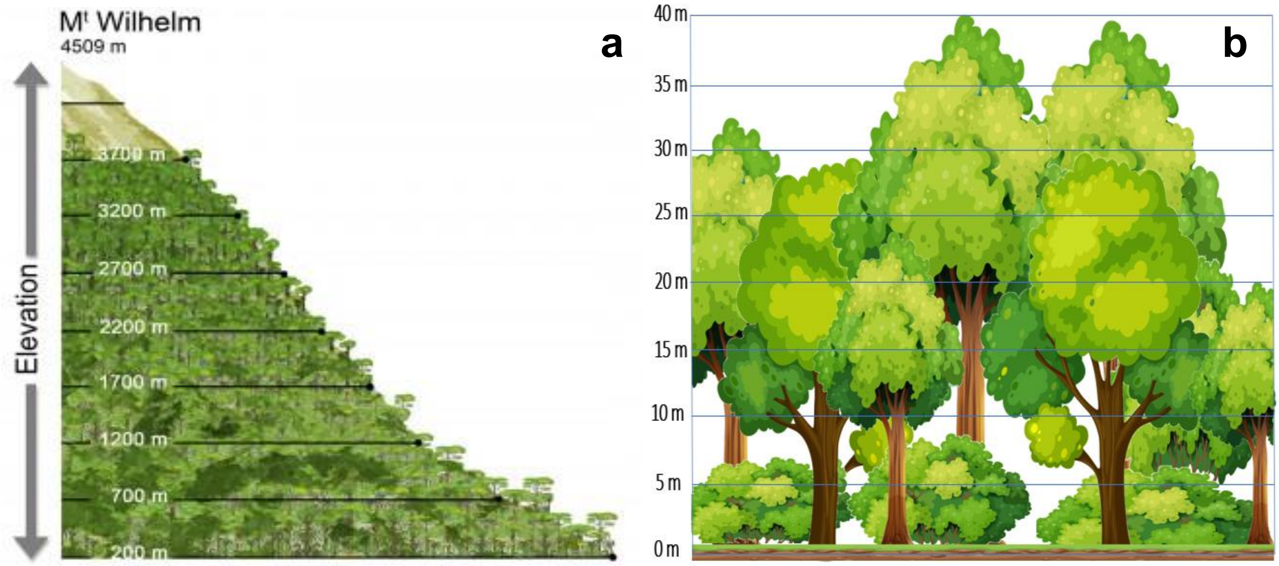

Figure 1. Depictions of the ecological gradients used in this thesis a) The transect of Mt. Wilhelm showing the full elevational gradient (credit: Maurice Leponce) and b) The vertical forest gradient in Cameroon, segmented into 5 m strata (credit: Sam Finnie).

Vertical forest gradients

A vertical forest gradient refers to the changes in environmental conditions and biodiversity that occur from the forest floor to the upper canopy. This gradient can be divided into multiple strata (**Fig. 1b**), each with unique conditions, allowing insect communities to be studied across the entire vertical span of the forest. Assessing the effects of vertical forest gradients on insect communities was, until relatively recently, extremely difficult due to the inaccessibility of the canopy, particularly in remote tropical forests (Lowman, 2009). However, recent innovations such as the use of canopy cranes (e.g., Nakamura et al., 2017; Sivault, et al., 2023), taking advantage of local logging in tropical countries (Volf et al., 2019), and fogging (Leponce et al., 2021) has allowed canopy-related entomology, and studies on how insect communities change across vertical forest gradients to flourish over the past four decades (Lowman, 2009). Numerous studies have shown that assemblages of target insect groups can vary greatly across forest strata (Ashton et al., 2016; Basset et al., 2003; de Souza Amorim et al., 2022; Grimbacher & Stork, 2007; Schulze et al., 2001; Stork et al., 2016). The drivers behind these distributional changes in their communities have been attributed to a myriad of factors. The most obvious of these is the escalating divergence in abiotic conditions between the forest floor and the upper canopy. Notably, there is a marked decrease in light penetration and throughfall, the portion of rainfall that passes through the canopy and reaches the forest floor, due to interception and evaporation from the dense intermediate foliage (Carlyle-Moses & Gash, 2011). This foliage also serves as a protective barrier, shielding the forest floor from adverse weather conditions such as strong winds and ultraviolet (UV) radiation from direct sunlight. These combined factors contribute to an increase in humidity within the understory (Pickering et al., 2021). These distinctions are the most extreme in tropical rainforests, where the foliage is denser and the canopy is typically higher and more layered (Basset, 2001).

The variation in different abiotic factors across vertical forest gradients shapes the distributions of different insect groups that may favour the conditions in the upper canopy, the understory, or the intermediate conditions in between (Prasad, 2022). For example, Stork et al. (2016) found that herbivorous beetles were proportionally more abundant in the upper canopy, whereas saprophagous beetles were restricted to the lower strata. Similarly, Ashton et al. (2016) found distinct differences between adult moth assemblages in the understory and canopy. Finally, in a large-scale study, which included multiple insect orders, de Souza Amorim et al. (2022) found that Diptera, Hymenoptera, and Coleoptera had their greatest abundance at the ground level, whereas Lepidoptera and Hemiptera were more abundant in the upper levels of the canopy. Unravelling the vertical preferences of insect communities is a difficult task, as certain species likely prefer to occupy different strata during different stages in their life cycle, weather conditions, and time of year. As previously underscored, studies that span entire vertical forest gradients are a relatively novel concept in science. Therefore, there is a pressing need for additional research so that we may better understand the factors that shape the vertical distributions of different insects, particularly in the tropics where studies are more limited and insect diversity is extremely high (**Chapter 2**).

1.2. Focal insect groups

This section serves as an overview of the diverse insect groups that form the core of the research presented in the manuscripts that constitute this doctoral thesis. The inclusion of this section aims to provide readers with a clear understanding of the specific insect groups under study, their unique characteristics, and their significance within the broader context of ecological research.

Tephritid fruit flies

Tephritid fruit flies (Tephritidae) (**Fig 2a**), are a large family within the order Diptera. They are globally dispersed with an impressive diversity of over 500 genera and 5000 species (Scolari et al., 2021). The majority of tephritid larvae are plant-eaters. Female flies lay their eggs in healthy plant tissues using their extendable ovipositors, providing a food source for the emerging larvae. Depending on the species, the larvae develop in various parts of the host plant, including leaves, stems, flowers, seeds, fruits, and roots. Some species are monophagous, feeding on a single plant species, while others are polyphagous, feeding on several related plant species.

These flies are infamous for causing substantial economic damage due to their infestation of a wide range of commercial fruits and vegetables (Carroll et al., 2002). They are known for their invasive nature in foreign agricultural landscapes and their rapid spread (Huan et al., 2019), resulting in significant destruction and financial loss to commercial fruit farmers (He et al., 2023). The most damaging tephritid pests belong to the Dacini (Tephritidae: Dacinae), specifically the *Bactrocera*, *Dacus*, and *Zeugodacus* genera (White & Elson-Harris, 1992). Which are extremely prevalent in the tropics. In efforts to manage pest tephritid species, farmers employ fruit fly lures, typically a blend of insecticide and attractant, with alternatives such as liquid traps where the pest drowns, or sticky traps that retain the insect until it perishes (Clarke et al. 2011). Additionally, the release of fruit fly parasitoids is sometimes used in conjunction with these methods, which have been demonstrated to decrease pest populations by up to 40% (Clarke et al. 2022).

Of all countries, PNG has the highest diversity of Dacinea (Clarke et al., 2004), and over 200 species in the *Bactrocera* and *Dacus* genera have been described, with over 60 newly discovered species awaiting description (Drew & Romig, 2013). Approximately 75% of these described species are endemic to PNG, with the rest primarily found in West Papua, the Solomon Islands, Torres Strait, and far north Queensland (Clarke et al., 2004; Drew, 2004; Drew & Romig, 2013).

Research into the variation of tephritid communities across elevational gradients remains a relatively unexplored area of study, with existing literature presenting a complex and somewhat contradictory picture. For instance, a study conducted by Salazar-Mendoza et al. (2021) reported that both the richness and abundance of tephritids and their parasitoids were found to be highest at lower elevations. This finding contrasts with the results of a study by Berrones-Morales et al. (2019), which identified a peak in tephritid diversity at an elevation of 900 meters above sea level. These disparate findings underscore the need for further research into the effects of elevation on tephritid communities.

Given these complexities, it becomes evident that more comprehensive studies are needed to fully understand these dynamics. In this context, PNG, with its remarkable tephritid diversity, presents an excellent location for such studies. The high diversity and endemism of tephritids in PNG not only provide a unique opportunity to study these species within their ecosystems but also aid in enhancing our understanding of their diversity. This, in turn, can contribute significantly to the development of effective strategies for agricultural pest management and control (**Chapter 1**).

Parasitoids

Parasitoids are functionally essential organisms to terrestrial ecosystems. Their sheer diversity, abundance and ecological importance have been well documented, and they represent over 10% of all known insect species (Heraty, 2017). Parasitoids are insects whose larvae feed exclusively on, and kill, a single arthropod host. The developmental strategies of parasitoids are categorised into either koinobiont or idiobiont. Koinobiont parasitoids allow their hosts to continue feeding and growing after being parasitised and the parasitoid larva are typically endophagous. Idiobionts will paralyse the host, halting further development, these parasitoids are generally ectoparasitoids, feeding externally on the host (Quicke, 2014).

Parasitoids occur in many insect orders however the preponderant species are the Hymenopteran, parasitoid wasps (**Fig. 2b**) and the Dipteran, tachinid flies (**Fig. 2c**). Hymenoptera are arguably the most speciose order of insects of which the parasitoid wasps may contribute to an estimated 90% of all parasitoid species, with the vast majority of these remaining undescribed (Forbes et al., 2018). The two largest superfamilies, the Chalcidoidea and the Ichneumonoidea have an estimated, combined total of 650,000 species (Quicke, 2014). These wasps prey on all life stages of their hosts: egg, larvae, pupae, and occasionally adults (Boppré, 2008). The majority of parasitoid hosts are from the order Lepidoptera, although Coleoptera, Diptera, Hemiptera, Neuroptera, Araneae, and other Hymenoptera are also obligate hosts. The tachinid flies exclusively belonging to the family Tachinidae collectively comprise over 60% of all dipteran parasitoids with over 10,000 species currently described and the true diversity being estimated at many thousands higher than this (O’Hara, 2008). Like their hymenopteran counterparts, the majority of tachinid species target lepidopteran caterpillars as hosts and they have been found to be the largest mediators of caterpillar populations in many environments (Gentry & Dyer, 2002; Lewis et al., 1994).

The functional importance of parasitoids among upper trophic levels has gained acknowledgement over the past few decades (Godfray, 2016). Arguably the most significant is the incorporation and appreciation of parasitoids in agriculture. Many economically significant, herbivorous agricultural pests are hosts to parasitoid species (Hajek & Eilenberg, 2018). Due to the specific nature of a parasitoid’s lifecycle, they make ideal natural enemies as they do not contribute to intra-guild predation like generalist predators (Müller & Brodeur, 2002). Adult parasitoids further benefit agriculture as pollinators, feeding almost exclusively on nectar (Zemenick et al., 2019). The commercial rearing of parasitoids has also become a key component in integrated pest management

(Clarke et al. 2022). Parasitoids that specialise in a certain host can be mass-reared in cultures and released in situ can successfully reduce pest populations by over 70% (van Lenteren et al., 2020). Moreover, the use of parasitoids as indicators in forensic entomology has emerged in recent years, further proving their anthropocentric value (Rivers, 2016). Parasitoids are also extremely important mediators of their host species in more natural settings. As they naturally reduce the abundance of their predominantly herbivorous hosts, they are indirectly beneficial to plants (Connahs et al., 2011). They can be significant suppressors of invasive outbreak species like the oak processionary moth (*Thaumetopoea processionea*) reducing their populations by as much as 50% (Kitson et al., 2019). This relationship has driven the evolution of unique strategies among parasitoids to access and parasitise their hosts effectively. These strategies have facilitated parasitoids in infiltrating hosts that are located deep within trees, beyond the reach of other predators, and in enduring the potent toxins of poisonous hosts (Fischer et al., 2001; Gentry & Dyer, 2002).

Research on parasitoid assemblages across ecological gradients reveals distinct trends. Elevational studies show varied responses, with Tachinidae diversity decreasing with elevation (Corcos et al., 2018), while ichneumon species richness peaks at mid- and high-elevation sites (Veijalainen et al., 2014). Peck et al. (2008) further observed an increase in parasitoid species richness with elevation. In vertical forest gradients, tachinid species richness was found to peak at 8 m heights above the forest floor (de Souza Amorim et al., 2022), whereas ichneumon species richess highest in the canopy (Giovanni et al., 2014). Given the vast diversity of parasitoids, and their conspicuous lifestyle, they remain significantly understudied, particularly in tropical regions. More comprehensive studies are required to elucidate the complex interactions between parasitoids and their hosts and to enhance our understanding of their many roles within the ecosystems they inhabit (**Chapter 2, Chapter 3**).

Lepidopteran caterpillars

The famously hungry caterpillars (Carle, 2016) are an ecologically and economically important group of herbivores in nearly all natural and agricultural terrestrial ecosystems. They are the larval stages of butterflies and moths from the order Lepidoptera (**Fig 2c**, **Fig 3**). Currently, at least 160,000 species have been described, with the vast majority found in tropical regions, and there are likely thousands more yet to be identified (Ghazanfar et al., 2016). Lepidoptera are also very widespread, occupying every continent except Antarctica (James, 2017). Caterpillar species are almost exclusively herbivorous, feeding on a wide variety of plant matter, including leaves, stems, and flowers. However, there are exceptions, with some species exhibiting carnivorous behaviour, such as preying on ants or other caterpillars, and others functioning as detritivores (Bodner et al., 2015; Montgomery, 1983).

Caterpillar species can be specialists (feeding on a single plant species) or generalists (feeding on multiple plant species). They are prodigious herbivores representing as much as 45% of all phytophagous insects in tropical insect communities (Hawkins, 1994). Their voracity and prevalence can lead to significant herbivory damage to plants, making many caterpillars a pest species in an agricultural setting (Ode, 2022). Conversely, they are also significant hosts and food sources for parasitoids and predators respectively. The adults are also vital pollinators in many ecosystems (Ghazanfar et al., 2016; James, 2017).

Caterpillars have evolved a wide range of behavioural, physical, and chemical defensive traits. Making them an ideal insect group for ecological studies focusing on predator-prey and parasitoid-host interactions (Marquis & Koptur, 2022). Furthermore, caterpillars are typically located on the specific host plant they feed on and due to their sessile behaviour, they can be easily sampled in the field. This makes them the ideal insect group for research focusing on herbivore-host plant interactions or vertical stratification in forests (Seifert et al., 2020).

Research on lepidopteran communities, primarily focused on adults, has revealed distinct patterns across elevational gradients. For instance, richness and diversity maxima varied among

Lepidoptera families, with each peaking at different elevations (Brehm et al., 2007), and geometrid moths consistently showed a pattern of reaching their highest richness at mid-elevations (Beck et al., 2017). However, the Sphingidae family showed a distinct richness peak at lower elevations (Sublett et al., 2019). Studies on caterpillar communities across vertical forest gradients are scant, but have revealed significant variations in feeding guilds and species composition across strata (Seifert et al., 2020), with the highest caterpillar abundance in the midstory (Sigut et al., 2018), and the greatest abundance and richness within the canopy (Neves et al., 2013). Given their remarkable diversity, extensive distribution, and significant ecological roles, it is imperative to conduct further research on caterpillar communities. This is particularly crucial in tropical forests, where many species are likely yet to be discovered (**Chapter 3**).

Galling insects

Galling insects are a unique group of insects that induce the formation of galls, which are abnormal growths of plant tissue (**Fig 2d, 2e)**. These insects have a symbiotic relationship with their host plants, often manipulating the plant’s growth and development to create a specialised structure that serves as both a habitat and a food source for the insect (Redfern, 2011). Some galls act as "physiologic sinks", concentrating resources in the gall from the surrounding plant parts. Additionally, they provide the galling insect with physical protection from predators and parasitoids (Ozaki et al., 2006).

Gallers are extremely diverse with 13,000 species currently described and estimates as high as 211,000 globally (Espírito-Santo & Fernandes, 2007), although the sheer abundance of galling insects in tropical regions has been described as inestimable (Gagné & Jaschhof, 2021). There are several groups of insects known to induce gall formation. They include gall wasps (Cynipidae), scales (Coccoidea), gall midges (Cecidomyiidae), aphids (Aphidoidea), psyllids (Psyllidae), and certain species of leafminer flies (Agromyzidae). Each of these insects interacts with the plant in a specific way to induce gall formation, often involving the secretion of chemicals that stimulate or regulate plant growth (Redfern, 2011). The most speciose galling insects are the cecidomyiids and the cynipids with 6,600 and 1,400 species described respectively (Gagné & Jaschhof, 2021; Ronquist et al., 2015) which have been the focal insect groups for many ecological studies.

Gall-inducing insects are known for being indicators of ecosystem health (Fernandes et al., 2010). In addition, they are known for being highly specialised on their host plant species, as well as specific plant organs, although few species are known to induce galls on multiple closely related plant species (Butterill & Novotny, 2015). Furthermore, these highly specialized interactions are closely linked to their interactions with parasitoids. Certain gall species or structures host larger assemblages of parasitoids. Additionally, some parasitoids may exhibit more or less specialisation than others in relation to specific gall species or taxa (de Araújo & Maia, 2021; Paniagua et al., 2009), although these interactions remain largely understudied.

Studies on galling insect communities across elevational gradients are scant, but informative. Serrano-munoz (2022) found that the abundance and richness of gall-inducing insects and their associated parasitoids increased with increasing elevation in central Mexico. Other studies have highlighted the importance of water availability (i.e. mesic and xeric habitats) for shaping gall communities. For example, Lara et al. (2002) found that in xeric habitats, increasing elevation reduced gall richness, but this pattern did not extend to mesic habitats. Moreover, Blanche & Ludwig (2001) found that gall richness and abundance was highest at intermediate, mesic elevations.

For vertical forest gradients, many studies highlight the surprising abundance and diversity of galls in the upper canopy (Juliao et al. 2014). Thought to be a product of increased survivability in upper forest strata where they avoid death by fungi, parasitoids or accidental chewing from leaf herbivores. The findings of Paniagua et al. (2009) further support this, finding the highest species richness of gall parasitoids in the understory whereas gall richness was highest in the canopy. Basset (2003) also discussed the clear vertical stratification of gall communities, shaped by differences in foliage quality and the narrow host ranges of most galling insects. Future research focusing on the specialisation and assemblages of parasitoids of galling insects are necessary to uncover the drivers and patterns behind these intricate ecological interactions (**Chapter 2**).

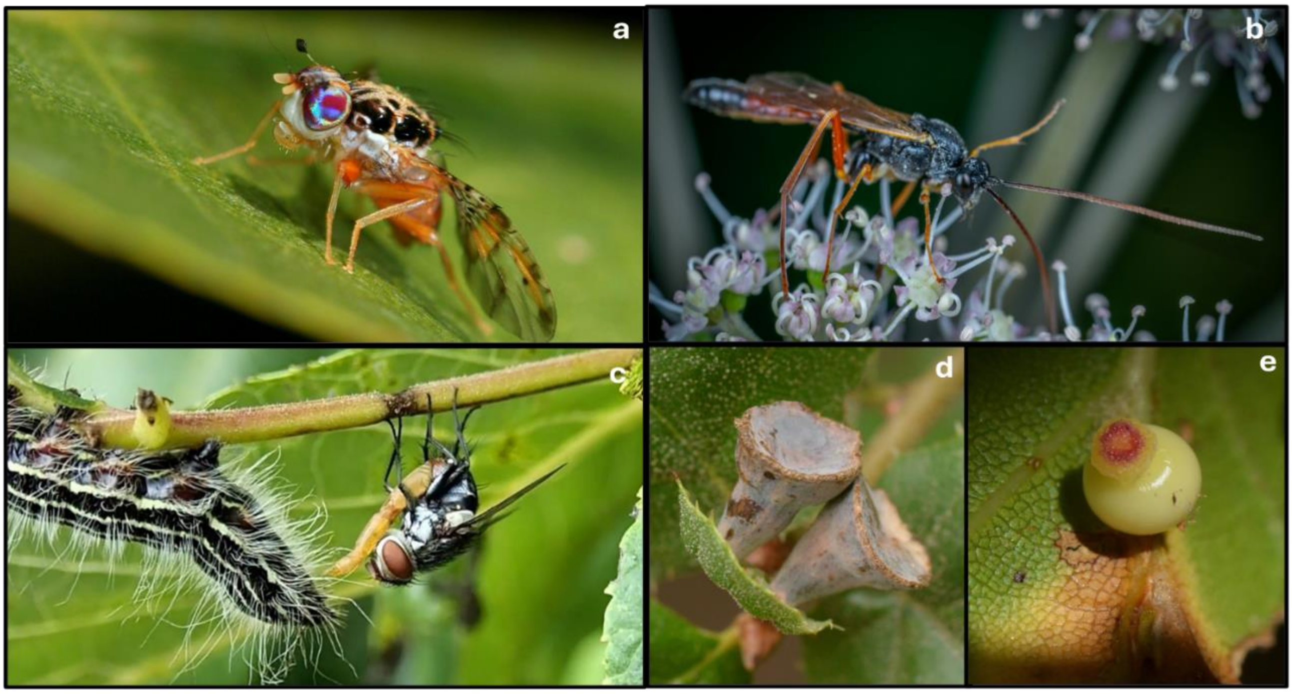

Figure 2. Images of insects from all the focal insect groups, a fly from the family Tephritidae (*Ceratitis capitata*) (**a**), a parasitoid wasp (Ichneumonidae sp.) (**b**), a tachinid fly (unknown sp.) ovipositing on a caterpillar (*Datana sp.*) (**c**), galls from the Live Oak Apple Gall Wasp (*Callirhytis quercuspomiformis*) (**d**), and the Kernel Flower Gall Wasp (*Callirhytis serricornis*) (**e**) (Image credit: © 2016 Mark Kummel).

1.3. Host-parasitoid interaction

This section delves into the intricate dynamics of host-parasitoid interactions, a central theme in the research presented in this doctoral thesis. The purpose of this section is to equip readers with a comprehensive understanding of the specific aspects of these interactions under investigation, including parasitism rates, defensive traits of parasitoid hosts, and the host range of parasitoids. These topics are presented as an overview, providing a broad perspective on their unique characteristics and their significance in the context of ecological research.

Parasitism rates

Parasitism rates are defined as the proportion of hosts within a population that have been successfully parasitised, expressed as a percentage of the total population. These rates serve as an effective measure for assessing the susceptibility of a host group to parasitoids, as well as identifying specific traits or locations of host species or individuals that increase their vulnerability to being parasitised. Previous research comparing parasitism rates has identified that shelter-building caterpillar hosts have higher parasitism rates than exposed feeders and that gregarious host species experience higher rates of parasitism than solitary (Rodovalho et al., 2007; Stireman, 2016). Further, both Connahs et al. (2011) and Sobek et al. (2009) found higher parasitism rates in the canopy than understory despite similar densities of caterpillars and Šigut et al. (2018) found that parasitism rates in caterpillars differed significantly between the host plants of the caterpillars. Parasitism rates are also used to compare how successful a particular parasitoid species is at controlling its host species, which can prove invaluable when they are used as biological control agents of pest species in agriculture (Hawkins & Cornell, 1994). In a recent study, Lampert et al. (2010) proposed the "*Safe Haven"* (SH) hypothesis regarding the interaction between chemically defended hosts and parasitoids. They suggested that hosts with chemical defences, which deter predators due to their toxicity, may in fact serve as ideal sanctuaries for parasitoids. This is because parasitoids, being susceptible to intra-guild predation, could benefit from the host's defences, reducing their likelihood of being consumed. Similarly, this concept extends to the "*Enemy-Free Space"* (EFS) theory (Jeffries & Lawton, 1984) which states that an organism can minimise their likelihood of mortality by inhabiting spaces or adopting behaviours that reduce their exposure to predators or other natural enemies, thereby exploiting EFS. In the context of larval parasitoids, ETS can refer to host choice, where parasitoids may selectively (or evolutionarily) infect hosts that are better defended from predators, and are therefore a safer environment for their development (Murphy et al., 2014). EFS has been shown to be a strong selective force in a variety of herbivore systems (e.g., Diamond & Kingsolver, 2010; Murphy, 2005), and the degree to which they may shape host-parasitoid interactions and parasitism rates needs to be further explored. Such studies could provide valuable insights into whether theories such as SH and EFS influence the susceptibility of hosts to parasitoids, and could prove invaluable to the use of parasitoids as natural enemies of pest species in agriculture (**Chapter 3**).

Defensive traits of parasitoid hosts

The hosts of parasitoids have evolved a myriad of different defence traits to avoid predation and parasitism, these traits may have trade-offs and be more successful at deterring one type of natural enemy than another (Baer & Marquis, 2020). For the relevance of this thesis, I will discuss three of the most significant defensive traits utilised by external and semi-concealed caterpillars: aposematism, crypsis, and shelter-building. All of which are thematic to **Chapter 3**.

Aposematism

Aposematism is a biological mechanism by which an organism advertises its dangerous or noxious nature to potential natural enemies typically with bright contrasting colouration increasing conspicuity (**Fig 3a**). This warning system can deter predators from attacking or eating the organism. The unprofitability of the prey may consist of any defences which make the prey difficult to kill and eat, such as toxicity, venom, foul taste or smell, sharp spines, or aggressive nature. Aposematism is strongly linked with caterpillar species that sequester toxins, advertising their unpalatability, or in some cases, Batesian mimics imitating such species (Willmott et al., 2011). This defensive trait has been shown to reduce predation rates in insects (Aslam et al., 2020; Lichter-Marck et al., 2015). Conversely, it has been shown to increase parasitism rates in caterpillars (Gentry & Dyer, 2002; Greeney et al., 2012; Murphy et al., 2014) although, there some studies supporting the opposite (Singer et al., 2004). Such contradictions necessitate the need for further research on the effects of aposematism on host-parasitoid interaction.

Crypsis

Cryptic insects utilise a biological mechanism, to avoid observation or detection. This strategy is primarily an antipredation adaptation, although it can also be used as an effective predation strategy too (Brakefield, 2009). Crypsis can involve visual, olfactory (with pheromones), or auditory concealment. The majority of cryptic caterpillars are generic in colouration, often shades of green or brown allowing them to blend in with the foliage they inhabit. Some will utilise a form of crypsis known as mimesis, in which they mimic features in their surroundings such as leaves, twigs (**Fib 3b**), bars, or even bird droppings (**Fig 3c**) (Suzuki & Sakurai, 2015). Research has shown that whilst crypsis can be a very effective antipredator adaptation, certain cryptic insects may have higher predation rates than other defence traits such as aposematism and shelter-building (e.g. Aslam et al., 2020; Lichter-Marck et al., 2015; Tvardikova & Novotny, 2012). Conversely, there is some evidence to suggest they also have fewer incidences of parasitism (Gentry & Dyer, 2002; Hrcek et al., 2013), suggesting a trade-off between the efficiently of predator and parasitoid avoidance. However, studies addressing these trade-offs are lacking. Furthermore, crypsis encompasses a variety of defensive traits that fall under different subcategories. These traits can have diverse impacts on host-parasitoid interactions and anti-predator success, likely with varying degrees of effectiveness across a range of complex ecological scenarios.

Shelter-building

Shelter-building is a defensive trait exhibited by tens of thousands of caterpillar species from at least 25 families (Jones, 1999). Shelters are built using silk (sometimes by cutting, rolling, and folding leaves) to manipulate the portions of their host plants into positions, allowing the caterpillar to take refuge (**Fig. 3d**). Shelter architecture ranges from complex, origami-like tents (Greeney, 2009) to simple sheets of silk covering the leaf surface (e.g., Rota & Wagner, 2008). Two of the proposed benefits of shelters are protection from natural enemies and protection from desiccation (Diniz et al., 2012). Evidence certainly suggests that shelters built by caterpillars offer enhanced protection against predators (Baer & Marquis, 2020). However, these shelters may paradoxically serve as EFS for parasitoids, although the literature is inconclusive. Both Hrcek et al. (2013) and Connahs et al. (2011) found that shelter-builders had significantly higher parasitism rates than exposed caterpillars in tropical forests, whereas both Diniz et al., (2012) and Rodovalho et al. (2007) found the opposite in the Brazilian Cerrado. Interestingly, LoPresti & Morse (2013) found that whilst shelters protected caterpillars from parasitoids, shelter-builders were far more vulnerable to attack from parasitoids than predators. Given these contrasting findings, further research is crucial to better understand the specific contexts under which shelter-building provides an advantage against parasitoids, and when it inadvertently increases their vulnerability.

Host range

Host range refers to the degree of host specificity within a given parasitoid species (i.e. how specialised the parasitoid is).It is defined as the number and diversity of hosts used by a parasitoid species. These ranges can span from completely monophagous specialists, that parasitise a single species, to oligophagous generalists with multiple host species. There appears to be a trade-off between host range and host-use efficiency (i.e. the success rate of oviposition), with specialists being more efficient than generalists (Straub et al., 2011). This efficiency makes specialist parasitoids ideal for biological control programs in agriculture, although, in more natural settings, their effectiveness is limited by their dependence on a specific host, making them vulnerable to changes in host availability (Stilmant et al., 2008). Establishing the true host ranges of parasitoids is challenging. Yet, it is generally observed that a majority of parasitoids tend to attack a narrow range of hosts (Stireman & Singer, 2003). An exception to this trend is found in certain species, particularly among certain species of tachinid. These species exhibit broad host ranges, a phenomenon likely facilitated by their oviposition strategy that allows them to bypass the physiological defences of their hosts (Stireman et al., 2017). Although some studies found that tachinids were more host-specific than the hymenopteran parasitoids in specific locations(Hrcek et al., 2013; Šigut et al., 2018). Moreover, some tachinids that were previously considered generalists, were in fact found to be multiple cryptic specialist species (Smith et al., 2007).

One of the main drivers behind parasitoid host range is thought to be host taxonomy (Askew & Shaw, 1986). For example, in Panama, leaf miners were shown to be host to predominantly generalist parasitoids (Lewis et al., 2002), whereas gall-forming insects had mostly specialists (Paniagua et al., 2009). Similarly, semi-concealed caterpillars had more specialist parasitoids than exposed caterpillars in a Papua New Guinean rainforest (Hrcek et al., 2013). The other main driver of host range is thought to be ecological factors, like habitat structure. Šigut et al. (2018) found that generalist parasitoids were more prevalent in the lower canopy, whereas generalists were more abundant in strata where their hosts were more abundant. Additionally, Bellone et al. (2020) found that generalist parasitoids preferred areas with higher tree density, likely increasing their likelihood of finding a suitable host. These studies highlight the variation and nuance in determining host ranges in parasitoids, highlighting the need for further research to fully understand the factors contributing to these contrasts **(Chapter 2**).

Locating the host

Host location in female parasitoids is a multifaceted process involving habitat identification, microhabitat selection, and host recognition. This requires extensive mobility, especially for species with solitary larval development which have to locate a new host individual for each oviposition. The foraging process, encompassing both habitat and host location, often involves long-range movements, typically by flight, transitioning to short-range foraging upon nearing hosts (Woiwood et al., 2001). Once located, host selection is typically accepted or rejected using cues obtained from physical contact with the host (Henneman, 2008).

During the process of host location, parasitoids utilise a blend of visual and chemical cues. The reliance on these cues varies significantly across different stages of location, host species, and parasitoid species. The visual cues can range from broadly identifying potential host habitat (Woiwood et al., 2001), to determining whether the size of the host is suitable once it has been located (Morehead & Feener, 2000). Chemical cues detected from from parasitoid hosts range from chemical footprints, left behind by the host as it forages, to sex pheromones left by the egg-laying female of the host species in egg parasitoids (Colazza et al., 2009). Parasitoids are capable of identifying the species of host plants based on the volatiles they emit. Furthermore, they also respond to specific volatiles that are released by these plants when they are being consumed by a host species (De Moraes et al., 1998). Moreover, there is substantial evidence that parasitoids possess the ability to learn and improve their recognition of visual and chemical cues over the course of their adult lifespan (Wäckers & Lewis, 1994).

Host location processes in parasitoids are likely based on multisensory responses to a variety of cues, which are integrated in accordance with the habitat and environmental conditions (Kroder et al., 2007). For example, Canale et al.2013) demonstrated that a species of Braconidae exhibited an optimal response when both visual and chemical cues were presented concurrently, suggesting a synergistic function of vision and olfaction in this species. The reliance on visual and chemical cues can significantly vary across different parasitoid taxa and host groups. Dipteran parasitoids, for instance, have been shown to rely more heavily on visual cues (Yamawaki et al., 2002), while hymenopteran parasitoids typically utilise more chemical cues. When considering host groups, studies on leaf miners have shown that parasitoids are more attracted to leaves with multiple mines (Low, 2008). Furthermore, Hawkins & Gagné (1989) demonstrated that more conspicuous galls typically host larger parasitoid assemblages, suggesting that visual cues play a crucial role for parasitoids when locating these host groups, although that has not been explicitly tested. The role of chemical cues in host location has already been well documented (Powell & Poppy, 2001). However, the significance of visual cues in this process is not as thoroughly explored, warranting the need for further visually orientated studies on parasitoid host location (**Chapter 2**).

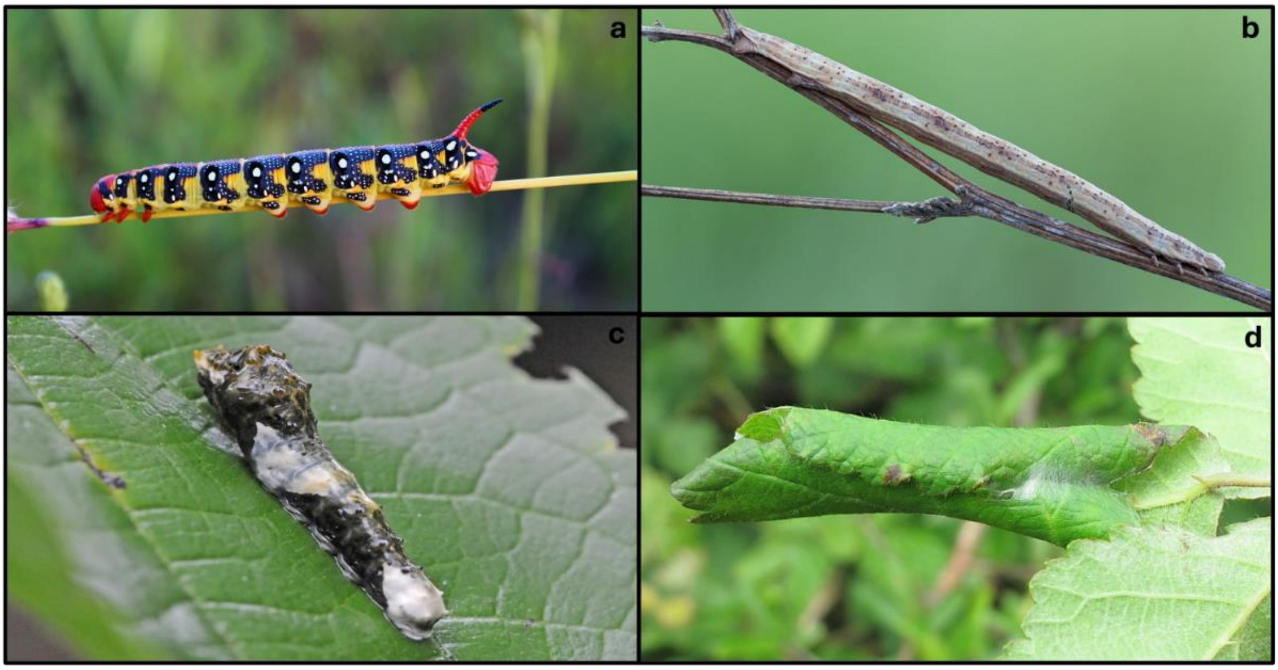

Figure 3. Images showing examples of defensive traits in caterpillars, the Spurge Hawk-moth (*Hyles euphorbiae*) with typical aposematic colouration (**a**), a caterpillar (Geometridae sp.) exhibiting crypsis, mimicking a branch (Image credit: Crisian Arghius, 2012) (**b**), a Giant Swallowtail caterpillar (*Papilio cresphontes*), employing crypsis by mimicking a bird dropping (Image credit: © 2013 Michael J. Raupp) (**c**), the leaf roll of a shelter-building caterpillar (Tortrix sp.) (**d**).

1.4. Thesis Overview

In this thesis, I delve into the intricate dynamics of tropical insect communities, examining the significant role of various biotic and abiotic conditions in shaping these assemblages. The exploration underscores the profound influence of ecological gradients and host-parasitoid interactions in significantly altering the structure and composition of insect communities.

In **Chapter 1**, I conducted a comprehensive survey of fruit fly species along an elevational gradient on Mt Wilhelm in Papua New Guinea, a region renowned for its diverse dacine fruit flies. The study involved the collection and identification of fruit flies, and the examination of how their abundance, species richness, and assemblages were influenced by elevation and the type of lure used. This research serves as a foundation for understanding the geographical and elevational distribution of these significant agricultural pests and sets the stage for further exploration.

In **Chapter 2**, I focused on the how the specialisation, physical, and phylogenetic traits of gall-inducing insects effect the assemblages of their associated parasitoids across multiple sites in Papua

New Guinea. This research contributes significantly to our understanding of these complex ecological interactions within tropical rainforests, particularly in the context of a large and diverse plant-gall-parasitoid network.

In **Chapter 3**, I investigate the intricate interactions between caterpillars and their parasitoids across a vertical forest gradient in a tropical forest in Cameroon. This study focuses on how the various defensive traits of caterpillars effect both their vertical stratification and their interactions with parasitoids. This research provides valuable insight into the factors shaping host-çparasitoid interactions, and the vertical structure of caterpillar assemblages in a diverse tropical forest.

1.5. References

Anslow, F. S., & Shawn, M. J. (2002). An investigation of local alpine terrestrial lapse rates in the Canadian Rockies. *Proceedings of the 32nd Annual Arctic Worshop, INSTAAR, University of Colorado, Boulder*, *1*.

Ashton, L. A., Nakamura, A., Basset, Y., Burwell, C. J., Cao, M., Eastwood, R., Odell, E., De Oliveira, E. G., Hurley, K., Katabuchi, M., Maunsell, S., McBroom, J., Schmidl, J., Sun, Z., Tang, Y., Whitaker, T., Laidlaw, M. J., McDonald, W. J. F., & Kitching, R. L. (2016). Vertical stratification of moths across elevation and latitude. *Journal of Biogeography*, *43*(1), 59–69. https://doi.org/10.1111/jbi.12616

Askew, R. R., & Shaw, M. R. (1986). *Parasitoid communities: Their size, structure and development.* https://www.cabidigitallibrary.org/doi/full/10.5555/19870540695

Aslam, M., Nedvěd, O., & Sam, K. (2020). Attacks by predators on artificial cryptic and aposematic insect larvae. *Entomologia Experimentalis et Applicata*, *168*(2), 184–190. https://doi.org/10.1111/eea.12877

Baer, C. S., & Marquis, R. J. (2020). Between predators and parasitoids: Complex interactions among shelter traits, predation and parasitism in a shelter-building caterpillar community. *Functional Ecology*, *34*(10), 2186–2198. https://doi.org/10.1111/1365-2435.13641

Barry, R. G. (2008). *Mountain weather and climate.* Cambridge University Press. https://www.cabdirect.org/cabdirect/abstract/20093032476

Basset, Y. (2001). Invertebrates in the canopy of tropical rain forests How much do we really know? In K. E. Linsenmair, A. J. Davis, B. Fiala, & M. R. Speight (Eds.), *Tropical Forest Canopies: Ecology and Management* (Vol. 69, pp. 87–107). Springer Netherlands. https://doi.org/10.1007/978-94-017-3606-0_8

Basset, Y., Hammond, P. M., Barrios, H., Holloway, J. D., & Miller, S. E. (2003). Vertical stratification of arthropod assemblages. *Arthropods of Tropical Forests*, 17–27.

Beck, J., McCain, C. M., Axmacher, J. C., Ashton, L. A., Bärtschi, F., Brehm, G., Choi, S.-W., Cizek, O., Colwell, R. K., Fiedler, K., Francois, C. L., Highland, S., Holloway, J. D., Intachat, J., Kadlec, T., Kitching, R. L., Maunsell, S. C., Merckx, T., Nakamura, A., … Novotny, V. (2017). Elevational species richness gradients in a hyperdiverse insect taxon: A global meta-study on geometrid moths. *Global Ecology and Biogeography*, *26*(4), 412–424. https://doi.org/10.1111/geb.12548

Bellone, D., Björkman, C., & Klapwijk, M. J. (2020). Top-down pressure by generalist and specialist natural enemies in relation to habitat heterogeneity and resource availability. *Basic and Applied Ecology*, *43*, 16–26. https://doi.org/10.1016/j.baae.2019.10.005

Bodner, F., Brehm, G., & Fiedler, K. (2015). Many caterpillars in a montane rain forest in Ecuador are not classical herbivores. *Journal of Tropical Ecology*, *31*(5), 473–476.

Boppré, M. (2008). Adult Lepidoptera are not parasitised—Or are they. *Antenna*, *32*, 26–27.

Brakefield, P. M. (2009). Crypsis. In *Encyclopedia of insects* (pp. 236–239). Elsevier. https://www.sciencedirect.com/science/article/pii/B9780123741448000710

Butterill, P. T., & Novotny, V. (2015). Gall-forming insects in a lowland tropical rainforest: Low species diversity in an extremely specialised guild. *Ecological Entomology*, *40*(4), 409–419. https://doi.org/10.1111/een.12198

Canale, A., Benelli, G., & Lucchi, A. (2013). Female-borne cues affecting *Psyttalia concolor* (Hymenoptera: Braconidae) male behavior during courtship and mating. *Insect Science*, *20*(3), 379–384. https://doi.org/10.1111/j.1744-7917.2012.01516.x

Carle, E. (2016). *The Very Hungry Caterpillar*. Penguin.

Carlyle-Moses, D. E., & Gash, J. H. C. (2011). Rainfall Interception Loss by Forest Canopies. In D. F. Levia, D. Carlyle-Moses, & T. Tanaka (Eds.), *Forest Hydrology and Biogeochemistry: Synthesis of Past Research and Future Directions* (pp. 407–423). Springer Netherlands. https://doi.org/10.1007/978-94-007-1363-5_20

Carroll, L. E., White, I. M., Freidberg, A., Norrbom, A. L., Dallwitz, M. J., & Thompson, F. C. (2002). Pest fruit flies of the world. *DELTA–DEscription Language for Taxonomy. Retrieved from Http://Www. Delta-Intkey. Com/Www/Data. Htm*. https://www.delta-intkey.com/ffa/refs.htm

Clarke, A.R, Balagawi, S., Clifford, B., Drew, R., Leblanc, L., Mararuai, A., McGuire, D., Putulan, D., Romig, T., Sar, S., & Tenakanai, D. (2004). Distribution and Biogeography of Bactrocera and Dacus Species (Diptera: Tephritidae) in Papua New Guinea. *Austral Entomology*, *43*, 148–156

Clarke, A. R., Harris, C., Kay, B. J., Mainali, B. P., McLay, L. K., Strutt, F., & Cunningham, J. P. (2022). Opiine parasitoids (Hymenoptera: Braconidae) and biological control of fruit flies (Diptera: Tephritidae) in Australia: Past, present and future. *Annals of Applied Biology*, *180*(1), 44–72. https://doi.org/10.1111/aab.12724

Clarke, A. R., Powell, K. S., Weldon, C. W., & Taylor, P. W. (2011). The ecology of Bactrocera tryoni (Diptera: Tephritidae): what do we know to assist pest management? *Annals of Applied Biology*, *158*(1), 26–54. https://doi.org/10.1111/j.1744-7348.2010.00448.x

Colazza, S., Peri, E., Salerno, G., & Conti, E. (2009). Host Searching by Egg Parasitoids: Exploitation of Host Chemical Cues. In F. L. Consoli, J. R. P. Parra, & R. A. Zucchi (Eds.), *Egg Parasitoids in Agroecosystems with Emphasis on Trichogramma* (pp. 97–147). Springer Netherlands. https://doi.org/10.1007/978-1-4020-9110-0_4

Colwell, R. K., Gotelli, N. J., Ashton, L. A., Beck, J., Brehm, G., Fayle, T. M., Fiedler, K., Forister, M. L., Kessler, M., Kitching, R. L., Klimes, P., Kluge, J., Longino, J. T., Maunsell, S. C., McCain, C. M., Moses, J., Noben, S., Sam, K., Sam, L., … Novotny, V. (2016). Midpoint attractors and species richness: Modelling the interaction between environmental drivers and geometric constraints. *Ecology Letters*, *19*(9), 1009–1022. https://doi.org/10.1111/ele.12640

Connahs, H., Aiello, A., Van Bael, S., & Rodríguez-Castañeda, G. (2011). Caterpillar abundance and parasitism in a seasonally dry versus wet tropical forest of Panama. *Journal of Tropical Ecology*, *27*(1), 51–58.

Corff, J. Le., & Marquis, R. J. (1999). Differences between understorey and canopy in herbivore community composition and leaf quality for two oak species in Missouri. *Ecological Entomology*, *24*(1), 46–58. https://doi.org/10.1046/j.1365-2311.1999.00174.x

Dahl, C., Richards, S. J., Basien, I., Mungkaje, A. J., & Novotny, V. (2024). Local and regional diversity of frog communities along an extensive rainforest elevation gradient in Papua New Guinea. *Biotropica*, *56*(1), 90–97. https://doi.org/10.1111/btp.13283

de Araújo, W. S., & Maia, V. C. (2021). Topological structure of a tritrophic network composed of host plants, gall-inducing insects and parasitoids in a restinga area in Brazil. *Entomological Science*, *24*(2), 201–216. https://doi.org/10.1111/ens.12468

De Moraes, C. M., Lewis, W. J., Pare, P. W., Alborn, H. T., & Tumlinson, J. H. (1998). Herbivore-infested plants selectively attract parasitoids. *Nature*, *393*(6685), 570–573.

de Souza Amorim, D., Brown, B. V., Boscolo, D., Ale-Rocha, R., Alvarez-Garcia, D. M., Balbi, M. I. P. A., de Marco Barbosa, A., Capellari, R. S., de Carvalho, C. J. B., Couri, M. S., de Vilhena Perez Dios, R., Fachin, D. A., Ferro, G. B., Flores, H. F., Frare, L. M., Gudin, F. M., Hauser, M., Lamas, C. J. E., Lindsay, K. G., … Rafael, J. A. (2022). Vertical stratification of insect abundance and species richness in an Amazonian tropical forest. *Scientific Reports*, *12*(1), Article 1. https://doi.org/10.1038/s41598-022-05677-y

Diamond, S., & Kingsolver, J. (2010). Fitness consequences of host plant choice: A field experiment. *Oikos*, *119*, 542–550. https://doi.org/10.1111/j.1600-0706.2009.17242.x

Dianzinga, N. T., Moutoussamy, M., Sadeyen, J., Ravaomanarivo, L. H. R., & Frago, E. (2020). The interacting effect of habitat amount, habitat diversity and fragmentation on insect diversity along elevational gradients. *Journal of Biogeography*, *47*(11), 2377–2391. https://doi.org/10.1111/jbi.13959

Diniz, I. R., Hay, J. D., Rico-Gray, V., Greeney, H. F., & Morais, H. C. (2012). Shelter-building caterpillars in the cerrado: Seasonal variation in relative abundance, parasitism, and the influence of extra-floral nectaries. *Arthropod-Plant Interactions*, *6*(4), 583–589. https://doi.org/10.1007/s11829-012-9207-2

Drew, R. (2004). Biogeography and Speciation in the Dacini (Diptera: Tephritidae: Dacinae). *Bishop Museum Bulletin in Entomology*, *12*.

Drew, R. A. I., & Romig, M. C. (2013). *Tropical Fruit Flies (Tephritidae Dacinae) of South-East Asia: Indomalaya to North-West Australasia*. CABI.

Espírito-Santo, M. M., & Fernandes, G. W. (2007). How Many Species of Gall-Inducing Insects Are There on Earth, and Where Are They? *Annals of the Entomological Society of America*, *100*(2), 95–99. https://doi.org/10.1603/0013-8746(2007)100[95:HMSOGI]2.0.CO;2

Fernandes, G. W., Almada, E. D., & Carneiro, M. A. A. (2010). Gall-inducing insect species richness as indicators of forest age and health. *Environmental Entomology*, *39*(4), 1134–1140.

Fischer, S., Samietz, J., Wäckers, F., & Dorn, S. (2001). Interaction of vibrational and visual cues in parasitoid host location. *Journal of Comparative Physiology A*, *187*(10), 785–791. https://doi.org/10.1007/s00359-001-0249-7

Forbes, A. A., Bagley, R. K., Beer, M. A., Hippee, A. C., & Widmayer, H. A. (2018). Quantifying the unquantifiable: Why Hymenoptera, not Coleoptera, is the most speciose animal order. *BMC Ecology*, *18*(1), 21. https://doi.org/10.1186/s12898-018-0176-x

Gagné, R. J., & Jaschhof, M. (n.d.). *Cecidomyiidae (Diptera) of the World*. Retrieved April 17, 2024, from https://www.ars.usda.gov/ARSUserFiles/80420580/Gagne_Jaschhof_2021_World_Cat_5th_Ed.pdf

Gentry, G. L., & Dyer, L. A. (2002). On the conditional nature of neotropical caterpillar defenses against their natural enemies. *Ecology*, *83*(11), 3108–3119. https://doi.org/10.1890/0012-9658(2002)083[3108:OTCNON]2.0.CO;2

Ghazanfar, M., Malik, M. F., Hussain, M., Iqbal, R., & Younas, M. (2016). Butterflies and their contribution in ecosystem: A review. *Journal of Entomology and Zoology Studies*, *4*(2), 115–118.

Godfray, H. C. J. (2016). Four decades of parasitoid science. *Entomologia Experimentalis et Applicata*, *159*(2), 135–146. https://doi.org/10.1111/eea.12413

González, G., Willig, M. R., & Waide, R. B. (2013). Ecological gradient analyses in a tropical landscape: Multiples perspectives and emerging themes. *Ecological Bulletins*, *54*, 13–20.

Greeney, H. F. (2009). A revised classification scheme for larval hesperiid shelters, with comments on shelter diversity in the Pyrginae. *Journal of Research on the Lepidoptera*, *41*(2002), 53–59.

Greeney, H. F., Dyer, L. A., & Smilanich, A. M. (2012). Feeding by lepidopteran larvae is dangerous: A review of caterpillars' chemical, physiological, morphological, and behavioral defenses against natural enemies. *Invertebrate Survival Journal*, *9*(1), 7–34.

Grimbacher, P. S., & Stork, N. E. (2007). Vertical stratification of feeding guilds and body size in beetle assemblages from an Australian tropical rainforest. *Austral Ecology*, *32*(1), 77–85. https://doi.org/10.1111/j.1442-9993.2007.01735.x

Hajek, A. E., & Eilenberg, J. (2018). *Natural enemies: An introduction to biological control*. Cambridge University Press. https://books.google.com/books?hl=en&lr=&id=Y-RfDwAAQBAJ&oi=fnd&pg=PR9&dq=-%09Hajek,+A.E.+and+Eilenberg,+J.,+2018.+Natural+enemies:+an+introduction+to+biological+control.+Cambridge+University+Press.&ots=ixpnnXGaEQ&sig=e1izoXUO9KKNBkWCAgXoGpEmKZs

Hawkins, B. A. (1994). *Pattern and process in host-parasitoid interactions*. Cambridge University Press. https://www.cabdirect.org/cabdirect/abstract/19941107169

Hawkins, B. A., & Cornell, H. V. (1994). Maximum Parasitism Rates and Successful Biological Control. *Science*, *266*(5192), 1886–1886. https://doi.org/10.1126/science.266.5192.1886

Hawkins, B. A., & Gagné, R. J. (1989). Determinants of assemblage size for the parasitoids of Cecidomyiidae (Diptera). *Oecologia*, *81*(1), 75–88. https://doi.org/10.1007/BF00377013

He, Y., Xu, Y., & Chen, X. (2023). Biology, Ecology and Management of Tephritid Fruit Flies in China: A Review. *Insects*, *14*(2), 196. https://doi.org/10.3390/insects14020196

Henneman, M. L. (2008). Host Location in Parasitic Wasps. In J. L. Capinera (Ed.), *Encyclopedia of Entomology* (pp. 1862–1863). Springer Netherlands. https://doi.org/10.1007/978-1-4020-6359-6_1407

Heraty, J. (2017). Parasitoid Biodiversity and Insect Pest Management. In R. G. Foottit & P. H. Adler (Eds.), *Insect Biodiversity* (1st ed., pp. 603–625). Wiley. https://doi.org/10.1002/9781118945568.ch19

Hodkinson, I. D. (2005). Terrestrial insects along elevation gradients: Species and community responses to altitude. *Biological Reviews*, *80*(3), 489–513. https://doi.org/10.1017/S1464793105006767

Hrcek, J., Miller, S. E., Whitfield, J. B., Shima, H., & Novotny, V. (2013). Parasitism rate, parasitoid community composition and host specificity on exposed and semi-concealed caterpillars from a tropical rainforest. *Oecologia*, *173*, 521–532.

Huan, L. I. U., ZHANG, D., XU, Y., Lei, W., CHENG, D., QI, Y., Ling, Z., & Yongyue, L. U. (2019). Invasion, expansion, and control of Bactrocera dorsalis (Hendel) in China. *Journal of Integrative Agriculture*, *18*(4), 771–787.

Iziomon, M. G., & Mayer, H. (2002). Characterisation of the shortwave radiation regime for locations at different altitudes in south-west Germany. *Climate Research*, *20*(3), 203–209.

James, D. G. (2017). *The Book of Caterpillars: A Life-Size Guide to Six Hundred Species from Around the World*. University of Chicago Press.

Jeffries, M. J., & Lawton, J. H. (1984). Enemy free space and the structure of ecological communities. *Biological Journal of the Linnean Society*, *23*(4), 269–286.

Jones, M. T. (1999). *Leaf shelter-building and frass ejection behavior in larvae of Epargyreus clarus (Lepidoptera: Hesperiidae), the Silver-spotted Skipper* [PhD Thesis]. Georgetown University.

Kitson, J. J. N., Hahn, C., Sands, R. J., Straw, N. A., Evans, D. M., & Lunt, D. H. (2019). Detecting host–parasitoid interactions in an invasive Lepidopteran using nested tagging DNA metabarcoding. *Molecular Ecology*, *28*(2), 471–483. https://doi.org/10.1111/mec.14518

Körner, C. (2007). The use of 'altitude'in ecological research. *Trends in Ecology & Evolution*, *22*(11), 569–574.

Lampert, E. C., Dyer, L. A., & Bowers, M. D. (2010). Caterpillar Chemical Defense and Parasitoid Success: Cotesia congregata Parasitism of Ceratomia catalpae. *Journal of Chemical Ecology*, *36*(9), 992–998. https://doi.org/10.1007/s10886-010-9840-0

Le Cesne, M., Wilson, S. W., & Soulier-Perkins, A. (2015). Elevational gradient of Hemiptera (Heteroptera, Auchenorrhyncha) on a tropical mountain in Papua New Guinea. *PeerJ*, *3*, e978.

Leponce, M., Dejean, A., Mottl, O., & Klimes, P. (2021). Rapid assessment of the three-dimensional distribution of dominant arboreal ants in tropical forests. *Insect Conservation and Diversity*, *14*(4), 426–438. https://doi.org/10.1111/icad.12486

Lewis, O. T., Memmott, J., Lasalle, J., Lyal, C. H. C., Whitefoord, C., & Godfray, H. C. J. (2002). Structure of a diverse tropical forest insect–parasitoid community. *Journal of Animal Ecology*, *71*(5), 855–873. https://doi.org/10.1046/j.1365-2656.2002.00651.x

Lewis, W. J., Sheehan, W., & Tumlinson, J. H. (1994). *Unravelling the story of how parasitoids find their hosts.* https://www.cabidigitallibrary.org/doi/full/10.5555/19951107125

Libra, M., Tulai, S., Novotny, V., & Hrcek, J. (2019). Elevational contrast in predation and parasitism risk to caterpillars in a tropical rainforest. *Entomologia Experimentalis et Applicata*, *167*(11), 922–931. https://doi.org/10.1111/eea.12851

Lichter-Marck, I. H., Wylde, M., Aaron, E., Oliver, J. C., & Singer, M. S. (2015). The struggle for safety: Effectiveness of caterpillar defenses against bird predation. *Oikos*, *124*(4), 525–533. https://doi.org/10.1111/oik.01515

LoPresti, E. F., & Morse, D. H. (2013). Costly leaf shelters protect moth pupae from parasitoids. *Arthropod-Plant Interactions*, *7*(4), 445–453. https://doi.org/10.1007/s11829-013-9261-4

Low, C. (2008). Grouping increases visual detection risk by specialist parasitoids. *Behavioral Ecology*, *19*(3), 532–538.

Lowman, M. D. (2009). Canopy research in the twenty-first century: A review of arboreal ecology. *Tropical Ecology*, *50*(1), 125.

Marquis, R. J., & Koptur, S. (2022). *Caterpillars in the Middle: Tritrophic Interactions in a Changing World*. Springer Nature. https://books.google.com/books?hl=en&lr=&id=OXtsEAAAQBAJ&oi=fnd&pg=PR10&dq=caterpillars+in+the+middle&ots=Qrw3iuBjTg&sig=aGmNBFiCOk4gynmP8hruuaYKrxs

McCain, C. M., & Grytnes, J.-A. (2010). Elevational gradients in species richness. *eLS*. https://citeseerx.ist.psu.edu/document?repid=rep1&type=pdf&doi=14b2e9494524f65d229e2274cc41e5617c84fc7d

Montgomery, StevenL. (1983). Carnivorous caterpillars: The behavior, biogeography and conservation of Eupithecia (Lepidoptera: Geometridae) in the Hawaiian Islands. *GeoJournal*, *7*(6). https://doi.org/10.1007/BF00218529

Morehead, S. A., & Feener, D. H. (2000). Visual and Chemical Cues Used in Host Location and Acceptance by a Dipteran Parasitoid. *Journal of Insect Behavior*, *13*(4), 613–625. https://doi.org/10.1023/A:1007875921705

Moses, J., Fayle, T. M., Novotny, V., & Klimes, P. (2021). Elevation and leaf litter interact in determining the structure of ant communities on a tropical mountain. *Biotropica*, *53*(3), 906–919. https://doi.org/10.1111/btp.12914

Müller, C. B., & Brodeur, J. (2002). Intraguild predation in biological control and conservation biology. *Biological Control*, *25*(3), 216–223.

Murphy, S. (2005). Enemy-free space maintains swallowtail butterfly host shift. Proceedings of the National Academy of. *Proceedings of the National Academy of Sciences of the United States of America*, *101*, 18048–18052. https://doi.org/10.1073/pnas.0406490102

Murphy, S. M., Lill, J. T., Bowers, M. D., & Singer, M. S. (2014). Enemy-free space for parasitoids. *Environmental Entomology*, *43*(6), 1465–1474.

Nakamura, A., Kitching, R. L., Cao, M., Creedy, T. J., Fayle, T. M., Freiberg, M., Hewitt, C. N., Itioka, T., Koh, L. P., & Ma, K. (2017). Forests and their canopies: Achievements and horizons in canopy science. *Trends in Ecology & Evolution*, *32*(6), 438–451.

Noroozi, J., Talebi, A., Doostmohammadi, M., Rumpf, S. B., Linder, H. P., & Schneeweiss, G. M. (2018). Hotspots within a global biodiversity hotspot-areas of endemism are associated with high mountain ranges. *Scientific Reports*, *8*(1), 10345.

Ode, P. J. (2022). Caterpillars, Plant Chemistry, and Parasitoids in Natural vs. Agroecosystems. In R. J. Marquis & S. Koptur (Eds.), *Caterpillars in the Middle: Tritrophic Interactions in a Changing World* (pp. 395–423). Springer International Publishing. https://doi.org/10.1007/978-3-030-86688-4_12

O'Hara, J. E. (2008). Overview of the Tachinidae (Diptera). *Tachinidae Resources.* https://www.lsuagcenter.com/~/media/system/5/5/6/4/55646c0ef9404ad2073c46b006e13b51/tachinidae%20fliesdocx.docx

Ozaki, K., Yukawa, J., Ohgushi, T., & Price, P. W. (Eds.). (2006). *Galling Arthropods and Their Associates*. Springer Japan. https://doi.org/10.1007/4-431-32185-3

Paniagua, M. R., Medianero, E., & Lewis, O. T. (2009). Structure and vertical stratification of plant galler–parasitoid food webs in two tropical forests. *Ecological Entomology*, *34*(3), 310–320. https://doi.org/10.1111/j.1365-2311.2008.01079.x

Pickering, B. J., Duff, T. J., Baillie, C., & Cawson, J. G. (2021). Darker, cooler, wetter: Forest understories influence surface fuel moisture. *Agricultural and Forest Meteorology*, *300*, 108311. https://doi.org/10.1016/j.agrformet.2020.108311

Powell, W., & Poppy, G. (2001). Host location by parasitoids. *Insect Movement. Mechanisms and Consequences. Oxon, CABI Publishing*, 111–128.

Prasad, K. V. H. (2022). Factors Affecting Growth and Metabolism of Insects. In K. V. H. Prasad (Ed.), *Insect Ecology: Concepts to Management* (pp. 23–35). Springer Nature. https://doi.org/10.1007/978-981-19-1782-0_5

Quicke, D. L. (2014). *The braconid and ichneumonid parasitoid wasps: Biology, systematics, evolution and ecology*. John Wiley & Sons.

Quicke, D. L. J., Ghafouri Moghaddam, M., & Butcher, B. A. (2023). Dietary Challenges for Parasitoid Wasps (Hymenoptera: Ichneumonoidea); Coping with Toxic Hosts, or Not? *Toxins*, *15*(7), Article 7. https://doi.org/10.3390/toxins15070424

Rahbek, C., Borregaard, M. K., Antonelli, A., Colwell, R. K., Holt, B. G., Nogues-Bravo, D., Rasmussen, C. M. Ø., Richardson, K., Rosing, M. T., Whittaker, R. J., & Fjeldså, J. (2019). Building mountain biodiversity: Geological and evolutionary processes. *Science*, *365*(6458), 1114–1119. https://doi.org/10.1126/science.aax0151

Redfern, M. (2011). *Plant Galls (Collins New Naturalist Library, Book 117)*. HarperCollins UK.

Rivers, D. B. (2016). Parasitic Hymenoptera as forensic indicator species. *Forensic Analysis: From Death to Justice. London: IntechOpen Limited*, 67–83.

Rodovalho, S. R., Laumann, R. A., & Diniz, I. R. (2007). Ecological aspects of lepidopteran caterpillar parasitoids from Caryocar brasiliense Camb. (Caryocaraceae) in a cerrado sensu stricto of Central Brazil. *Biota Neotropica*, *7*, 239–243. https://doi.org/10.1590/S1676-06032007000300026

Ronquist, F., Nieves-Aldrey, J.-L., Buffington, M. L., Liu, Z., Liljeblad, J., & Nylander, J. A. (2015). Phylogeny, evolution and classification of gall wasps: The plot thickens. *PLoS One*, *10*(5), e0123301.

Rota, J., & Wagner, D. L. (2008). Wormholes, sensory nets and hypertrophied tactile setae: The extraordinary defence strategies of Brenthia caterpillars. *Animal Behaviour*, *76*(5), 1709–1713.

Sam, K., Koane, B., Bardos, D. C., Jeppy, S., & Novotny, V. (2019). Species richness of birds along a complete rain forest elevational gradient in the tropics: Habitat complexity and food resources matter. *Journal of Biogeography*, *46*(2), 279–290. https://doi.org/10.1111/jbi.13482

Sam, K., Koane, B., & Novotny, V. (2015). Herbivore damage increases avian and ant predation of caterpillars on trees along a complete elevational forest gradient in Papua New Guinea. *Ecography*, *38*(3), 293–300. https://doi.org/10.1111/ecog.00979

Schulze, C. H., Linsenmair, K. E., & Fiedler, K. (2001). Understorey versus canopy: Patterns of vertical stratification and diversity among Lepidoptera in a Bornean rain forest. In K. E. Linsenmair, A. J. Davis, B. Fiala, & M. R. Speight (Eds.), *Tropical Forest Canopies: Ecology and Management* (Vol. 69, pp. 133–152). Springer Netherlands. https://doi.org/10.1007/978-94-017-3606-0_11

Scolari, F., Valerio, F., Benelli, G., Papadopoulos, N. T., & Vaníčková, L. (2021). Tephritid fruit fly semiochemicals: Current knowledge and future perspectives. *Insects*, *12*(5), 408.

Seifert, C. L., Lamarre, G. P. A., Volf, M., Jorge, L. R., Miller, S. E., Wagner, D. L., Anderson-Teixeira, K. J., & Novotný, V. (2020). Vertical stratification of a temperate forest caterpillar community in eastern North America. *Oecologia*, *192*(2), 501–514. https://doi.org/10.1007/s00442-019-04584-w

Šigut, M., Šigutová, H., Šipoš, J., Pyszko, P., Kotásková, N., & Drozd, P. (2018). Vertical canopy gradient shaping the stratification of leaf-chewer–parasitoid interactions in a temperate forest. *Ecology and Evolution*, *8*(15), 7297–7311. https://doi.org/10.1002/ece3.4194

Singer, M. S., Carrière, Y., Theuring, C., & Hartmann, T. (2004). Disentangling Food Quality from Resistance against Parasitoids: Diet Choice by a Generalist Caterpillar. *The American Naturalist*, *164*(3), 423–429. https://doi.org/10.1086/423152

Sivault, E., Amick, P. K., Armstrong, K. N., Novotny, V., & Sam, K. (2023). Species richness and assemblages of bats along a forest elevational transect in Papua New Guinea. *Biotropica*, *55*(1), 81–94. https://doi.org/10.1111/btp.13161

Sivault, E., Kollross, J., Jorge, L. R., Finnie, S., Diez-Méndez, D., Garzon, S. F., Maraia, H., Lenc, J., Libra, M., Murakami, M., Nakaji, T., Nakamura, M., Sreekar, R., Sam, L., Abe, T., Weiss, M., & Sam, K. (2023). *Insectivorous birds and bats outperform ants in the top-down regulation of arthropods across strata of a Japanese temperate forest* (p. 2023.11.07.565745). bioRxiv. https://doi.org/10.1101/2023.11.07.565745

Smith, M. A., Wood, D. M., Janzen, D. H., Hallwachs, W., & Hebert, P. D. N. (2007). DNA barcodes affirm that 16 species of apparently generalist tropical parasitoid flies (Diptera, Tachinidae) are not all generalists. *Proceedings of the National Academy of Sciences*, *104*(12), 4967–4972. https://doi.org/10.1073/pnas.0700050104

Sobek, S., Tscharntke, T., Scherber, C., Schiele, S., & Steffan-Dewenter, I. (2009). Canopy vs. understory: Does tree diversity affect bee and wasp communities and their natural enemies across forest strata? *Forest Ecology and Management*, *258*(5), 609–615.

Souto-Vilarós, D., Houadria, M., Michalek, J., Sisol, M., Isua, B., Kuyaiva, T., Weiblen, G. D., Novotny, V., & Segar, S. T. (2020). Contrasting patterns of fig wasp communities along Mt. Wilhelm, Papua New Guinea. *Biotropica*, *52*(2), 323–334. https://doi.org/10.1111/btp.12763

Souto-Vilarós, D., Proffit, M., Buatois, B., Rindos, M., Sisol, M., Kuyaiva, T., Isua, B., Michalek, J., Darwell, C. T., Hossaert-McKey, M., Weiblen, G. D., Novotny, V., & Segar, S. T. (2018). Pollination along an elevational gradient mediated both by floral scent and pollinator compatibility in the fig and fig-wasp mutualism. *Journal of Ecology*, *106*(6), 2256–2273. https://doi.org/10.1111/1365-2745.12995

Stilmant, D., Van Bellinghen, C., Hance, T., & Boivin, G. (2008). Host specialization in habitat specialists and generalists. *Oecologia*, *156*(4), 905–912. https://doi.org/10.1007/s00442-008-1036-8

Stireman III, J. O. (2016). Community ecology of the ‘other’parasitoids. *Current Opinion in Insect Science*, *14*, 87–93.

Stireman, J. O., Dyer, L. A., & Greeney, H. F. (2017). Specialised generalists? Food web structure of a tropical tachinid-caterpillar community. *Insect Conservation and Diversity*, *10*(5), 367–384. https://doi.org/10.1111/icad.12238

Stireman, J. O., & Singer, M. S. (2003). What determines host range in parasitoids? An analysis of a tachinid parasitoid community. *Oecologia*, *135*(4), 629–638. https://doi.org/10.1007/s00442-003-1235-2

Stork, N. E., Stone, M., & Sam, L. (2016). Vertical stratification of beetles in tropical rainforests as sampled by light traps in North Queensland, Australia. *Austral Ecology*, *41*(2), 168–178. https://doi.org/10.1111/aec.12286

Straub, C. S., Ives, A. R., & Gratton, C. (2011). Evidence for a Trade-Off between Host-Range Breadth and Host-Use Efficiency in Aphid Parasitoids. *The American Naturalist*, *177*(3), 389–395. https://doi.org/10.1086/658177

Suzuki, T. N., & Sakurai, R. (2015). Bent posture improves the protective value of bird dropping masquerading by caterpillars. *Animal Behaviour*, *105*, 79–84.

Tito, R., Vasconcelos, H. L., & Feeley, K. J. (2020). Mountain Ecosystems as Natural Laboratories for Climate Change Experiments. *Frontiers in Forests and Global Change*, *3*. https://doi.org/10.3389/ffgc.2020.00038

Toko, P. S., Koane, B., Molem, K., Miller, S. E., & Novotny, V. (2023). Ecological trends in moth communities (Geometridae, Lepidoptera) along a complete rainforest elevation gradient in Papua New Guinea. *Insect Conservation and Diversity*, *16*(5), 649–657. https://doi.org/10.1111/icad.12663

Tvardikova, K., & Novotny, V. (2012). Predation on exposed and leaf-rolling artificial caterpillars in tropical forests of Papua New Guinea. *Journal of Tropical Ecology*, *28*(4), 331–341. https://doi.org/10.1017/S0266467412000235

van Lenteren, J. C., Alomar, O., Ravensberg, W. J., & Urbaneja, A. (2020). Biological Control Agents for Control of Pests in Greenhouses. In M. L. Gullino, R. Albajes, & P. C. Nicot (Eds.), *Integrated Pest and Disease Management in Greenhouse Crops* (pp. 409–439). Springer International Publishing. https://doi.org/10.1007/978-3-030-22304-5_14

Vetaas, O. R. (2021). Mountain biodiversity and elevational gradients. *Frontiers of Biogeography*, *13*(3). https://doi.org/10.21425/F5FBG54146

Volf, M., Klimeš, P., Lamarre, G. P., Redmond, C. M., Seifert, C. L., Abe, T., Auga, J., Anderson-Teixeira, K., Basset, Y., & Beckett, S. (2019). Quantitative assessment of plant-arthropod interactions in forest canopies: A plot-based approach. *PLoS One*, *14*(10), e0222119.

Wäckers, F. L., & Lewis, W. J. (1994). Olfactory and visual learning and their combined influence on host site location by the parasitoid Microplitis croceipes (Cresson). *Biological Control*, *4*(2), 105–112.

Weiss, M., Zimová, K., Mogia, M., & Novotny, V. (2024). Bark and ambrosia beetles on native and transplanted dead wood along an altitudinal tropical forest gradient. *Biotropica*, e13316. https://doi.org/10.1111/btp.13316

White, I. M., & Elson-Harris, M. M. (1992). *Fruit Flies of Economic Significance: Their Identification and Bionomics*. CAB International.

Willmott, K. R., Elias, M., & Sourakov, A. (2011). Two possible caterpillar mimicry complexes in Neotropical danaine butterflies (Lepidoptera: Nymphalidae). *Annals of the Entomological Society of America*, *104*(6), 1108–1118.

Woiwood, I., Reynolds, D. R., & Thomas, C. D. (2001). *Insect Movement: Mechanisms and Consequences: Proceedings of the Royal Entomological Society's 20th Symposium*. CABI. https://books.google.com/books?hl=en&lr=&id=YeEq-797_2YC&oi=fnd&pg=PA1&dq=insect+Movement:+Mechanisms+and+Consequences&ots=QHmTAyh6-u&sig=dbLcaEwvAMkOXFf84m39AfP4Ghs

Yamawaki, Y., Kainoh, Y., & Honda, H. (2002). Visual control of host pursuit in the parasitoid fly Exorista japonica. *Journal of Experimental Biology*, *205*(4), 485–492. https://doi.org/10.1242/jeb.205.4.485

Zemenick, A. T., Kula, R. R., Russo, L., & Tooker, J. (2019). A network approach reveals parasitoid wasps to be generalized nectar foragers. *Arthropod-Plant Interactions*, *13*(2), 239–251. https://doi.org/10.1007/s11829-018-9642-9

2.Results

Chapter 1

Assemblages of fruit flies (Diptera: Tephritidae) along an elevational gradient in the rainforests of Papua New Guinea

Sam Finnie, Katerina Sam, Maurice Leponce, Yves Basset, Dick Drew, Mark K. Schutze, Chris Dahl, Micah Damag, Mary Dilu, Bradley Gewa, Borenke Kaupa, Martin Keltim, Bonny Koane, Joseph Kua, Roll Lilip, Martin Mogia, Frank Philip, Bryan Ray, Legi Sam, Salape Tulai, Collin Uma, Ruma Umari, Joseph Valeba, Joachim Yalang, and Vojtech Novotny

Insect Conservation and Diversity 14, no. 3 (2021): 348-355.

DOI: 10.1111/icad.12456

(IF = 4.7)

Assemblages of fruit flies (Diptera: Tephritidae) along an elevational gradient in the rainforests of Papua New Guinea

Sam Finnie[1,2], Katerina Sam[1,2,*], Maurice Leponce[3,4], Yves Basset[1,2,5,6], Dick Drew[7], Mark K. Schutze[8], Chris Dahl[1], Micah Damag[9], Mary Dilu[10], Bradley Gewa[9], Borenke Kaupa[9], Martin Keltim[9], Bonny Koane[9], Joseph Kua[9], Roll Lilip[9], Martin Mogia[9], Frank Philip[9], Bryan Ray[9], Legi Sam[1], Salape Tulai[9], Collin Uma[9], Ruma Umari[9], Joseph Valeba[9], Joachim Yalang[9], and Vojtech Novotny[1,2]

[1] Biology Centre CAS, Institute of Entomology, Ceske Budejovice, 37005 Czech Republic

[2] University of South Bohemia, Faculty of Sciences, Ceske Budejovice, 37005 Czech Republic

[3] Biodiversity Monitoring & Assessment, Royal Belgian Institute of Natural Sciences, Brussels, Belgium

[4] Evolutionary Biology & Ecology, Université Libre de Bruxelles, Brussels, Belgium

[5] ForestGEO, Smithsonian Tropical Research Institute, Balboa, Ancon, Panama

[6] Maestria de Entomologia, Universidad de Panama, Panama

[7] School of Environment and Science, Griffith University, Queensland, Australia

[8] Queensland Department of Agriculture and Fisheries, Brisbane, Queensland, Australia

[9] The New Guinea Binatang Research Centre, Madang, Papua New Guinea

[10]deceased, formerly The New Guinea Binatang Research Centre, Madang, Papua New Guinea

Abstract

1. Papua New Guinea (PNG) has the greatest diversity of dacine fruit fly species (Diptera: Tephritidae: Dacinae) in the world, many of which are significant agricultural pests.
2. Although their taxonomy is relatively well known, there is limited research on their geographical and elevational distribution.

3. We undertook a survey of PNG's fruit fly fauna along a complete elevational gradient on Mt Wilhelm (175 – 3,700 m a.s.l.) to determine the elevational species distribution and turnover of fruit fly communities. Fruit flies were sampled using a selection of male parapheromone lures, including Zingerone, a new and promising male attractant.

4. In total, we collected 10,700 fruit flies representing 77 species. The total abundance and species richness of fruit flies decreased with increasing elevation. Species richness was similarly high at 175 and 200 m a.s.l. (30 and 27 species) and at 700 and 1,200 m a.s.l. (16 and 20 species respectively) but dropped suddenly to only two species at 1,700 m a.s.l. and to zero at all four sites above this elevation. There were no flies attracted exclusively to Zingerone. One species (*Bactrocera recurrens*) exhibited dramatic, temporal changes in abundance during the study period. Fruit fly assemblages were significantly influenced by both the lure type and elevation. Similarity of fruit fly communities decayed linearly with increasing elevational distance.

5. We concluded that the upper limit of fruit fly distribution in PNG occurs between 1,700 and 2,200 m a.s.l. and the centre of diversity occurs between 0 and 700 m above sea level.

Introduction

A number of tephritid fruit fly species are major agricultural pests with some causing significant losses in the yields of fruit and other plant crops every year (Carroll *et al.*, 2006). Throughout tropical Asia, the South Pacific and Australia the most significant tephritid pests are the Dacini (Tephritidae: Dacinae), specifically those belonging to genera *Bactrocera*, *Dacus*, and *Zeugodacus* (White & Elson-Harris, 1992). Studying these flies is therefore essential if we are to better understand their ecology and reduce the negative impacts that they have on agriculture.

Papua New Guinea (PNG), is extremely rich in tephritid species, containing the highest diversity of Dacinae in the world (Clarke *et al.*, 2004). Over 200 species in the genera *Bactrocera* Macquart (including *Zeugodacus*, which is now considered a separate genus; Virgilio *et al.*, 2015) and *Dacus* Fabricius have been described and more than sixty newly-discovered species are awaiting description (Drew & Romig, 2013; R.A.I. Drew, pers. comm.). Approximately 75% of the described species are endemic to PNG, with the remainder largely restricted to Papua, West Papua, the Solomon Islands, Torres Strait and far north Queensland (Clarke *et al*., 2004, Drew, 2004, Drew & Romig, 2013).

The taxonomy of fruit flies in PNG is relatively well known, largely due to the work of R.A.I. Drew (e.g. Drew, 1989, Drew & Romig, 2001, 2013) and A. R. Clarke (e.g. Clarke *et al.*, 2004). However, literature on their geographical distribution and elevational limits is scarce. Drew's (1989) monograph contains limited distributional data, where the majority of species are reported from only one or two lowland localities. To date large areas of PNG remain that are yet to be surveyed for tephritids.

Research on fruit fly communities at higher altitudes is also limited. In Papua New Guinea, an exceptional study by Clarke *et al*. (2004) showed that very few species were restricted to higher elevations, while a subset of lowland species also occurred at higher altitudes. Only *Zeugodacus daulus* (Drew, 1989) occurred in the highlands of PNG (Western Highlands, Eastern Highlands, and Simbu provinces) but was absent from lowland regions (Clarke *et al.*, 2004). In Tanzania, Guerts *et al*., (2012) found that four of the five most abundant species occurred more frequently at lower elevations (581 m a.s.l.) with the remaining species being most abundant at 1,650 m above sea level. They concluded that fruit fly survival with a restricted temperature tolerance and narrow host range was not possible along the entire 581-1,650 m elevational range studied.

The male attractants methyl eugenol (ME) and Cue lure have been successfully used to control and monitor target fruit fly pest populations over the last 50 years (Royer, 2015). Papua New Guinea has several pest fruit fly species most of which are known to respond to Cue lure (e.g. *Z. cucurbitae* and *B. trivialis*) or ME (e.g. *B. dorsalis*). However, over half of the Dacini are nonresponsive to these lures (Royer *et al.*, 2017). Zingerone or vanillyl acetone is a relatively new male attractant that has proven to be invaluable for attracting both non- or weakly responsive pests and non-pest fruit fly species both in Australia (Fay, 2012; Dominiak *et al.*, 2015; Hanssen, 2015; Royer, 2015) and PNG (Royer *et al.*, 2016, 2018). Zingerone has been shown to attract dacine species that are non-responsive to traditional Cue and ME lures. Royer *et al.*, (2018) captured five undescribed fruit fly species using Zingerone lures in PNG. Therefore, the use of Zingerone alongside other traditional attractants may be necessary to catch a higher number of species and consequently, a more accurate representation of the natural community.

Elevation trends in tropical insect communities and their ecological determinants are difficult to study due to insufficient taxonomic knowledge of tropical insects and low sampling effort. Focusing on a relatively well-known taxon of prominent pests, such as dacine fruit flies, with better than average taxonomic resolution and multiple bait attractants, is a promising and potentially profitable method of advancing our understanding of these communities along elevational gradients in rainforest ecosystems. We undertook the first survey of PNG's fruit fly fauna along a complete rainforest elevation gradient (175 – 3,700 m a.s.l.) to determine the elevational distribution of species and species similarity in fruit fly communities along an elevational gradient by placing a combination of different attractants at incremental altitudes along Mt. Wilhelm.

Materials and Methods

Study sites

Data on fruit-fly elevational distribution were collected during two surveys. The first was a short-term survey, part of the Our Planet Reviewed - Papua New Guinea project (OPR-PNG), (Leponce *et al.*, 2016), a large multi-taxa sampling exercise following the approach of IBISCA (Investigating the Biodiversity of Soil and Canopy Arthropods; Leponce *et al.*, 2012). The second was a long-term survey part of the Arthropod Initiative of the ForestGEO project (Anderson-Teixeira *et al.*, 2015). Both surveys shared the lowest lowland site of Wanang.

Short-term fruit fly sampling

The short-term study was performed on the slopes of Mt Wilhelm (4,509 m a.s.l.) in the Central Range and in Wanang Conservation Area (5°13.5’ S, 145°04.9’ E, 175 m a.s.l.) in Papua New Guinea (Tvardikova and Novotny, 2012, Leponce *et al.*, 2016). The complete Mt. Wilhelm rain forest gradient spanned from the lowland floodplains of the Ramu river (ca. 200 m a.s.l., 5° 44’ S 145° 20’ E) to the timberline (3,700 m a.s.l., 5° 47’ S 145° 03’ E; (Leponce *et al.*, 2016, Marki *et al.*, 2016, Sam *et al.*, 2015, Sam & Koane, 2014)). Mt Wilhelm represents a 30 km long transect with eight sites (**Table S1**), evenly spaced at 500 m elevational increments. Average annual precipitation is 3,288 mm in the lowlands, rising to 4,400 mm at 3,700 m a.s.l., with a distinct condensation zone around 2,500 – 2,700 m a.s.l.. Mean annual temperature (measured by our data loggers) decreases from 27.4°C at the lowland site to 8.4°C at the tree line at a constant rate of 0.54°C per 100 elevational metres.

Fruit flies were attracted by male parapheromone lures exposed in Steiner traps (Leponce *et al.*, 2016). Five plots (A, B, C, D, E) were established in a grid with the minimum distance between traps ≈100 m at each elevational study site and in Wanang (i.e. 9 sites with 5 plots each). Each Steiner trap was fitted with one of the three baits (Leponce *et al.*, 2016). Cue lure (50% [4-(*p*-acetoxyphenyl)-2-butanone]) was used in three traps (A-C), ME (50% [4-allyl-1,2-dimethoxybenzene-carboxylate]) in trap (D) and Zingerone (i.e. Vanillyl acetone, 50%, [4-(4-Hydroxy-3-methoxyphenyl)butan-2-one]) in trap (E). The traps also contained cotton balls soaked in a synthetic pyrethroid insecticide (Belltek Bifenthrin 1% EC, active compound Bifenthrin).

Steiner traps were suspended from tree branches in the forest understorey 1.5 m above the ground. The rope used to hang the trap was coated with Tanglefoot® glue to prevent ants from accessing the trap and removing the flies. We ensured that new gloves were used between handling different types of lure to avoid cross-contamination of attractants. All traps at all nine study sites (eight along elevational gradient and one in Wanang) were surveyed daily for 16 days.

All flies were layered in dry tissue paper and placed in carton boxes that were stored in a waterproof container lined with silica gel. Samples were identified to species by R.A.I. Drew, using Drew (1989) at the Environmental Futures Research Institute, Griffith University, Australia where they currently remain. Steiner traps for collecting fruit-flies amounted to a total of 720 trap-days (i.e. 16 collections from each trap x 5 traps/study site x 9 study sites) with three different types of baits: Cue, ME, and Zingerone - representing 432, 144 and 144 trap-days.

We used generalized linear models (function *glm,* (R Core Team, 2017) to analyse the effect of elevation and lure on the species richness and abundance of fruit flies. We used constrained canonical analysis (function *cca*, package *vegan*) to analyse effect of elevation and lure on the composition of fruit fly communities and function *orditorp* (package *vegan*) to plot species names in the graphic results (air = 0.8, which replaces part of the overlapping labels by points).

Long-term fruit fly sampling in Wanang

The Wanang site (5°13.5’ S, 145°04.9’ E, 175m a.s.l.) is located approximately 63 km north-east from the bottom of the Mt. Wilhelm transect (200 m a.s.l.). Long-term monitoring of fruit flies was initiated in 2014, with a slightly different protocol. We used McPhail traps (Agency, 2003); model from Biobest, (www.biobestgroup.com), baited with ME and Cue lures. At each sampling location we placed two traps, baited with wicks that contained one of each attractant as well as Malathion insecticide (Bugs for Bugs, Glenvale Qld 4350, Australia, www.bugsforbugs.com.au). Traps were set up at approximately 100 m and 400 m increments along a 500 m transect. Traps were placed in vegetation and not in direct sunlight, at 3-4 m from the ground. Ten such transects within and near the permanent ForestGEO plot of Wanang were set up (Anderson-Teixeira *et al.*, 2015), with a total of 20 traps that were at least 250m apart (the attraction range of baits is < 100-200m (Cunningham & Couey, 1986)). Traps were run for one week during the months of January, April, July and October between

July 2014 and October 2016 amounting to nine sampling weeks. A total of 20 x 7 x 9 = 1260 trap-days were obtained.

Results

Short-term fruit fly sampling

We collected 10,700 fruit flies representing 77 species over a total of 720 traps days (**Table S2**). The total abundance of fruit flies decreased with increasing elevation, with particularly sharp decline above 1,200 m a.s.l.. The total number of individuals collected was 6,722, 1,064, 398 and 11 respectively at 200, 700, 1,200 and 1,700 a.s.l. of the elevational gradient of Mt. Wilhelm. Only 2,505 individuals were trapped at the Wanang site (175 m a.s.l.) during the short-term survey.

Total species richness (pooled data per site, mean value per one Cue trap from plots A-C were considered) was similarly high at 175 and 200 m a.s.l. (30 and 27 species) and at 700 and 1,200 m a.s.l. (16 and 20 species respectively; **Fig. 1a**) but dropped suddenly to only two species at 1,700 m a.s.l. and to zero above this elevation. Mean daily number of species recorded per trap (averaged for the three different lures) was highest at the two lowland sites (175 and 200 m a.s.l.) and decreased steeply with increasing elevation (**Fig. 1b**).

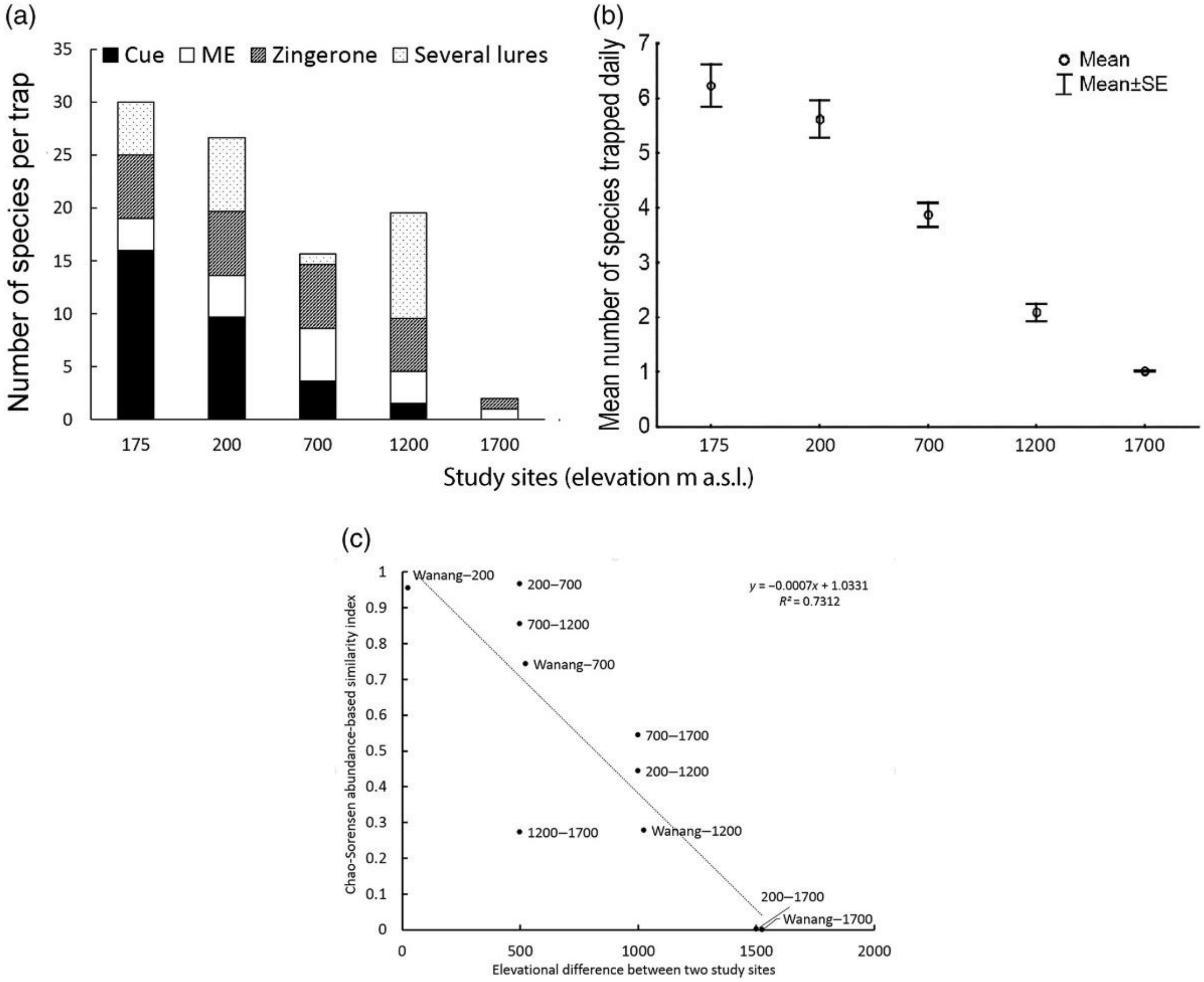

Figure 1. Number of species per trap after 16 days of sampling with Cue, methyl eugenol, or Zingerone lures, or >1 lures simultaneously **(a)**. Mean (±SE) number of species per trap and day, and lure **(b)**. Chao-Sorensen similarity decay with increasing elevational difference between pairs of study sites along the Mt. Wilhelm gradient (elevation in m a.s.l.) and Wanang site (175 m a.s.l. and 63 km distance from the 200 m a.s.l. site of the gradient) **(c)**.

The similarity of fruit-fly communities between the study sites (Chao-Sorensen abundance-based index, all lures combined) was highest between 200 and 700 m a.s.l. and between Wanang (175 m a.s.l.) and 200 m a.s.l. (**Fig. 1c**). The similarity then decreased with increasing elevational distance. Thirty-seven species were observed at a single study site, 17 at two sites, 10 at three and 13 at four elevation sites. Not a single species was found at all five study sites. The similarity between communities attracted to different lures was very low.

Composition of the fruit fly samples was significantly influenced by both type of lure ($F_{1,11}$ = 2.17, $P < 0.05$) and elevation ($F_{1,11}$ = 1.86, $P < 0.05$). Elevation and lure type explained 26.86% of the variance in the canonical analysis (**Fig. 2**). Relative success of different lure types also changed with elevation (**Fig. S3**). At lowland sites (200 and 175 m a.s.l) an average Cue lure trap (from plots A-C) attracted daily 11 and 17 individuals respectively (i.e. 60 – 65% of all individuals trapped daily per site, **Fig. 3**). Attractiveness of Cue lure for fruit flies then decreased to 2.2, 1.5 and 0% (of individuals per trap) at 700, 1,200 and 1,700 m a.s.l. respectively. Methyl eugenol was the most effective lure at 700 and 1700 m a.sl. (**Fig. 3**) while Zingerone was the most successful at 1200 m a.s.l. (**Fig. 3**). Cue lure at 200 m a.s.l. had unusually high standard deviation of the mean (61.5 in comparison to 0.4 – 8.9 for other lures at individual elevations). This was driven by an unusual abundance of *Bactrocera recurrens* with 1,154 individuals found in the three traps with Cue lure during the last day of sampling (9^{th} Nov 2012).

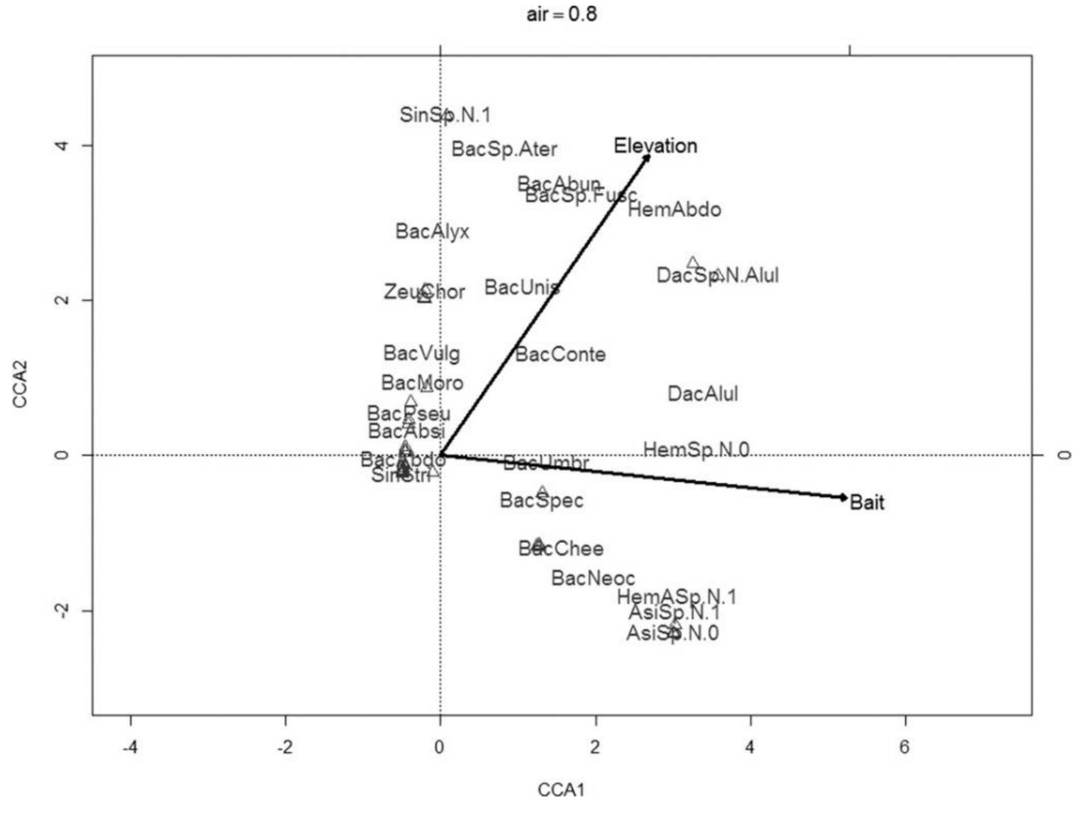

Figure 2. Ordination diagram for the canonical constrained analysis, where elevation and lure type were used as explanatory environmental variables. Function orditorp (air = 0.8) was used to declutter the graph (i.e. species which would otherwise overlap were replaced by a triangle). Species abbreviations as in **Table S2**.

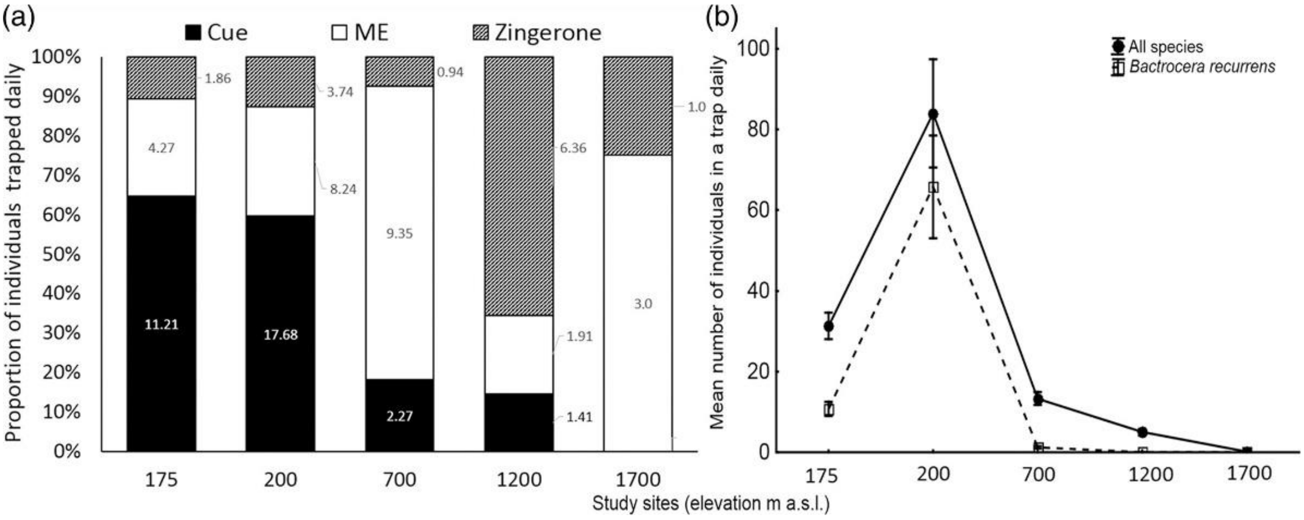

Figure 3. Mean proportion (%) and number of individuals (values inside bars) attracted daily per trap and lure **(a)**, and mean number of all individuals of all species and of *B. recurrens* attracted per trap **(b)**.

Based on the best model selected, both elevation and lure type had a significant effect on the number of species trapped daily, both individually and in combination (**Table S3**).. Cue was the most successful lure, attracting 5.46 ± SE 0.22 species, ME attracted 3.71 ± SE 0.36 and Zingerone attracted 2.33 ± SE 0.37 species per day in an average trap when elevations (200 – 1700 m a.s.l.) were pooled.

Three genera (*Bactrocera, Dacus* and *Zeugodacus*) were detected in our samples. *Bactrocera* were represented by 10,663 individuals while only 22 and 15 individuals belonged to the genera of *Zeugodacus* and *Dacus* respectively. Both genera were found at all study sites up to 1,700 m but *Dacus* species were attracted only to Zingerone. The majority of all collected individuals, 6,226 (58%) belonged to a single species – *Bactrocera recurrens* (Hering, 1941). We found an average of 109 (± 12.73 S.E.) *B. recurrens* individuals per Cue lure trap at 200 m a.s.l. per day in St. Wilhelm although the abundance increased over time (**Fig. S1**). Furthermore, we found an average of only 3 and 1.3 *B. recurrens* individuals daily at 700 and 1200 m a.s.l respectively. Contrastingly, we found an average of 17.9 (± 1.76 S.E.) individuals of this species in the Cue lure sample in Wanang where fluctuations in abundance over time were negligible (**Fig. S1**). The other two most abundant species represented less than 4% of all collected individuals were *Bactrocera musae* (n = 467) and *Bactrocera contermina* (n = 445). Twenty-four species out of 80 (i.e. 30%) were represented by less than three individuals. Sixty-one species were attracted to a single lure only. Five species were recorded both from ME and Cue lure, which is likely to be a result of contamination (**Table S2**, possible contaminations marked by asterisk).

Fifty-six of the 77 species were trapped from at least one of the lowland study sites (i.e. at or below 200 m a.s.l.). Twenty-six (or less than half of 77) species were found also at higher elevations (**Fig. 4, Table S2**). Twenty-one species were observed only above 700 m a.s.l. The species that only occurred at higher elevations usually had very low abundances (1-8 individuals in total). The exceptions were *Bactrocera* sp. n. nr *obliqua* (192 individuals), *Bactrocera* sp. n. nr *aterrima (*44 individuals) and *Bactrocera abdomininigra* (31 individuals).

Out of the 77 species recorded, only four are known to be pest species according to Carroll *et al.* (2006). These species were detected mostly at the lowest elevations (175, 200 and 700 m a.s.l.) with only two individuals trapped at 1200 m above sea level. *Bactrocera bryoniae* (Tryon) was trapped at 200 m and 700 m only (attracted to Cue), *B. musae* (Tryon) at 175 m and 200 m only (attracted to all three lures in our study but reported to be attracted only to ME by (Carroll *et al.*, 2006), *B. umbrosa* (Fabricius) between 175 m and 1200 m a.s.l. (attracted to ME), *B. trivialis* (Drew) between 175 m and 1200 m a.s.l. (attracted to Cue). No pest species were attracted exclusively to Zingerone.

Long-term fruit fly sampling in Wanang

Overall, 14,796 individuals were trapped and identified into 106 (morpho)species from the ForestGEO long-term sampling in Wanang. While species lists and trap design from both projects are not fully comparable (see Methods for details), at least 21 species occurred in both the long-term and short-term surveys (out of 106 and 44 species respectively). Species richness estimates based on long-term data were about 2.5 times higher than those based on short-term data (**Fig. S2**).

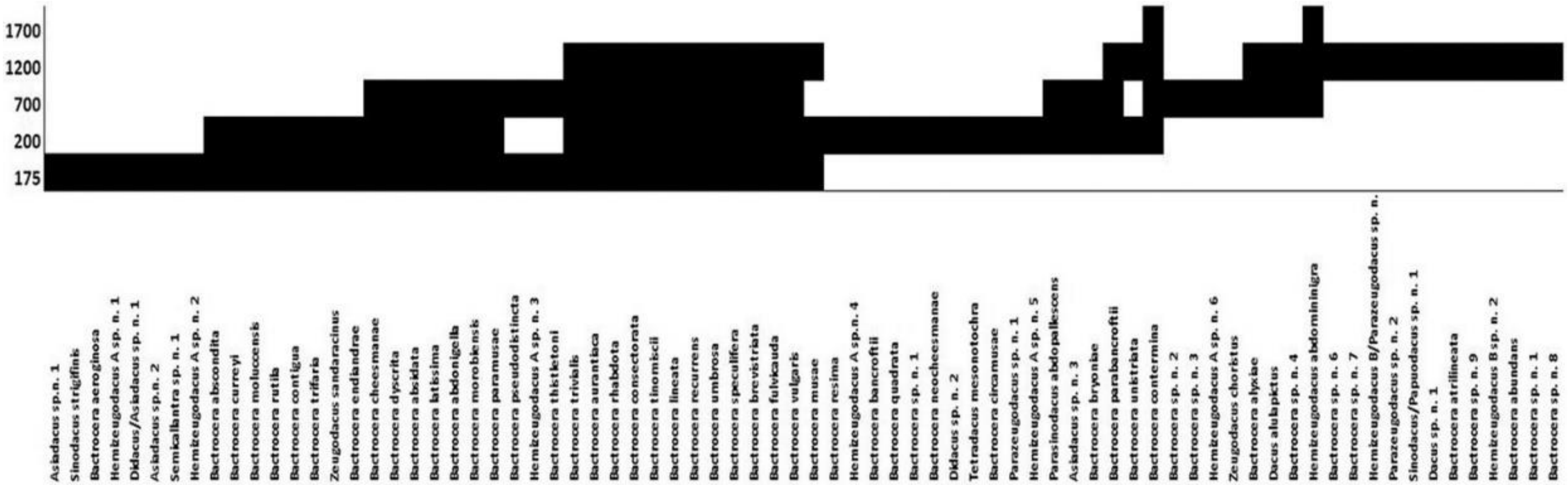

Figure 4. Distribution of the fruit fly species at the five study sites (175, 200, 700, 1200 and 1700 m a.s.l.) along the elevational gradient of Mt. Wilhelm.

Discussion

We detected a very steep decrease both in the species richness and abundance of fruit flies with increasing elevation in Papua New Guinea. We did not detect any fruit flies at elevations between 2,200 and 3,700 m above sea level, despite significant sampling effort at four sites.

Our results thus confirm the assumptions of Clarke *et al.* (2004) who did not sample at high-elevations (above 1,800 m a.s.l.) because they did not expect any species there. The vast majority of species (73%) only occurred at the sites with the lowest elevations (175 and 200 m a.s.l.). In total ≈ 50% of all species occurred only in the lowlands (defined as sites below 700 m a.s.l.). The rapid elevational decrease in the species richness of fruit flies and their entire absence in the upper montane forest in Mt. Wilhelm resembled a trend in ants (50 and 60 ant species, at 200 and 700 m a.s.l. respectively followed by a steep decrease towards 2,200 m a.s.l., (Colwell *et al.*, 2016)). Butterflies also exhibited a monotonous, but slower decrease with elevation from 150 species at 200 m a.s.l. to three species at 3,700 m a.s.l. (Colwell *et al.*, 2016). Conversely, Toko (unpublished) found that geometrid moths exhibited a different, unimodal, diversity pattern with peak diversity at 1200 m above sea level. Previous studies on herbivorous taxa along altitudinal gradients have found that although diversity decreases with altitude, it is never completely absent if the host plants are present (e.g. Colewell *et al.* 2016). However, this cannot be the constraining variable in this case as tephritid host plants (i.e. fruiting trees) are still present at 2200 m a.s.l. and above which may be indicative that instead, the elevational range of tephritids is constrained by thermal tolerance.

Similarity decay of fruit fly communities along the gradient was steep, with similarity typically above 70% between study sites that were 500 m apart and no common species shared between study sites 1,500 m a.s.l. apart. These results are also comparable with butterfly communities that exhibited zero species overlap between communities that were 2,000 m a. s. l. apart in Mt. Wilhelm (Legi Sam, unpublished). Geographical distance between the study sites had little effect on the fruit-fly community composition, at least in the lowlands where two study sites 63 km apart exhibited Chao-

Sorensen similarity > 0.9. These values are similar to those reported by Novotny *et al.* (2007) (Chao-Sorensen >0.95) for fruit fly communities in undisturbed Papua New Guinean rainforest separated by almost 1,000 km.

The decreasing abundance of fruit flies with increasing elevation was driven mainly by the most abundant species, *B. recurrens*, which was already known from the Madang region of Papua New Guinea. This result is consistent with previously published information (Drew & Romig, 2013). Temporal changes in the number of trapped individuals has been described for several species (Dominiak *et al.*, 2015); however, the increase in the number of individuals trapped has never been so high, fluctuating between 0 and 100 individuals for several species in traps checked fortnightly. The mechanism behind these fluctuations is unknown and any theories would be overly speculative, however, future research looking at how *B. recurrens* populations are influenced by environmental variables could prove to be insightful.

Species composition of fruit flies attracted to traps was strongly influenced by the type of lure used. We detected six species (**Table S2**) attracted to all lures, four species attracted to Cue and Zingerone, and five attracted to ME and Zingerone. Contrary to Royer *et al.* (2018), we did not find any species that were attracted exclusively to Zingerone. According to the literature, some ME-responsive species may be attracted to Zingerone and other Cue-responsive species may be attracted to Zingerone, yet there are currently no studies that found species that are responsive to both ME and Cue lure simultaneously. However, in our samples, *Bactrocera tinomiscii* was recorded from all lures (Cue 106, ME 26, Zingerone 1 individuals), and to date, this species has only ever been recorded as being attracted to Cue lure. We suspect that at least some of the observations of species crossover between different lures might be due to accidental cross-contamination. An alternative theory, however, is that there are one or multiple cryptic species similar to *B. tinomiscii* collected in the ME and Zingerone at the 200 m a.s.l. site (**Table S2**). We confirmed that the majority of the species and individuals were attracted to Cue lure, followed by ME and then Zingerone, with the latter two lures being more successful at higher elevations (1200 and 1700 m a.s.l.) than Cue. As there are significantly fewer tephritid species at higher elevations, the use of Zingerone as an attractant may only be necessary in elevational studies, although in this particular study, using just Cue lure and ME would have still yielded the same total number of species.

Papua New Guinea was reported to have several common pest fruit fly species most of which are known to respond to Cue lure (e.g. *Z. cucurbitae* and *B. trivialis* [Drew]) or ME (e.g. *B. dorsalis*) (Carroll *et al.*, 2006, Royer *et al.*, 2017). While our sampling confirmed the presence of *B. trivialis* (between 175 and 1200 m a.s.l.) and its attraction to Cue lure, we did not record the other two most common pests. We detected a further three minor pests (*Bactrocera bryoniae* – a pest of more than 300 plant species worldwide, *B. musae* – a major pest of some banana varieties, and *B. umbrosa* – a pest of genus *Artocarpus)* which are known to respond to male attractants at low elevations. We recorded *B. musae* up to 1,200 m a.s.l. which correlates with the current distribution of banana gardens in PNG, while the majority of species of genus *Musa* (native banana plants) grow naturally up to 950 m a.s.l. (Bourke, 1985). Distribution of *B. umbrosa* (up to 1,200 m a.s.l.), a pest of bread fruit (*Artocarpus altilis,* 0-1,500 m a.s.l) and jackfruit (*A. heterophyllus,* 0-100 m a.s.l.), also corresponded with the distribution of the host-plants in PNG (Yukawa, 1984). However, we did not record any other known minor non-responsive pest species, specifically we did not record *B. obliqua* (Malloch), *Z. atrisetosus* (Perkins) nor *Z. decipiens* (Drew) (Royer *et al.*, 2017). *Bactrocera obligua* is a known pest of several plants commonly distributed across PNG, including: guava (*Myrtaceae*), mountain apple (*Syzygium malaccense* (Myrtaceae) and water apple (*Syzygium aqueum*). *Zeugodacus atrisetosus* and *Z. decipiens* are pests of Cucurbitaceae which are planted along a wide range of elevations in PNG, generally up to 2,300 m a.s.l. (Bourke, 1985).

Species estimate curves (**Chao 1, Fig. S2**) were asymptotic for both long-term and short-term sampling. However, the estimated number of species differed significantly between the short-term and long-term surveys with long-term having much more species. We therefore argue that long-term surveys, or surveys of at least twice the sample size we have achieved here, are necessary for accurate sampling of fruit-fly communities and that their seasonality should be considered even in tropical areas where seasonal fluctuations are assumed to be low.

We found that fruit flies in PNG are likely to have an upper elevational range limit and distribution somewhere between the elevational band of 1,700 and 2,200 m above sea level. This study also confirmed that the centre of diversity for fruit-flies is in the lowlands (up to 700 m a.s.l.) and that the composition and structure of their communities changes significantly with increasing elevation. Additionally, we recorded previously unknown interactions between attractant lures and tephritid species. We found Zingerone to be useful attractant in higher elevations only, with Cue and ME yielding majority of the fruit flies in lowlands. Further, no pest species were attracted exclusively to Zingerone. We highlighted distinct temporal changes in the local abundance of *B. recurrens*, drastically different to those recorded in other studies and therefore prompting the need for future research.

Acknowledgements

We are thankful to villagers from Wanang, Kausi, Numba, Memeku, Degenumbu, Bananumbu, Sinopass, Bruno Sawmill, and Kegesugl for their approval for this research on their lands and for the assistance provided. We also thank the staff of the New Guinea Binatang Research Center for logistical support. The short-term study along the elevational gradient was conducted in the framework of “Our Planet Reviewed Papua-New-Guinea 2012-2013” set up by Pro-Natura International, the National Museum of Natural History (MNHN, France), the Institut de Recherche pour le Développement (IRD, France) in partnership with the Royal Belgian Institute of Natural Sciences, the New Guinea Binatang Research Center, the University of Papua New Guinea, and the Divine Word University of Madang and with core funding of Prince Albert II of Monaco Foundation, the Stavros Niarchos Foundation, the Total Foundation, the Fondation d’entreprise EDF, the Fonds Pacifique, Spiecapag, Entrepose Contracting, the New-Caledonia Government, the Reef Foundation, FNRS (Belgium) and the Belgian

National Lottery. The IBISCA expert network, the patron for this project, Prof. R.L. Kitching AM, and all other participants in this collective effort are thanked for their contribution. We express our gratitude to the Department of Environment and Conservation (DEC, Port Moresby) for issuing the permit # 012297, allowing us to export the material collected during this project. We also thank Biobest (www.biobestgroup.com) for providing us with McPhail Traps for the long-term monitoring of tephritids at Wanang. We acknowledge the Grant Agency of the Czech Republic project No. 18-23794Y (K. Sam) and 20-10205S (V. Novotny).

References

Agency, I.A.E. (2003) *Trapping guidelines for area-wide fruit fly programmes.* International Atomic Energy Agency Vienna, Austria.

Anderson-Teixeira, K.J. (2015) CTFS-ForestGEO: a worldwide network monitoring forests in an era of global change. *Global Change Biology,* **21,** 528-549.

Carroll, L.E., White, I.M., Freidberg, A., Norrbom, A.L., Dallwitz, M.J. & Thompson, F.C. (2006) *Pest fruit flies of the world.* <http://delta-intkey.com> 2nd April 2019.

Clarke, A.R., Balagawi, S., Clifford, B., Drew, R.A., Leblanc, L., Mararuai, A., McGuire, D., Putulan, D., Romig, T., Sar, S. and Tenakanai, D. (2004) Distribution and biogeography of Bactrocera and Dacus species (Diptera: Tephritidae) in Papua New Guinea. *Austral Entomology,* **43,** 148-156.

Colwell, R.K., Gotelli, N.J., Ashton, L.A., Beck, J., Brehm, G., Fayle, T.M., Fiedler, K., Forister, M.L., Kessler, M., Kitching, R.L. and Klimes, P. (2016) Midpoint attractors and species richness: Modelling the interaction between environmental drivers and geometric constraints. *Ecology letters,* **19,** 1009-1022.

Cunningham, R. & Couey, H. (1986) Mediterranean fruit fly (Diptera: Tephritidae): distance/response curves to trimedlure to measure trapping efficiency. *Environmental entomology*, **15,** 71-74.

Dominiak, B.C., Fay, H.A. & Fanson, B.G. (2015) Field evaluation of the efficacy of zingerone and other chemical lures on seven species of fruit fly in Sydney, New South Wales, Australia. *Plant Protection Quarterly*, **30,** 67.

Drew, R.A.I. (2004) Biogeography and speciation in the Dacini (Diptera: Tephritidae: Dacinae). *Bishop Museum Bulletin in Entomology,* **12,** 165-178.

Drew, R.A.I. & Hooper, G. (1983) Population studies of fruit flies (Diptera: Tephritidae) in south-east Queensland. *Oecologia*, **56,** 153-159.

Drew, R.A.I. & Romig, M.C. (1997) Overview-Tephritidae in the Pacific and Southeast Asia. ACIAR Proceedings, Australian Centre for International Agricultural Research, 46-53.

Drew, R.A.I. (1989) The tropical fruit flies (Diptera: Tephritidae: Dacinae) of the Australasian and Oceanian regions. *Memoirs of the Queensland Museum,* 26.

Drew, R.A.I. & Romig, M.C. (2001) The fruit fly fauna (Diptera: Tephritidae: Dacinae) of Bougainville, the Solomon Islands and Vanuatu. *Austral Entomology,* **40,** 113-150.

Drew, R.A.I. & Roming, M.C. (2013) *Tropical fruit flies (Tephritidae Dacinae) of South-East Asia: Indomalaya to North-West Australasia,* CABI.

Fay, H.A. (2012) A highly effective and selective male lure for *Bactrocera jarvisi* (Tryon) (Diptera: Tephritidae). *Austral Entomology,* **51,** 189-197.

Fletcher, B.S. (1998) Dacine fruit flies collected during the dry season in the lowland rainforest of Madang Province, Papua New Guinea (Diptera: Tephritidae). *Austral Entomology,* **37,** 315-318.

Geurts, K., Mwatawala, M., De Meyer, M., & Heckel, D. (2012Indigenous and invasive fruit fly diversity along an altitudinal transect in Eastern Central Tanzania. *Journal of Insect Science,* **12,** 12.

Hanssen, B.L. (2015) Synthesis and analysis of zingerone analogues as fruit fly attractants.

Leblanc, L., Balagawi, S., Mararuai, A., Putulan, D., & Clarke, A.R. (2001) Fruit flies in Papua New Guinea. *Pest advisory leaflet*.

Leponce, M., Corbara, B., & Bassett, Y. (2012) IBISCA - a collaborative programme to study the diversity and distribution of arthropods from canopy to forest floor,. *In:* LOWMAN M., S. T. F. J. (ed.) *Forest Canopy Methods, University of California Press.* University of California Press: University of California Press.

Leponce, M., Novotny, V., Pascal, O., Robillard, T., Legendre, F., Villemant, C., Munzinger, J., Molino, J.F., Drew, R., Odegaard, F. & Schmidl , J. (2016) Land module of Our Planet Reviewed - Papua New Guinea: aims, methods and first taxonomical results. *In:* Robillard T., Legendre F., Villemant C., & Leponce, M. (ed.) *Insects of Mount Wilhelm, Papua New Guinea.* Mémoires du Muséum national d'Histoire naturelle.

Marki, P.Z., Sam, K., Koane, B., Kristensen, J.B., Kennedy, J.D., & Jønsson, K.A. (2016) New and noteworthy bird records from the Mt. Wilhelm elevational gradient, Papua New Guinea. *Bulletin of the British Ornithologists' Club,* **137,** 263-271.

Novotny, V., Clarke, A.R., Drew, R.A., Balagawi, S., & Clifford, B. (2005). Host specialization and species richness of fruit flies (Diptera: Tephritidae) in a New Guinea rain forest. *Journal of Tropical Ecology,* **21,** 67-77.

Novotny, V., Miller, S.E., Hulcr, J., Drew, R.A., Basset, Y., Janda, M., Setliff, G.P., Darrow, K., Stewart, A.J., Auga, J. and Isua, B. (2007) Low beta diversity of herbivorous insects in tropical forests. *Nature,* **448,** 692-695.

R Core Team (2017) R: A language and environment for statistical computing.

Royer, J.E. (2015) Responses of fruit flies (Tephritidae: Dacinae) to novel male attractants in north Queensland, Australia, and improved lures for some pest species. *Austral Entomology,* **54,** 411-426.

Royer, J.E., Agovaua, S., Bokosou, J., Kurika, K., Mararuai, A., Mayer, D.G. and Niangu, B. (2017) Responses of fruit flies (Diptera: Tephritidae) to new attractants in Papua New Guinea. *Austral Entomology* **57**, 40-49.

Sam, K. & Koane, B. (2014) New avian records along the elevational gradient of Mt. Wilhelm, Papua New Guinea. *Bulletin of the British Ornithologists' Club,* **134,** 116-133.

Sam, K., Koane, B. & Novotny, V. (2015) Herbivore damage increases avian and ant predation of caterpillars on trees along a complete elevational forest gradient in Papua New Guinea. *Ecography,* **38,** 293-300.

Tan, K.H. & Nishida, R. (2000) Mutual reproductive benefits between a wild orchid, *Bulbophyllum patens*, and *Bactrocera* fruit flies via a floral synomone. *Journal of Chemical Ecology,* **26,** 533-546.

Tan, K.H. & Nishida, R. (2007) Zingerone in the floral synomone of *Bulbophyllum baileyi* (Orchidaceae) attracts *Bactrocera* fruit flies during pollination. *Biochemical Systematics and Ecology,* **35,** 334-341.

Tvardikova, K. & Novotny, V. (2012) Predation on exposed and leaf-rolling artificial caterpillars in tropical forests of Papua New Guinea. *Journal of Tropical Ecology,* **28,** 331-341.

Virgilio, M., Jordaens, K., Verwimp, C. & De Meyer, M. (2015) Higher phylogeny of frugivorous fruit flies (Diptera, Tephritidae, Dacini) as inferred from mitochondrial and nuclear gene fragments. *Molecular Phylogenetics and Evolution doi,* 10.

White, I.M. & Elson-Harris, M.M. 1992. *Fruit flies of economic significance: their identification and bionomics,* CAB International.

Yukawa, J. (1984) Fruit flies of the genus Dacus (Diptera: Tephritidae) on the Krakatau Islands in Indonesia, with special reference to an outbreak of *Dacus albistrigatus* De Meijere. *Japanese Journal of Ecology,* **34,** 281-288.

Zalucki, M., Drew, R. & Hooper, G. (1984) Ecological studies of Eastern Australian fruit flies (Diptera: Tephritidae) in their endemic habitat. *Oecologia,* **64,** 273-279.

Supplementary material

Table S1. Study sites, their elevation, location and mean daily temperature.

Study site name	Elevation (m a.s.l.)	Latitude	Longitude	Mean temperature (°C)
Wanang	175	05°15’10”S	145°16’01”E	26.0
Kausi	200	05°44’33”S	145°20’01”E	24.5
Numba	700	05°44’14”S	145°16’12”E	22.3
Memeku	1,200	05°43’18”S	145°16’17”E	19.5
Bananumbu	1,700	05°45’21”S	145°14’11”E	18.4
Sinopass	2,100	05°45’34”S	145°10’49”E	15.6
Bruno Sawmill	2,700	05°48’57”S	145°09’02”E	13.1
Kombuno Mambuno	3,200	05°48’18”S	145°04’20”E	9.9
Lake Piunde	3,700	05°47’10”S	145°03’32”E	7.9

Table S2. List of taxa (subgenus and species or morphospecies) and their abundance recorded at individual study sites and lures.

Subgenus	Species	Sp. Code	Cue Lure					Methyl eugenol					Zingerone				
			200	700	1200	1700	Wanang	200	700	1200	1700	Wanang	200	700	1200	1700	Wanang
Apodacus	*cheesmanae*	ApoChee						6	1			4	2				
Asiadacus	*sp. n. 2*	AsiSp.2															11
Asiadacus	*sp. n. 3*	AsiSp.3											14	1			
Asiadacus	*sp.n. 1*	AsiSp.1															2
Bactrocera	*abdonigella*	BacAbdo	19	14			150										
Bactrocera	*abscondita*	BacAbsc	1				3										
Bactrocera	*absidata*	BacAbsi	11	4			4										
Bactrocera	*abundans**	BacAbun			3					1					2		
Bactrocera	*aeroginosa*	BacAero					4										
Bactrocera	*alyxiae*	BacAlyx		2	1												
Bactrocera	*atriliniellata*	BacAtri			2												
Bactrocera	*aurantiaca*	BacAura	16	1	1		12										
Bactrocera	*bancroftii*	BacBanc						2									
Bactrocera	*brevistriata*	BacBrev	17	151	13		5										
Bactrocera	*bryoniae*	BacBryo	1	21													
Bactrocera	*circamusae*	BacCirc	19														
Bactrocera	*consectorata*	BacCons	7	2	2		5										
Bactrocera	*contermina*	BacConte						4	396	43						2	
Bactrocera	*contigua*	BacConti	3				141										
Bactrocera	*curreyi*	BacCurr	1				11										
Bactrocera	*dyscrita*	BacDysc	45	1			184										
Bactrocera	*endiandrae*	BacEndi						54				23					
Bactrocera	*fulvicauda**	BacFulv					6	106	52	14		84	2				1
Bactrocera	*latissima*	BacLati	147	12			7										

Bactrocera	*lineata*	BacLine	37	75	2		48						1	6			1
Bactrocera	*moluccensis*	BacMolu	1				31										
Bactrocera	*morobiensis*	BacMoro	9	17			6						1				
Bactrocera	*musae*	BacMusa						332		1		131	3				
Bactrocera	*neocheesmanae*	BacNeoc						3					2				

Table S2. Continuation

Subgenus	Species	Sp. Code	Cue Lure					Methyl eugenol					Zingerone				
			200	700	1200	1700	Wanang	200	700	1200	1700	Wanang	200	700	1200	1700	Wanang
Bactrocera	*parabancroftii*	BacParab	26	2	3												
Bactrocera	*paramusae*	BacParam	234	33			48										
Bactrocera	*pseudodistincta*	BacPseu		1			2										
Bactrocera	*quadrata*	BacQuad	2														
Bactrocera	*recurrens*	BacRecur	5262	99	4		860						1				
Bactrocera	*resima*	BacResi	1														
Bactrocera	*rhabdota*	BacRhab	3	3	1		12										
Bactrocera	*rutila*	BacRuti	3				5										
Bactrocera	*sp. n. 1 (nr. obliqua)*	BacSp.1			16					3				3	170		
Bactrocera	*sp. n. 2 (nr. rhabdota)*	BacSp.2		1													
Bactrocera	*sp. n. 3 (nr. strigifinis/univittata)*	BacSp.3		1													
Bactrocera	*sp. n. 4 (nr. obliqua)*	BacSp.4			8												
Bactrocera	*sp. n. 5*	BacSp.5											4				
Bactrocera	*sp. n. 6 (nr. Fuscouswings caliginosa)*	BacSp.6								1							
Bactrocera	*sp. n. 7 (nr. nigella)*	BacSp.7			1												
Bactrocera	*sp. n. 8 (nr. aterrima)*	BacSp.8			35					1					9		
Bactrocera	*sp. n. 9*	BacSp.9			3												
Bactrocera	*speculifera*	BacSpec						56	25	6		52					
Bactrocera	*thistletoni*	BacThis		1			8										
Bactrocera	*tinomiscii**	BacTino	36	6	1		63	26							1		
Bactrocera	*trifaria*	BacTrif	5				4										
Bactrocera	*trivialis*	BacTriv	12	1	1		322										
Bactrocera	*umbrosa*	BacUmbr						71	59	4		11					
Bactrocera	*unistriata*	BacUnis	1							2							
Bactrocera	*vulgaris*	BacVulg	42	62	13		21								2		
Dacus	*alulapictus*	DacAlul												2	1		
Dacus	*sp. n. 1 (nr. alulapictus)*	DacSp.1													1		
Didacus	*sp. n. 2*	DidSp.2											5				
Didacus/Asiadacus	*sp. n. 1*	DidSp.1															6
Hemizeugodacus	*A sp. n. 1*	HemASp.1												1			

Hemizeugodacus	*A sp. n. 2*	HemASp.2										1					162
Hemizeugodacus	*A sp. n. 3*	HemASp.3															5
Hemizeugodacus	*A sp. n. 4*	HemASp.4												1			4
Hemizeugodacus	*A sp. n. 5*	HemASp.5											24				
Hemizeugodacus	*A sp. n. 6*	HemASp.6											1				
Hemizeugodacus	*abdomininigra**	HemAbdo			1						9			2	19		

Table S2. Continuation

Subgenus	Species	Sp. Code	Cue Lure					Methyl eugenol					Zingerone				
			200	700	1200	1700	Wanang	200	700	1200	1700	Wanang	200	700	1200	1700	Wanang
Hemizeugodacus	*B sp. n. 2*	HemBSp.2													3		
Hemizeugodacus	*B/Parazeugodacus sp. n. 1*	HemBSp.1													1		
Parasinodacus	*abdopallescens*	ParAbdo	1	1													
Parazeugodacus	*sp. n. 1*	ParSp.1											19				
Parazeugodacus	*sp. n. 2*	ParSp.2													1		
Semicallantra	*sp. n. 1*	SemSp.1															33
Sinodacus	*strigifinis*	SinStri					2										
Sinodacus/Papuodacus	*sp. n. 1*	SinSp.1			1												
Tetradacus	*mesonotochra*	TetMeso											9				
Zeugodacus	*choristus*	ZeuChor		4													
Zeugodacus	*sandaracinus*	ZeuSand	13				9										
	Species richness		31	25	25	1	29	11	6	13	2	7	15	8	12	2	9
	Abundance		6176	1215	1323	1700	1976	859	1233	1265	1709	304	287	716	1410	1702	225

Table S3. Akaike's second-order information criterion (dAICc) for regression models of observed richness and abundance of fruit flies along Mt. Wilhelm.

Richness	**AIC**	**dAICc**	**df**
poly(Elevation) :Trap	1377.6	0	9
poly(Elevation) + Trap + poly(Elevation) :Trap	1380.3	2.6	7
poly(Elevation) +Trap	1406.7	28.8	5
Elevation + Trap + Elevation:Trap	1467.7	89.9	6
Elevation + Trap	1486.2	108.3	4
Elevation	1624.9	246.9	2
Trap	2148.2	770.2	3
Null	2286.8	908.1	1
Abundance			
poly(Elevation) :Trap	12613	0	9
poly(Elevation) + Trap + poly(Elevation) :Trap	12718	108.5	7
poly(Elevation) +Trap	13999	417.8	6
Elevation + Trap + Elevation: Trap	13031	1385.2	5
Elevation + Trap	14065	1461.9	4
Elevation	16604	3990.6	2
Trap	24876	12262.3	3
Null	27415	14808.1	1

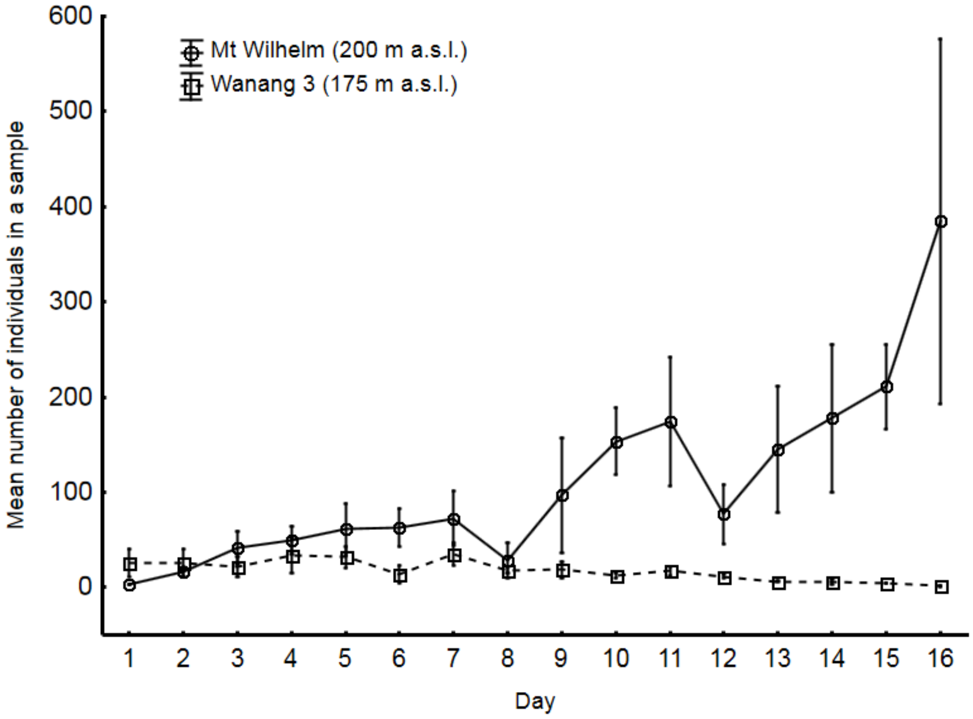

Figure S1. Mean number of individuals of *Bactrocera recurrens* trapped daily (days 1-16) per Cue lure trap (N = 3 per study site and day) at two lowland study sites in Papua New Guinea.

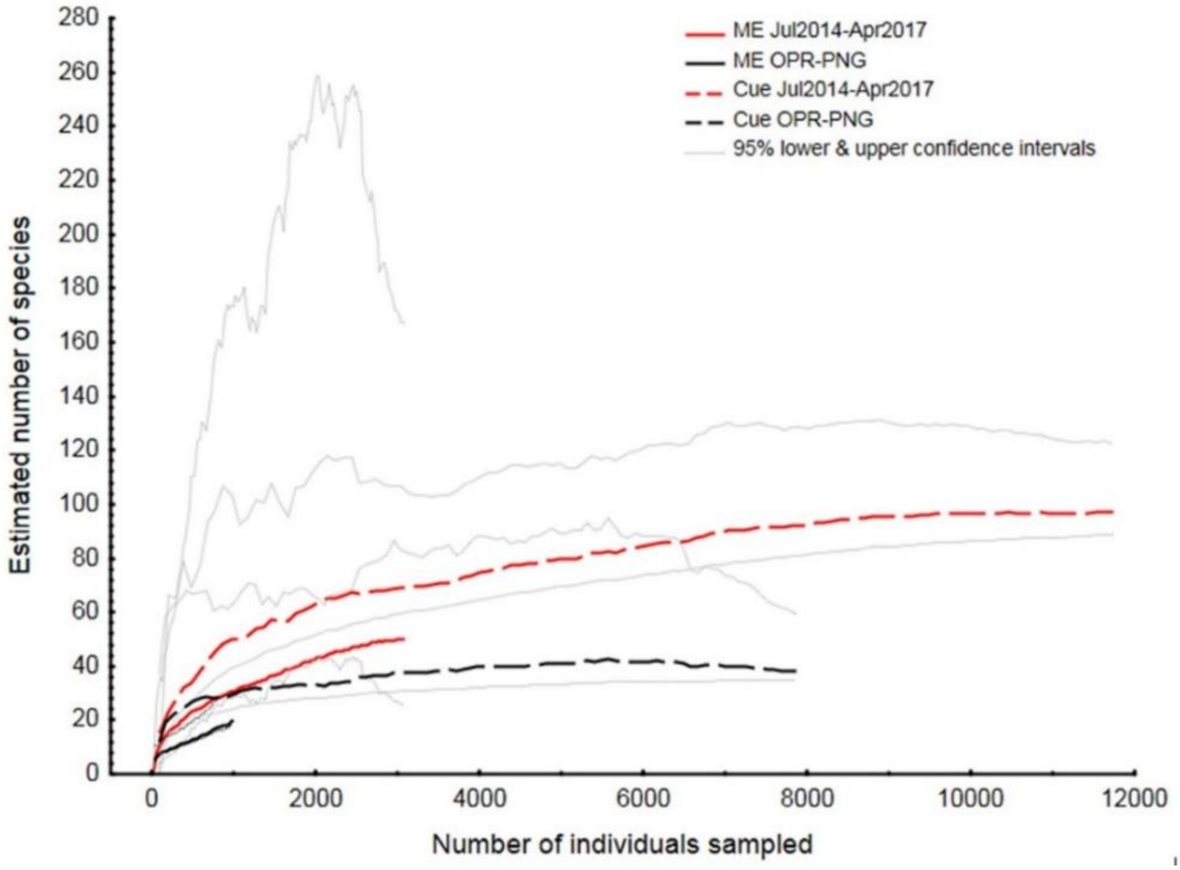

Figure S2. Chao 1 estimates (standard estimate ± 9% confidence intervals) of fruit fly species richness attracted to Cue and ME lure during the short-term (OPR-PNG) and long-term (Jul2014-Apr2017) ForestGEO surveys at Wanang, Papua New Guinea.

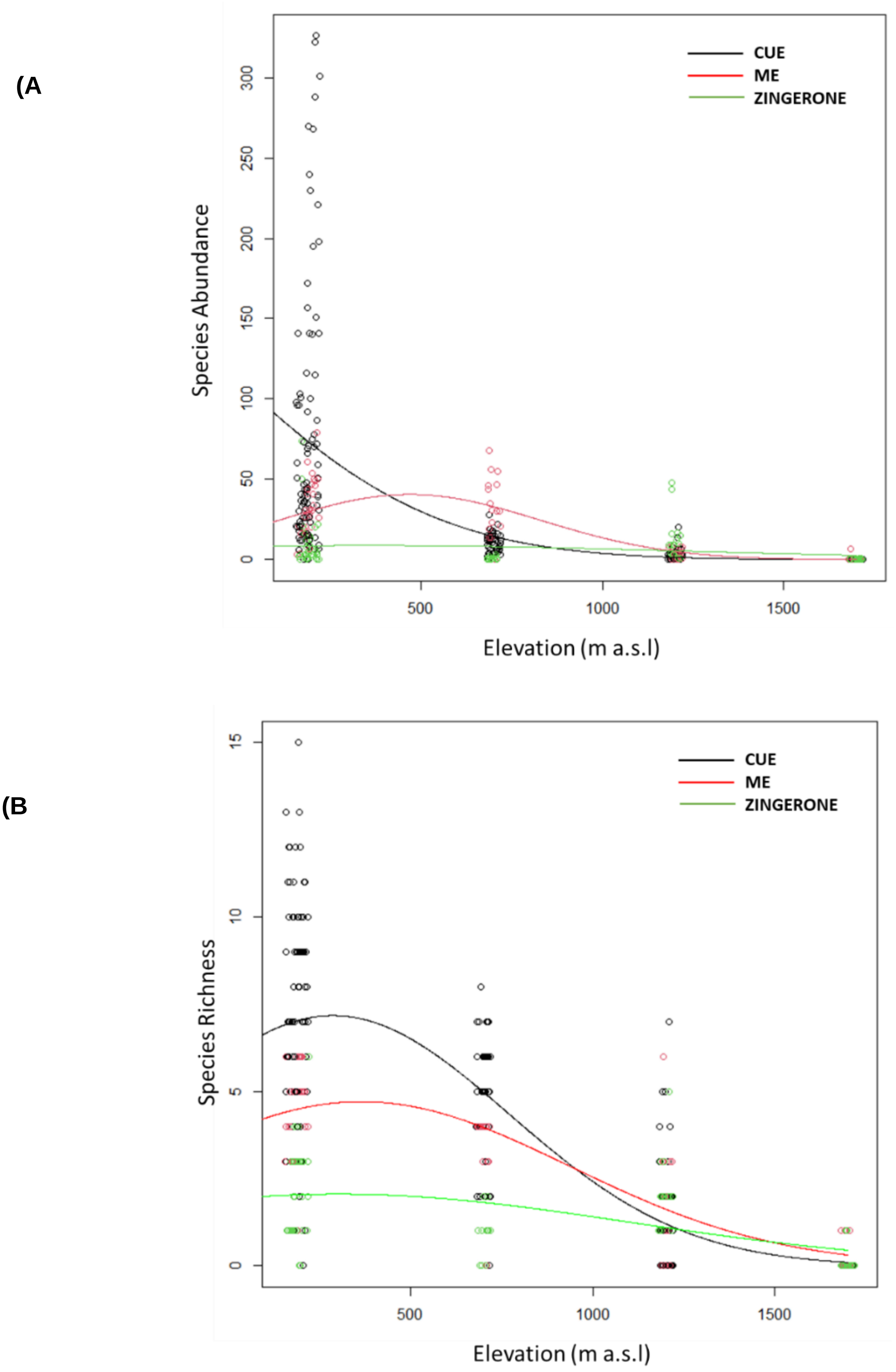

Figure S3. Scatter plots showing the relative abundance (**A**) and species richness (**B**) at different elevations. The lines represent the predicted trends resulting from the best model (see **Table S3**) for each lure type based. For graph (**A**), the outlying abundance of *Bactrocera recurrens (N = 824)* was omitted. Due to the similar elevations, data from Wanang (175 m a.sl.) was lumped with the data from 200 m a.sl. on Mt. Wilhelm for the purpose of these graphs.

Chapter 2

Gall apparency drives parasitoid richness in a highly specialised gall-parasitoid food web from a tropical rainforest

Philip T. Butterill, Sam Finnie, Katerina Sam, Tom Fayle, and Vojtech Novotny

Manuscript

Gall apparency drives parasitoid richness in a highly specialised gall-parasitoid food web from a tropical rainforest

Philip T. Butterill[1,2], Sam Finnie[1,2], Katerina Sam[1,2], Tom Fayle[1], Vojtech Novotny[1,2,3]

[1] University of South Bohemia, Faculty of Science, Branisovska 1760, 370 05 Ceske Budejovice, Czech Republic

[2] Biology Centre of the Czech Academy of Sciences, Institute of Entomology, Branisovska 31, 370 05 Ceske Budejovice, Czech Republic

[3] The New Guinea Binatang Research Centre, PO Box 604, Madang, Papua New Guinea

Abstract

1. Unravelling and understanding the complexity of interactions found in quantitative terrestrial food webs is one of the key challenges in ecology, especially in tropical habitats where reports on such networks are scarce in the published literature.
2. We sought to quantify the diversity and host specificity of the third trophic level (parasitoids) for a highly specialised guild of herbivorous insects – the gall formers. Further, we attempted to uncover potential drivers of parasitoid species richness using gall morphological traits and host plant phylogeny.
3. We sampled gall-forming insects from 32 targeted host plant species in tropical lowland rainforests of Papua New Guinea (PNG). We morphotyped the reared parasitoids and constructed, characterised, and tested a quantitative tri-trophic network for the potential for apparent competition (PAC). We used generalised linear models (GLMs) to test potential drivers of parasitoid species richness, such as gall apparency, gall forming taxon, and plant phylogeny.

4. Our interaction network included 102 species of parasitoid (820 individuals), 78 galling insect species, and 32 host plants. Monophagous species dominated both parasitoids and gallers, leading to high network specialisation, H2', which was 0.99 and 0.90 for the plant-gall and gall-parasitoid subwebs, respectively. Thus, the potential for apparent competition was minimal. One third of host gall species did not share any parasitoids and the remainder had a mean PAC of 0.07. The potential for intraspecific competition was greater, with a mean PAC of 0.76. After controlling for low host abundances, we found that gall apparency (i.e. conspicuousness) and gall-forming taxon significantly predicted parasitoid assemblage size. Specifically, highly apparent galls formed by gall midges (Cecidomyiidae) hosted a greater number of parasitoid species. We found no significant signal of parasitoid species richness in the plant phylogeny.
5. In our study, high network specialisation appears to be a structural property of a diverse plant-gall-parasitoid network in which specialist gall formers were attacked by specialist parasitoids. This is the largest such network, in terms of species and interaction count, reported from tropical rainforests to date.

Introduction

Studies of food webs have proliferated in recent years (Ings et al., 2009), culminating in a wealth of empirical networks (Araújo & Maia, 2021; Hrcek et al., 2013; Kaartinen & Roslin, 2011), meta-analyses (Joppa et al., 2009; Morris et al., 2014; Poisot et al., 2011), and metrics that measure various characteristics of web structure (Bersier et al., 2002; Blüthgen et al., 2006; Dormann et al., 2009). The importance of food web research is such that, in the future, food webs could provide the framework on which to reconcile the structure of biodiversity with ecosystem function. In this context, tritrophic networks, comprised of interactions on three trophic levels, are particularly valuable. They provide a comprehensive view of ecosystem dynamics by incorporating the effects of indirect interactions and top-down control mechanisms. Understanding these interactions can provide valuable insights into the mechanisms driving biodiversity, thereby enhancing our ability to predict and manage changes in ecosystem dynamics (Thompson et al., 2012).

Of fundamental importance in network ecology is specialisation and associated network statistics. In general, specialisation refers to the extent to which species within a network are restricted to particular dietary or habitat niches, either for individual species or between networks. The correlation between latitude, seasonality, and specialisation has long intrigued ecologists. It has traditionally been assumed that biotic specialisation is higher in more stable environments. Theories for this pattern include the idea that greater stability and reduced seasonality, such as in the tropics, lead to more stable population dynamics than those at higher latitudes, thereby allowing for narrower (and more specialised) feeding niches (MacArthur, 1972). Similarly, historically more constant environments reflect the time available for potential coevolution of species due to the temporal stability of local communities, which increases local adaptation and favours biotic specialisation (Dalsgaard et al., 2013; Schleuning et al., 2012). However, recent network studies have addressed the geographical variation in biotic specialisation and found inconclusive and highly idiosyncratic results (Moles & Ollerton, 2016).

One system that has been studied in relative depth, is the geographical variation in host-parasitoid specialisation. Similarly, there seems to be no general latitudinal pattern in host specificity (Galiana et al., 2019; Morris et al., 2014). For exposed insect hosts, Hawkins et al. (1990) found that parasitoids in the tropics were typically more host specific. On the other hand, Hopper et al. (2019) found no geographic pattern in parasitoid specialisation on aphids. These contrasting results indicate that patterns likely vary between host taxa and across different spatial scales.

One extremely diverse example of parasitoid host group is the gallers, these organisms pervade global ecosystems, existing wherever plants are found. Galling insects have the unique ability to manipulate plant tissues and induce gall formation. This ability is exclusive to them among insect herbivores (Miller & Raman, 2019). The close association between galling insects and their host plants leads to highly specialised interactions between these organisms (Araújo et al., 2019). The relationship between galling insects and their host plants extends to their interactions with parasitoids. The plant tissues manipulated to form galls provide a distinct ecological niche for parasitoids. However, despite the ubiquity of gall-forming insects, studies focusing on gall-parasitoid networks are still relatively rare, particularly in tropical regions (Cagnolo, 2018).

The specialisation of parasitoids in relation to their galling hosts has been shown to vary greatly. Studies from temperate regions find that parasitoids have a tendency towards being generalists (e.g. Chust et al., 2007; Kuzmanich et al., 2023; Maldonado-Lopez et al., 2023). Empirically, this appears to be the case where the taxonomic breadth of sampled host plants (Hawkins & Goeden, 1984) or herbivores (Nyman et al., 2007; Rott & Godfray, 2000; Schönrogge et al., 2000) are restricted to a single genus. However, when a greater diversity of host plants are sampled, monophagous parasitoids appear to be more prominent (Dawah et al., 1995; Leppänen et al., 2013; Memmott et al., 1994; Tscharntke et al., 2001), especially when conducted in the tropics (Araújo & Maia, 2021; López-Núñez et al., 2019; Luz et al., 2021; Paniagua et al., 2009).

Another distinction between the galler communities in tropical, compared to temperate regions, is the dominant galling taxa. In temperate regions, the most speciose group of galling insects are the gall wasps (Cynipidae), which are predominantly parasitised by generalist parasitoids (Askew, 1975; Hayward & Stone, 2005; Maldonado-López et al., 2022). Cynipid-induced galls are found almost exclusively on oak trees in the genus Quercus (Fagaceae) which mainly occur in the temperate zones of North America and Eurasia (Abe et al., 2007; Ronquist et al., 2015). In the tropics, however, where oaks are almost absent, gall midges (Cecidomyiidae) are most species rich taxon (Butterill & Novotny, 2015; Hanson & Nishida, 2014). Previous studies have found that cecidomyiids and cynipids are mostly host-specific, although the host range of cynipids is typically more varied (Carneiro et al., 2009; Araújo & Maia, 2021). However, the specificity of the parasitoids of these two galling taxa have never been directly compared. It has been suggested that highly specialised hosts are more likely to have specialised parasitoids (Van Oudenhove et al., 2017). This could indicate that networks dominated by cecidomyiid gallers harbour more specialised parasitoids than those with a higher abundance of cynipids.

In addition to galling taxa, parasitoid assemblages on gall hosts are influenced by other factors. One key factor is the ‘galled plant module’—the specific part of the plant, such as a leaf or stem, where the gall occurs. Gallers often specialise not just on host species, but also on particular modules, and these preferences are also exhibited by their parasitoids, with galls on certain modules attracting larger parasitoid assemblages (Prauchner & De Souza, 2024). Another is gall apparency, where a combination of size, structure and colouration of a gall, can make it more visually conspicuous to parasitoids (Joseph et al., 2011). Different gallers induce the formation of highly characteristic galls and the association between plants and gallers is highly specific, to the point that it is possible to identify the gall species based on the plant species and gall morphology alone (Redfern et al., 2002; Russo, 2006). The Enemy Hypothesis (Price et al., 1987) posits that top-down parasitoid pressure on gall hosts drives gall morphology, leading to larger, harder, or more elaborate forms that should prohibit or reduce overall parasitism. However, such adaptations do not guarantee enemy-free space (Price et al., 1987; Stone & Schönrogge, 2003). On the contrary, the formers of highly conspicuous galls support some of the richest parasitoid faunas known (Askew, 1980; Price et al., 1987), suggesting that visual cues may play a pivotal role for gall parasitoids when locating their hosts, which has been shown in previous studies (Graziosi, 2015).

Another important factor in shaping patterns of parasitoid diet breadth and species richness is host plant phylogeny. For example, a herbivore's host plant choice can influence the vulnerability of that insect to natural enemies (Lill et al., 2002). In their study, Lill et al. (2002) showed that most parasitoids were specialised to a single caterpillar species feeding on congeneric host plant species, despite the fact that the insect also fed on other plant genera, thus suggesting the third trophic level as a potentially important selective top-down force in evolutionary terms. Conversely, an influence of host plant phylogeny on parasitoid assemblages was observed in lepidopteran leaf miners (Ives & Godfray, 2006; Lopez-vaamonde et al., 2005). However, in a system of specialised herbivorous aphids and a parasitoid introduced as a biocontrol agent, parasitoid abundance was found to be influenced by the aphid phylogeny, but not the host plant phylogeny (Desneux et al., 2012).

A characteristic of food webs that is intrinsically linked to specialisation is the potential for indirect interactions among species, such as apparent competition. This allows us to calculate the effects on herbivores mediated by shared enemies (Frank Van Veen et al., 2006; Morris et al., 2004). The potential impact of apparent competition increases with the proportion of generalist (parasitoids with more than one host species) parasitoids in the food web, whereas specialists (parasitoids with a single host species) may impose top-down density-dependent regulation on their hosts. In both cases, the likelihood of an effect depends on the intensity of attack (Frank Van Veen et al., 2006; Morris et al., 2004).

In this study, we analyse a tritrophic network from a large host plant-gall-parasitoid food web from a tropical Papua New Guinean rainforest. We aim to adress the following questions and hypotheses:

(1) How specialised are the parasitoids in a community of gall-forming insects in our tropical system and how does this affect the potential for apparent competition between gallers? [H1a] We hypothesise that a greater proportion of parasitoids will be monophagous specialists. This will be due to the equatorial location of our sampling site, with previous studies finding predominantly specialist gall parasitoids in tropical regions (Luz et al., 2021; Paniagua et al., 2009). [H1b] The preponderance of specialists within our system will minimise the potential for interspecific competition but increase the potential for intraspecific competition.

(2) Do gall characteristics drive parasitoid richness in our system? [H2] We hypothesise that the size of the parasitoid assemblage will be significantly influenced by factors such as host plant

phylogeny, gall-forming taxa, and galled plant module. And specifically, that more apparent galls will host more parasitoid species than inconspicuous galls.

Materials and Methods

Study sites

The study was carried out in the vicinity of Madang, Madang Province, Papua New Guinea (PNG). Sampling took place in the villages of Baitabag, Mis, and Ohu (145°41–7 E, 5°08 –14 S, 0–200 m asl), each of which are surrounded by approximately 200 ha of mixed secondary and primary rainforest. The mean annual rainfall in the Madang area is 3,558 mm with minor seasonal variation and the mean air temperature is 26.5°C, varying little throughout the year (McAlpine et al., 1983).

Target host plants

Thirty-two species of locally common woody angiosperms were chosen such that all the major angiosperm clades (plus a single gymnosperm) were represented. Four plant species hosted zero galls between them and they were therefore removed from analyses in this study. The selection of 28 species included 14 congenerics, 6 confamilials, and 8 species from additional families (**Table S1**).

Sampling protocol

Sampling took place over an eight month period from August 2010 to March 2011. Sample effort was recorded as the time spent searching the foliage of each plant species for galls, which was approximately proportional to the leaf area examined. Each plant species was sampled with uniform effort, amounting to 13 sampling events (5 in Mis, and 4 each in Baitabag and Ohu) per species. A single event comprised approximately six hours of foliage searching on individuals of the target species. Therefore, total sampling effort per host species, in terms of time, was ~ 78 hours (13 events x 6 hours per event). The sample dates of each host species evenly across the sampling period, in order to mitigate for any seasonal effects.

Due to the impracticality of counting individual galls, gall abundance was measured as the number of plant organs galled (i.e. the number of galled leaves, stems, petioles etc.). Galled modules were counted exactly from one to ten, and then categorised as either between 11-100 or >100. Only galls formed by, or strongly suspected to be formed by insects were sampled, thus fungal, bacterial, and mite galls were ignored.

Morphotyping and rearing

Galls were morphotyped based on a combination of gall morphology and host plant. We then took photographs, and reared the galls in clear plastic “bread” bags for a minimum period of one month. Each rearing bag was filled to no more than one-third capacity with galled plant material to minimise condensation and not impede the observation of emergent insects. We imposed a limit of six rearing bags per morphospecies per sample unit to keep collections to a manageable number, and discarded any excess galled plant material. Rearing bags were checked for emergent insects every 1-2 days.

All emergent insects were transferred to vials of 95% EtOH for storage, except for any Lepidoptera, which were pinned. Besides rearings, we carried out ad-hoc dissections of galls to yield immature stages and parasitoids for potential DNA barcoding, and to aid in gall former identification. We morphotyped gall formers as in Butterill & Novotny (2015). We sorted reared parasitoids (Hymenoptera) to family before sending them to taxonomists for identification (see **Table S2** for species listings and Acknowledgements). Our network probably includes facultative or obligate hyperparasitoids, but we could not identify them due to the need for a more in-depth biological understanding of the system that was not practical in this study. Therefore, our network reflects total parasitism. For brevity, we refer to all morphospecies as “species" from here on. For the purposes of this study, we categorised all parasitoids that we found to parasitise a single host species as ‘specialists’, and those that we found to parasitise two or more host species as ‘generalists’.

Data analysis

Food-web statistics

We measured species- and network-wide specialisation using the indices d' and H2', respectively, as defined by Bluthgen et al. (2006). These indices yield values between 0 for extreme generalisation and 1 for extreme specialisation. We used the species-level index, d', to measure the exclusivity or complementarity of interactions. We interpreted this as the "deviation of the actual interaction frequencies from a null model which assumes that all partners are used in proportion to their availability" (Blüthgen et al., 2006). However, this index could potentially underestimate specialisation by treating a rare species (that could be truly specialised) on a commonly visited host as a generalist because it conformed to the null model. Importantly, we found both indices to be scale-independent, making them ideal for comparisons with other networks.

Other web metrics referred to in this study include vulnerability and generality, the mean numbers of parasitoid species per host and host species per parasitoid, respectively; linkage density, the mean of vulnerability and generality; interaction evenness, Shannon's evenness of interactions; network asymmetry, the balance between the numbers of species in each trophic level; number of compartments, the number of subwebs disconnected from the rest of the web; compartment diversity, a measure of compartment size heterogeneity; specialisation asymmetry, based on log-transformed specialisation values of species-level specialisation, d', for each species, where a positive value indicates higher specialisation of the higher trophic level (for further details, see Blüthgen, 2010; Blüthgen et al., 2007; Dormann et al., 2009).

Indirect interactions

We calculated the potential for apparent competition using the PAC function of the Bipartite package in R (Dormann et al., 2009), based on Müller et al. (1999). We created a H x H matrix of standardised values between zero and one, *dij,* where H is the number of host species, and *i* & *j* are individual host species. We define the term *dij* as the probability that a parasitoid attacking species *i* developed on species *j* (Morris et al., 2005). When *i* & *j* are the same species, i.e. the diagonal cells in the matrix, *dij* represents apparent intraspecific competition.

Gall traits

We characterised galls based on aspects of their appearance and the taxon forming the gall (**Table 1**). We based gall apparency on a combination of physical size and visual similarity (**Table 3**), representing how visually conspicuous or distinctive the gall was from the surrounding plant tissue. For example, we would put a large gall that was highly similar to the surrounding plant tissue in the apparency category 'MID'. We would place open galls, such as hemipteran pit galls, which are highly similar to the leaf tissue on which they are formed, in the similarity category 'MID'. This is because, when occupied by a nymph, they become visually distinct from the leaf itself and may serve as visual cues for parasitoids.

Table 1. Categories of gall traits or taxa, utilised as explanatory variables in GLMs. Size and similarity were not used in GLMs, since apparency was derived from them (see **Table 3**).

Trait name	Description	Factor levels
Apparency	Gall conspicuousness, based on size and similarity (see **Table 3**)	LOW, MID, HIGH
Size	Size of gall	SMALL, MID, LARGE
Similarity	Similarity of galled tissue to regular plant tissue	LOW, MID, HIGH
Former	The gall-forming taxon. CECI = Cecidomyiidae, HEMI = Hemiptera, OTHE = Others, UNKN = Unknown. Others includes Thysanoptera, Lepidoptera, Hymenoptera and Agromyzidae (Diptera)	CECI, HEMI, OTHE, UNKN
Module	The type of plant module galled: LEAF = leaves; OTHER = non-leaf modules; COMP = complex: multiple module types galled	LEAF, OTHER, COMP
Pubescence	Whether gall surface was covered in hairs or not	HAIRY, NOT HAIRY
Openness	Whether the gall had some form of opening (TRUE) or was fully enclosed (FALSE)	TRUE, FALSE

Table 3. Categories of gall apparency, designated according to categories of gall size and similarity (to the surrounding plant tissue).

Apparency		Similarity HIGH	 MID	 LOW
Size	SMALL	LO	LO	MID
	MID	LO	MID	HI
	LARGE	MID	HI	HI

The number of parasitoid species reared is clearly a function of the abundance of galls sampled – galls found in high numbers are more likely to yield parasitoids than infrequently sampled galls. The response variable (number of parasitoid species), therefore, contained many zeros as a consequence of scarce gall species found in low abundances. To mitigate this effect, we included the natural log of host gall abundance as a covariate in every model prior to simplification (note that gall abundance reflects the minimum number of galled plant modules per species, and not absolute numbers of either plant modules or individual galls). Log-transforming gall abundance helped to stabilise residuals in the models and reduced the leverage of certain data points, as well as reducing AIC. Using generalised linear models (GLMs), we modelled each explanatory variable separately with the abundance covariate, before combining them together in a full model. A negative binomial error distribution accounted for overdispersion in the original Poisson GLM, caused by high numbers of zeros in the response, and was preferred as a procedural step to 'quasipoisson' since it retains the AIC as a selective tool. We then carried out model simplification to establish whether the trait or covariate could be dropped from the model. The explained deviance (e.d.) of each model was calculated using the formula (Zuur et al., 2009).

$$e.d. = 100\left(\frac{null\ deviance - residual\ deviance}{null\ deviance}\right)$$

Finally, we produced scatterplots of the number of parasitoid species versus host gall abundance with fitted lines for each trait factor level, using predicted values from the m.

Host plant phylogeny

We used an ultrametric phylogenetic tree of the 28 host plant species that hosted galls to test for phylogenetic signal of mean parasitoid species richness. For details of the construction of the tree, which utilised branch length adjustment (Butterill & Novotny 2015). A randomisation test for phylogenetic signal was used, which compares observed trait data with data that have been randomly permuted across the tips of the tree (Blomberg et al., 2003).

Software

Data were analysed in R (R Core Team, 2013). Negative binomial GLMs were implemented with the glm.nb function in package MASS (Ripley et al., 2013). The test for phylogenetic signal was implemented using Phylocom (Webb et al., 2008) and the package, picante (Kembel et al., 2010). Network statistics were calculated in R package, bipartite (Dormann et al., 2009). The tripartite diagram (**Fig. 1**) was programmed in Python (Python Software Foundation) using package NetworkX (Hagberg et al., 2008)

Results

We reared a conservative estimate of 8,150 galled plant modules yielding 820 individual parasitoids of 102 species. Parasitoids were reared from 42 (out of 78) host gall species on 28 (out of 32) host plant species. Individuals and species in the parasitoid community were dominated by Chalcidoidea (92.4% & 83.3% respectively), followed by Platygastroidea (3.8% & 9.8%), Ichneumonoidea (3.4% & 3.9%), and Ceraphronoidea (0.4% & 2.9%). Eulophidae accounted for 46% of all species and 40% of individuals. By traditional definition, 81 of the 102 (79%) parasitoid species in the network were specialists, feeding on only a single host, a figure significantly boosted by the number of rare species observed (37 singletons and 16 doubletons). After the removal of singletons and doubletons, 44 out of 65 (68%) and 33 out of 49 (67%) monophagous specialists remained, respectively. Even after removing all parasitoid species with abundances of ten or below, the network remained dominated by monophages with 11 out of 22 (50%) species. When the complementary specialisation index, d' (Blüthgen et al., 2006), was applied to the parasitoid species in the network, 61% had a value of greater than 0.5, indicating a greater propensity for specialisation than generalisation. High specialisation at the species level informed network-wide specialisation, which was at the extreme end of the specialisation spectrum (H2' = 0.89).

Food web statistics

In the entire tritrophic web (**Fig. 1**), there were 5 discrete compartments that contained members from each trophic level. One large compartment comprised 158 species nodes, and the remaining four between 3-13 nodes. In the gall-parasitoid sub-network, there were 17 separate compartments: a main one with 98 species nodes, and sixteen smaller compartments with between 2-6 nodes. Thus, the main tritrophic compartment represents ~ 75% of species in the whole network. Five gall-parasitoid compartments were species pairs with a single exclusive interaction.

Indirect interaction.

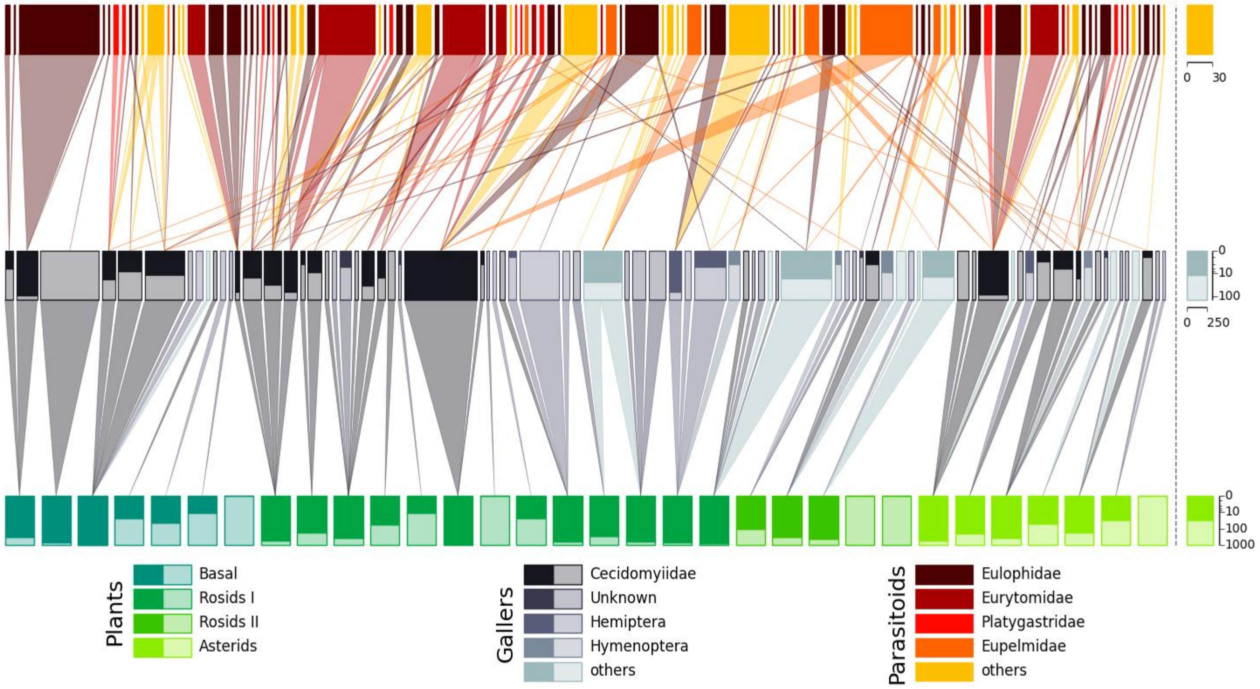

Figure 1. Quantitative tripartite network for a community of host plant species (lower level), galling insect species (middle levels), and parasitoid species (top level). Node (species) widths and interaction segments are proportional to the relative abundance per trophic level, i.e. uniform sampling of host plants, the minimum abundance of galled plant modules (see Methods for details), and parasitoid abundances, respectively. Node width scales are shown for herbivores and parasitoids. For host plants and herbivores, nodes are filled according to the abundance of the herbivores and parasitoids consuming them, respectively. Vertical log scales represent consumed abundances. The network is ordered by the host plant phylogeny (see supplementary), from basal species on the left to derived species on the right. The remaining trophic levels were ordered to minimise the number of interaction crossings.

The parasitoid overlap graph (**Fig. 2**) provides a visual representation of the potential for apparent competition (PAC) in the gall-parasitoid food web. Out of a possible 861 host gall pairs (42 x 42, minus same-species pairs), only 62 (7%) had non-zero potential (mean PAC of pairs with non-zero values was 0.069 (sd=0.12). Sixteen of the 42 host gall species had zero potential for competition due to not sharing any parasitoids with other species. The mean PAC among same-species pairs (i.e. the diagonal cells of the matrix, representing intraspecific PAC), in contrast, was 0.76 (sd=0.26). In this food web, therefore, there is significantly more potential for intraspecific competition among hosts than for interspecific competition.

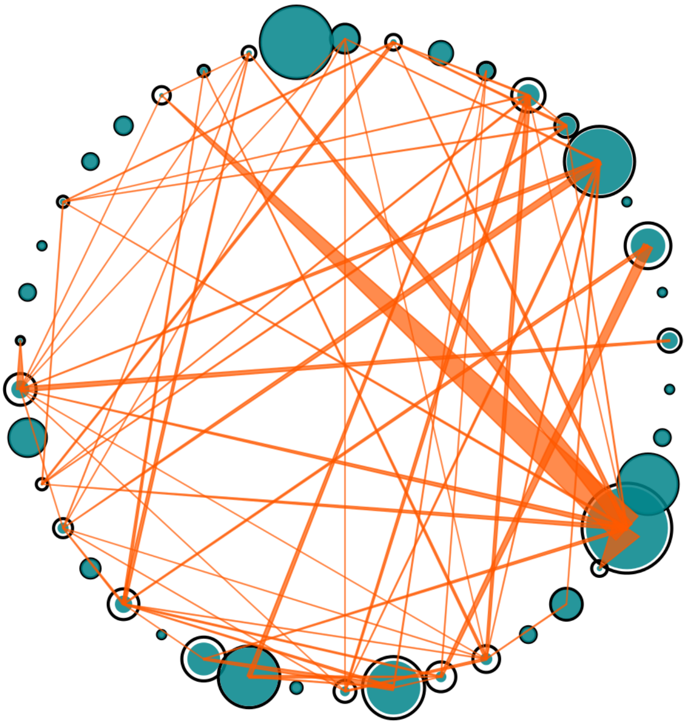

Figure 2. Parasitoid overlap graph for 42 host gall species, based on the potential for apparent competition (PAC) between hosts with shared natural enemies. The proportion of each numbered species node coloured black represents the extent to which that species is responsible for the supply of its own parasitoids. Connecting line segments represent shared enemies between pairs of hosts and the width of a segment at the widest end represents the extent to which the species at the widest end could be a potential supplier of the partner host's parasitoids. Species with no connecting segments have no shared parasitoids.

Gall traits and host plant phylogeny

The 78 gall species sampled were formed by Cecidomyiidae (41%), Sternorrhyncha (Hemiptera; 19%), Chalcidoidea (Hymenoptera; 5%), Agromyzidae & thysanoptera (4% each). Nineteen species (24%) remained unidentified. Only one species was found to gall more than one host plant species, the rest were monophagous (Butterill & Novotny, 2015).

In all GLMs, gall abundance was the most significant predictor of parasitoid species richness and abundance, as expected, but three gall traits were retained in their respective models: apparency (explained deviance or e.d. = 39.9%, d.f. = 74, s.e. = 0.54, $P < 0.001$), gall former (e.d. = 41.5%, d.f. = 73, s.e. = 0.54, $P < 0.001$) and gall openness (e.d. = 32.7%, d.f. = 75, s.e. = 0.35, $P < 0.05$) (**Table 2**). The natural log of host gall abundance, when modelled as the only predictor, explained 26.5% of the model deviance (e.d. = 26.5%, d.f. = 76, s.e. = 0.30, $P < 0.001$). The significant predictors are plotted in **Fig. 4**, with fitted lines as predicted by each model.

In a full model with all predictors, only apparency ($P < 0.05$), gall former ($P < 0.05$) and host abundance ($P < 0.001$) were retained after model simplification (**Table 3**). The optimal model explained 50.2 % deviance (e.d. = 43.5 %, d.f. = 71, AIC = 246.8).

The hypothesis that different gall-forming taxa support parasitoid species of varying richness is driven mainly by Cecidomyiidae, which hosted more species than the other taxa, although the three agromyzid gallers, grouped with ‘others’, actually hosted more species than cecidomyiids on average (**Fig. 4b**).

No phylogenetic signal of mean parasitoid species richness was discovered in the host plant phylogeny, either for all plants hosting galls (N = 28, K = 0.253, P = 0.22).

Table 2. Results of Generalised Linear Models (GLMs) for the number of parasitoid species against minimum host gall abundance (host qty) and several galls traits: gall-forming taxon (taxon), gall physical apparency (apparency), gall openness (open), galled plant module (module), and gall pubescence (pubescence). *P* values from analysis of deviance tests report the significance of dropping each parameter from the model. Significant parameters should, therefore, be retained in the model.

Model Parameters	Residual d.f.	Residual Deviance	Std. Err.	AIC	Explained deviance (%)	*P*
Full model	67	80.3	1.76	252.0	52.3	
Apparency						0.021 *
Taxon						0.009 **
Open						0.880
Module						0.242
Pubescence						0.828
Log(Host qty)						< 0.001 ***
Drop Open	68	80.3	1.76	250.0	52.3	
Apparency						0.020 *
Taxon						0.005 **
Module						0.241
Pubescence						0.812
Log(Host qty)						< 0.001 ***
Drop Pubescence	69	80.2	1.74	248.0	52.3	
Apparency						0.012 *
Taxon						0.005 **
Module						0.238
Log(Host qty)						< 0.001 ***
Drop Module (Final model)	71	80.2	1.44	246.8	50.2	
Apparency						0.009 **
Taxon						0.013 *
Log(Host qty)						< 0.001 ***

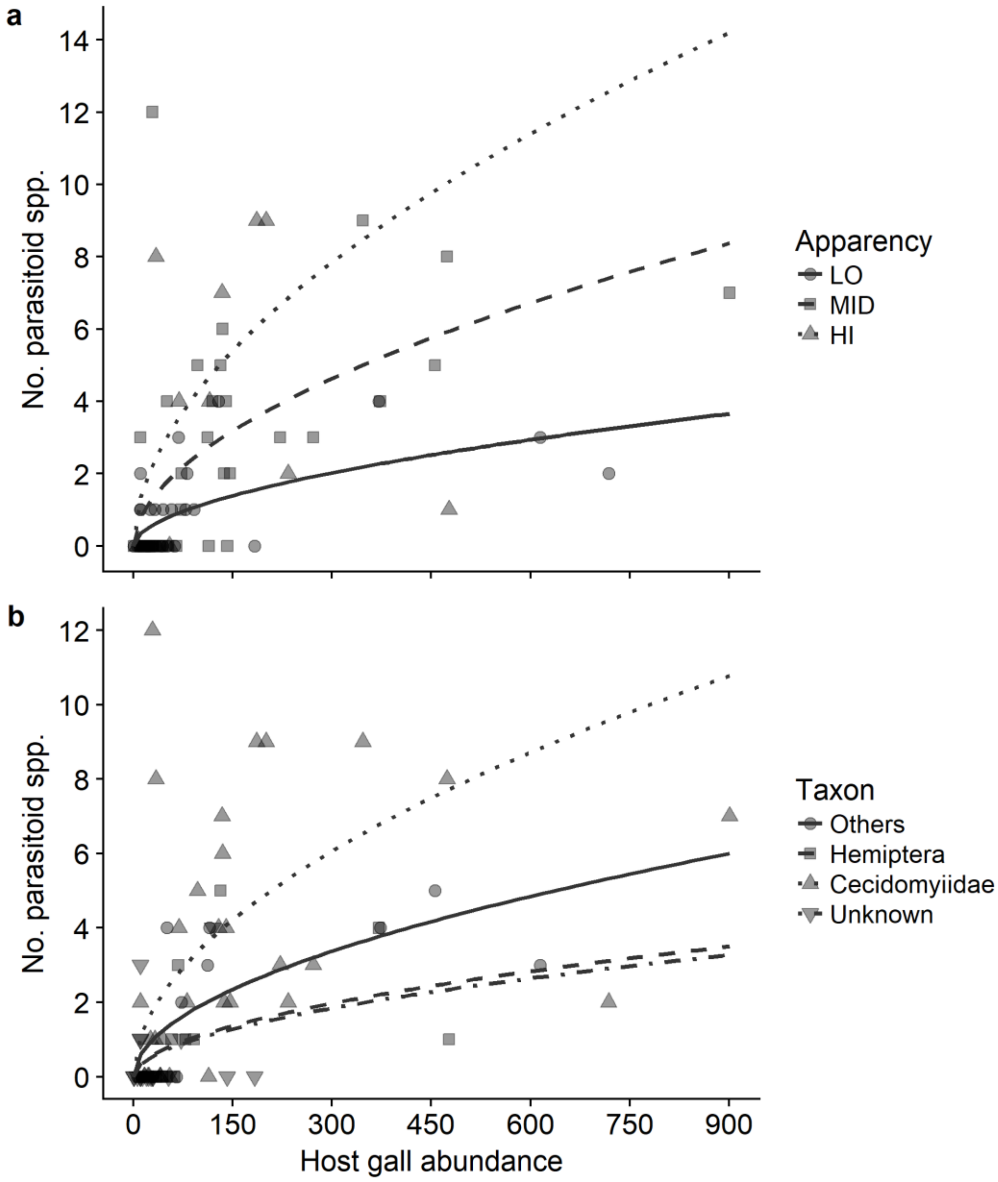

Figure 4. The relationship between the number of parasitoid species and (minimum) host gall abundance for **a)** gall apparency and **b)** gall-forming taxon. Curves were fitted as predicted by respective generalised linear models of both explanatory variables, in which they were significant components ($P < 0.001$; **Table S3**).

Discussion

Food web structure

Our study uncovers a highly diverse network of plant-gall-parasitoids, marked by significant specialisation. In our previous work, we presented the highly specialised system of gall forming insects on their host plants that were found in our study sites (Butterill & Novotny, 2015). Here, in line with our hypothesis [H1a], we further reveal that the parasitoids of these gall forming insects also exhibit a reciprocal level of specialisation. Moreover, the network's high compartmentalization and low connectance, which are driven by the high degree of specialisation, result in a limited potential for indirect interactions.

Highly specialised antagonistic networks are not uncommon in previous studies, however, they are typically characteristic of smaller networks with fewer species or fewer observed interactions. In contrast, our network represents the largest tropical plant-gall-parasitoid network known to date. A study by Morris et al. (2014) revealed that host-parasitoid networks had an average specialisation of H2' = 0.65. This underscores the unique level of specialisation in our network, which has a relative value of H2' = 0.90.

Extremely high levels of specialisation are not ubiquitous in gall-parasitoid networks. For instance, several studies have reported a predominance of generalist parasitoids. In studies by Chust et al. (2007) and Maldonado-López et al. (2022) more than half of the parasitoids were generalists with their study systems. Similarly, Kuzmanich et al. (2023) reported that 83% of parasitoid individuals and 46% of species were generalists in the Cordoba mountains in Argentina. Contrarily, López-Núñez et al. (2019) reported a higher percentage of specialists, with 64.6% of parasitoid species parasitizing a single host species along the Portuguese coast.

One clear distinction between these studies and ours is the equatorial location of our site. Studies conducted at high latitudes or altitudes, which have greater seasonal variation, tend to report a higher proportion of generalist parasitoids. In contrast, studies such as ours, conducted in tropical locations closer to the equator, tend to find a higher proportion of specialist parasitoids. For example, Paniagua et al. (2009) found that 91.2% of parasitoids were specialists in Panama, while Araújo & Maia (2021) and Luz et al. (2021) reported specialist percentages of 64.3% and 86.7% in southeast and southern Brazil respectively. Morris et al. (2014) showed that there appeared to be no increase in network specialisation for tropical host-parasitoid webs as opposed to those at more temperate latitudes. However, confidence in the lack of latitudinal trend is hampered by insufficient data, and subsequent studies over the last decade, particularly those focusing on gall-parasitoid networks, are challenging this notion, with our study being no exception.

In line with our hypothesis [H1b], interspecific PAC was low, whilst intraspecific PAC was high. Large numbers of specialised parasitoids could be the result of temporal niche availability and host insect voltinism. In gall-parasitoid systems, specialist parasitoids tend to be attackers of early-stage insects, whereas generalists tend to be late-stage (Askew, 1975). Furthermore, there is a trade-off dependent on the chosen strategy, with specialists having high fecundity and low competitive ability ("r" strategists), whereas generalists have low fecundity, but high competitive ability ("K" strategists) (Askew, 1975; Force, 1974). The large number of specialist parasitoid species in the food web, therefore, may reflect a lack of competition (**Fig. 2**) and indicate that resources are plentiful. In temperate regions, most gallers are either uni- or bivoltine, their generations being synchronised with either or both spring and autumn seasons. Thus, for both specialists and generalists, there are at most two temporal niches per year in which to prosper, leading to high competition for transitory resources. Although we know very little about galling insect life histories in the tropics, it seems reasonable to assume that most would be multivoltine, given the constant availability of their host plants, thus widening the temporal niche into one continuous one (Hawkins & Goeden, 1984). Gall formers are thus feeding asynchronously, and the ubiquity of resources could therefore favour early-stage specialist parasitoids as opposed to late-stage generalists.

Another criterion that has not been explicitly tested but appears to influence specialisation in gall-parasitoid networks is gall host taxa. The distribution of galling taxa, particularly the contrast between cynipid and cecidomyiid gallers, likely plays a pivotal role in this dynamic. Cynipids, whose diversity and abundance are highly correlated with the presence of Quercus trees, dominate gall communities in the northern hemisphere, especially at higher latitudes (Maldonado-López et al., 2016). This is due to Quercus trees constituting approximately 87% of cynipid host plants (Abrahamson et al., 2003). However, Quercus is mostly absent from the tropics, resulting in a vastly reduced cynipid fauna (other Fagaceae genera, Lithocarpus and Castanea, can host tropical cynipids; see Nieves-Aldrey & Butterill, 2014). Additionally, studies have shown that cynipid gallers can be more generalist than cecidomyiids which are almost exclusively host specialist (Araújo & Maia, 2021). The knock-on effect this has on the relative specialisation of their parasitoids is unknown, although it has been suggested that parasitoid specialisation can be reciprocal to that of their hosts (Van Oudenhove et al., 2017), which is certainly the case in our study.

Further, evidence for parasitoids of cecidomyiid gallers being highly specialised is supported by the literature. For example, the three quantitative food webs produced by Paniagua et al. (2009) from a tropical forest in Panama were dominated by monophagous parasitoids of predominantly cecidomyiid hosts. Similarly, studies by Araújo & Maia (2021), and Luz et al. (2021) observed that the majority of parasitoids were host-specific when sampling exclusively cecidomyiids. Indicating that parasitoids may be more specialised on cecidomyiid hosts, which accounted for approximately 70% of gall taxa in our study.

Due to a higher prevalence of cecidomyiids in the tropics it is difficult to ascertain whether the observed specialisation of cecidomyiid parasitoids is driven by latitudinal or taxonomic differences, or potentially by other unknown factors. However, the distribution of *Quercus* is not only diminished in the tropics, but also throughout the entire southern hemisphere. To eliminate latitudinal bias and better understand what is driving these differences, future studies should aim to compare host specificity of parasitoids in the northern and southern hemispheres at locations that are equidistant from the equator.

The current literature may suggests that host taxa, rather than latitude, primarily drive the specialisation of cynipid and cecidomyiid gall parasitoids. Lopez-Nunez et al. (2019) curiously found that the majority of gall parasitoids in their study system were specialists from a temperate region in the northern hemisphere. Notably in this study, cecidomyiids represented more than one-third of all the identified galling taxa. In contrast, a similar study conducted by Kuzmanich et al. (2023) in Argentina, found that out of 23 gall species, only 9 were cecidomyiids, and they found the majority of their parasitoids were generalists. These findings differ greatly from a study at similar latitude within the same hemisphere that focused exclusively on cecidomyiids, and found that parasitoids were almost exclusively host-specific (Luz et al., 2021).

Within our study, we have shown that galling taxa had a significant impact on parasitoid species richness. We cannot however, extrapolate this to parasitoid specialisation but tentatively suggest that the two may be linked. To decipher the drivers behind this trend and the apparent increase in specialisation in gall-parasitoid food webs towards the tropics, further research is required, particularly in intermediate latitudes in the northern hemisphere where there are comparable numbers of both cynipid and cecidomyiid gallers within the same communities. This would allow for a more comprehensive comparison of the specialisation of their parasitoids.

It is crucial to acknowledge that the quantification of specialist parasitoids in ecological studies may be subject to overestimation. This potential bias arises from the inherent definition of specialists and generalists. Specialists are characterised by their association with a single host, while generalists are known to interact with multiple hosts. Consequently, an organism identified as a specialist in a study may not be a true specialist; it is plausible that its interactions with other hosts have simply not been documented. This limitation is likely to be more pronounced in tropical environments, which are characterised by high species diversity. The sheer number of species in these regions makes comprehensive sampling of all gallers and parasitoids extremely difficult. However, the asymptotic nature of our parasitoid diversity and sample coverage accumulation curves suggest that we have documented the bulk of all the gall-parasitoid interactions across our three study sites, potentially mitigating these biases (**Fig. 2**). Furthermore, the lack of pre-existing taxonomic information and the difficulty in identifying all interactions exacerbate this challenge. In contrast, temperate regions, with their relatively lower species diversity, present fewer interactions and thus, are less likely to suffer from this drawback. Therefore, while interpreting the results of such studies, one must exercise caution and consider these potential sources of bias.

Gall traits and host plant phylogeny

Since sampling was standardised to host plants rather than host galls in this study, host gall abundance explained the greatest proportion of parasitoid species richness in all models. From our measured gall characteristics, both gall apparency, and gall forming taxa were similarly significant in explaining parasitoid richness, whilst gall openness, plant module, and pubescence had no effect (**Table 2**). Additionally, the phylogeny of gall host plants had no observed effect on parasitoid richness.

In line with our hypothesis [H2], more apparent galls had a tendency to host more parasitoid species. More apparent galls are those that 'stand out' from their host plants, usually due to relatively large size, contrasting colours, or both in the most apparent galls. If increased conspicuity of galls leads to increased parasitoid richness, this raises the question of the significance of visual location for parasitoids when finding their host galls. Gall apparency was previously identified as a key factor influencing the size of parasitoid assemblages in cecidomyiids (Hawkins & Gagné, 1989). Our study supports this finding, with two-thirds of the most apparent galls being formed by cecidomyiids. When we analysed data for cecidomyiids separately, apparency emerged as the most significant predictor of parasitoid richness, surpassing even gall abundance that was dropped from the model (**Table S5**). This underscores the potential role of gall apparency, and therefore the importance of visual host location in shaping parasitoid assemblages in cecidoymiids, and potentially all galling insects.

Apparent galls are often those with striking colouration. This distinctive colouration is a result of hijacked plant pigments such as carotenoids, anthocyanins, as well as tannins and other phenolic compounds (Lev-Yadun, 2016). Recently, “the aposematic gall hypothesis” was proposed by Inbar et al. (2010) suggesting that chemically protected galls, which are also visually conspicuous are aposematic, which could reduce their vulnerability to predators and parasitoids. However, in other host-parasitoid systems, such as caterpillar-parasitoids, it has been shown that aposematism, in caterpillars whilst decreasing predation rates, can actually cause increased parasitism rates and potentially increased parasitoid richness on aposematic hosts (Finnie et al., 2023). This has been justified with the “safe haven” hypothesis, which postulates that aposematic hosts that are better protected from predators actually provide ideal conditions for parasitoids (Lampert et al., 2010), and it is possible that this theory extends to gall forming hosts. Another possible justification may lie in certain galling species’ having the ability to silence or suppress parasitoid-attracting compounds during gall formation, which could emphasise the importance of using visual cues instead for host location (Tooker et al., 2008). While our study does not explicitly test these theories, it underscores the need for further targeted research to understand the mechanisms driving higher parasitoid richness in more apparent galls. This research should focus on the significance and justification of visual cues for gall parasitoids in both tropical and temperate locations.

Gall forming taxa were also a strong predictor of parasitoid richness in our study, with cecidoymiid gallers hosting the most species on average than any other galling taxon. The taxon of the gall former would seem to be of primary importance because it is intuitive that any associated parasitoids would have evolved in parallel to some extent with their hosts. Often, entire parasitoid genera are specific to a single gall-forming taxon (Medianero et al., 2014), so intuitively, the most abundant and speciose galling taxa are likely to have the largest number of parasitoid species. For cecidoymiids specifically, it is hard to determine what drives the high parasitoid richness in these galling species. It could be their highly apparent galls, their co-cladogenetic relationship with their parasitoids, or more likely, a combination of the two. To unravel the mechanisms behind this phenomenon, more targeted studies are required.

We also found no relationship between gall openness, galled plant module, and gall pubescence as predictors of parasitoid richness in the full model, although all of these were significant when modelled separately. This is likely a result of multicollinearity, in which these gall characteristics are actually more closely related to the significant predictors (e.g. pubescent galls being more apparent, or open galls being associated with a specific taxa) rather than with parasitoid richness directly. (Hawkins & Gagné, 1989) also found that gall pubescence had a minimal effect on parasitoid richness. Additionally, we found no evidence of phylogenetic signal in this study of parasitoid species being more attracted to galls on certain host plant species than others. While the influence of host plant phylogeny has been documented in studies of leaf miner-parasitoid associations (Ives & Godfray, 2006; Leppänen et al., 2013; Lopez-vaamonde et al., 2005), such influence does not appear to extend to our gall-parasitoid system.

Conclusion

In this study, we unveil a highly diverse tri-trophic plant-insect food web from a tropical forest, characterised by the unique interaction between highly specialised galling herbivores in our previous study (Butterill & Novotny, 2015) and their reciprocally specialised parasitoids. Our extensive sampling suggests that if generalist parasitoids were a dominant feature of tropical gall-parasitoid networks, we would have encountered a greater number of species. However, our findings indicate a marked prevalence of specialist parasitoids in our tropical gall-parasitoid food web. After comparing our findings with existing literature, we propose the potential existence of a latitudinal gradient in the specialisation of gall-parasitoid food webs, with specialisation intensifying towards the equator. This gradient may be influenced, in part, by a shift in the dominant galling taxa towards the tropics, with cecidomyiid gallers hosting more specialised parasitoid assemblages than cynipids. Additionally, our study reveals that galls with greater visual conspicuousness host a richer diversity of parasitoids. This finding implies a significant role of visual cues in parasitoid host location. Overall, our research provides valuable insights into the complex dynamics of tropical gall-parasitoid food webs and the factors influencing their structure and diversity.

Acknowledgements

For taxonomic identifications of parasitoids, we thank Christer Hansson (Chalcidoidea: Eulophidae and Tetracampidae), Gary Gibson (Chalcidoidea: Eupelmidae and Pteromalidae), Mike Gates (Chalcidoidea: Eurytomidae and Ormyridae), Petr Jansta (Chalcidoidea: Torymidae), and Peter Buhl (Platygastroidea). We also thank The European Union Center of Excellence for the global study of the function and biodiversity of forest ecosystems (CZ.1.07/2.3.00/20.0064), the Grant Agency of the Czech Republic (14-04258S), the Grant Agency of the University of South Bohemia (136/20101/P), the US National Science Foundation (Division of Environmental Biology 841885), the Christensen Fund (US), and the UK Darwin Initiative for the Survival of Species (14/054).

References

Abe, Y., Melika, G., & Stone, G. N. (2007). The diversity and phylogeography of cynipid gallwasps (Hymenoptera: Cynipidae) of the Oriental and eastern Palearctic regions, and their associated communities. Oriental Insects, 41(1), 169–212. https://doi.org/10.1080/00305316.2007.10417504

Abrahamson, W. G., Hunter, M. D., Melika, G., & Price, P. W. (2003). Cynipid gall-wasp communities correlate with oak chemistry. Journal of Chemical Ecology, 29(1), 209–223. https://doi.org/10.1023/A:1021993017237

Araújo, W. S. de, Freitas, É. V. D. de, Kollár, J., Pessoa, R. O., Corgosinho, P. H. C., Valério, H. M., Falcão, L. A. D., Fagundes, M., Pimenta, M. A. S., & Faria, M. L. de. (2019). Host specialization in plant-galling interactions: Contrasting mites and insects. Diversity, 11(10), 180.

Askew, R. R. (1975). The Organisation of Chalcid-Dominated Parasitoid Communities Centred upon Endophytic Hosts. In P. W. Price (Ed.), Evolutionary Strategies of Parasitic Insects and Mites (pp. 130–153). Springer US. https://doi.org/10.1007/978-1-4615-8732-3_7

Askew, R. R. (1980). The diversity of insect communities in leafmines and plant galls. The Journal of Animal Ecology, 817–829.

Bersier, L.-F., Banašek-Richter, C., & Cattin, M.-F. (2002). Quantitative Descriptors of Food-Web Matrices. Ecology, 83(9), 2394–2407. https://doi.org/10.1890/0012-9658(2002)083[2394:QDOFWM]2.0.CO;2

Blomberg, S. P., Garland, T., & Ives, A. R. (2003). Testing for phylogenetic signal in comparative data: behavioral traits are labile. Evolution, 57(4), 717–745. https://doi.org/10.1111/j.0014-3820.2003.tb00285.x

Blüthgen, N. (2010). Why network analysis is often disconnected from community ecology: A critique and an ecologist's guide. Basic and Applied Ecology, 11(3), 185–195.

Blüthgen, N., Menzel, F., & Blüthgen, N. (2006). Measuring specialization in species interaction networks. BMC Ecology, 6(1), 9. https://doi.org/10.1186/1472-6785-6-9

Blüthgen, N., Menzel, F., Hovestadt, T., Fiala, B., & Blüthgen, N. (2007). Specialization, constraints, and conflicting interests in mutualistic networks. Current Biology, 17(4), 341–346.

Butterill, P. T., & Novotny, V. (2015). Gall-forming insects in a lowland tropical rainforest: Low species diversity in an extremely specialised guild. Ecological Entomology, 40(4), 409–419. https://doi.org/10.1111/een.12198

Cagnolo, L. (2018). The Future of Ecological Networks in the Tropics. In W. Dáttilo & V. Rico-Gray (Eds.), Ecological Networks in the Tropics: An Integrative Overview of Species Interactions from Some of the Most Species-Rich Habitats on Earth (pp. 171–183). Springer International Publishing. https://doi.org/10.1007/978-3-319-68228-0_12

Carneiro, M. A. A., Branco, C. S., Braga, C. E., Almada, E. D., Costa, M., Maia, V. C., & Fernandes, G. W. (2009). Are gall midge species (Diptera, Cecidomyiidae) host-plant specialists? Revista Brasileira de Entomologia, 53, 365–378.

Chust, G., Garbin, L., & Pujade-Villar, J. (2007). Gall wasps and their parasitoids in cork oak fragmented forests. Ecological Entomology, 32(1), 82–91. https://doi.org/10.1111/j.1365-2311.2006.00850.x

Dalsgaard, B., Trøjelsgaard, K., Martín González, A. M., Nogués-Bravo, D., Ollerton, J., Petanidou, T., Sandel, B., Schleuning, M., Wang, Z., Rahbek, C., Sutherland, W. J., Svenning, J.-C., & Olesen, J. M. (2013). Historical climate-change influences modularity and nestedness of pollination networks. Ecography, 36(12), 1331–1340. https://doi.org/10.1111/j.1600-0587.2013.00201.x

Dawah, H. A., Hawkins, B. A., & Claridge, M. F. (1995). Structure of the parasitoid communities of grass-feeding chalcid wasps. Journal of Animal Ecology, 708–720.

De Araújo, W. S., & Maia, V. C. (2021). Topological structure of a tritrophic network composed of host plants, gall-inducing insects and parasitoids in a restinga area in BRAZIL. Entomological Science, 24(2), 201–216. https://doi.org/10.1111/ens.12468

De Araújo, W. S., & Maia, V. C. (2021). Topological structure of a tritrophic network composed of host plants, gall-inducing insects and parasitoids in a restinga area in Brazil. Entomological Science, 24(2), 201–216. https://doi.org/10.1111/ens.12468

Desneux, N., Blahnik, R., Delebecque, C. J., & Heimpel, G. E. (2012). Host phylogeny and specialisation in parasitoids. Ecology Letters, 15(5), 453–460. https://doi.org/10.1111/j.1461-0248.2012.01754.x

Dormann, C., Frund, J., Bluthgen, N., & Gruber, B. (2009). Indices, Graphs and Null Models: Analyzing Bipartite Ecological Networks. Open Journal of Ecology, 2, 7–24. https://doi.org/10.2174/1874213000902010007

Finnie, S., Butterill, P., Novotny, V., Redmond, C., Jorge, L. R., Abe, T., Lamarre, G. P., Maicher, V., & Sam, K. (2023). Vertical stratification and defensive traits of caterpillars against parasitoids in a lowland tropical forest in Cameroon. https://www.researchsquare.com/article/rs-3587026/latest

Force, D. C. (1974). Ecology of Insect Host-Parasitoid Communities: Competition may be the most vital factor in the structure and function of these systems. Science, 184(4137), 624–632. https://doi.org/10.1126/science.184.4137.624

Frank Van Veen, F. J., Morris, R. J., & Godfray, H. C. J. (2006). Apparent competition, quantitative food webs, and the structure of phytophagous insect communities. Annual Review of Entomology, 51(1), 187–208. https://doi.org/10.1146/annurev.ento.51.110104.151120

Galiana, N., Hawkins, B. A., & Montoya, J. M. (2019). The geographical variation of network structure is scale dependent: Understanding the biotic specialization of host–parasitoid networks. Ecography, 42(6), 1175–1187. https://doi.org/10.1111/ecog.03684

Graziosi, I. (2015). Invasiveness of an exotic gall wasp is influenced by interactions with exotic and endemic organisms. https://uknowledge.uky.edu/entomology_etds/15/

Hagberg, A., Swart, P., & S Chult, D. (2008). Exploring network structure, dynamics, and function using NetworkX. Los Alamos National Lab.(LANL), Los Alamos, NM (United States). https://www.osti.gov/biblio/960616

Hanson, P., & Nishida, K. (2014). Insect Galls of Costa Rica and Their Parasitoids. In G. W. Fernandes & J. C. Santos (Eds.), Neotropical Insect Galls (pp. 497–517). Springer Netherlands. https://doi.org/10.1007/978-94-017-8783-3_23

Hawkins, B. A., Askew, R. R., & Shaw, M. R. (1990). Influences of host feeding-niche and foodplant type on generalist and specialist parasitoids. Ecological Entomology, 15(3), 275–280. https://doi.org/10.1111/j.1365-2311.1990.tb00809.x

Hawkins, B. A., & Gagné, R. J. (1989). Determinants of assemblage size for the parasitoids of Cecidomyiidae (Diptera). Oecologia, 81(1), 75–88. https://doi.org/10.1007/BF00377013

Hawkins, B. A., & Goeden, R. D. (1984). Organization of a parasitoid community associated with a complex of galls on Atriplex spp. In southern California. Ecological Entomology, 9(3), 271–292. https://doi.org/10.1111/j.1365-2311.1984.tb00851.x

Hayward, A., & Stone, G. N. (2005). Oak gall wasp communities: Evolution and ecology. Basic and Applied Ecology, 6(5), 435–443. https://doi.org/10.1016/j.baae.2005.07.003

Hopper, K. R., Oppenheim, S. J., Kuhn, K. L., Lanier, K., Hoelmer, K. A., Heimpel, G. E., Meikle, W. G., O'Neil, R. J., Voegtlin, D. G., Wu, K., Woolley, J. B., & Heraty, J. M. (2019). Counties not countries: Variation in host specificity among populations of an aphid parasitoid. Evolutionary Applications, 12(4), 815–829. https://doi.org/10.1111/eva.12759

Hrcek, J., Miller, S. E., Whitfield, J. B., Shima, H., & Novotny, V. (2013). Parasitism rate, parasitoid community composition and host specificity on exposed and semi-concealed caterpillars from a tropical rainforest. Oecologia, 173, 521–532.

Inbar, M., Izhaki, I., Koplovich, A., Lupo, I., Silanikove, N., Glasser, T., Gerchman, Y., Perevolotsky, A., & Lev-Yadun, S. (2010). Why do many galls have conspicuous colors? A new hypothesis. Arthropod-Plant Interactions, 4(1), 1–6. https://doi.org/10.1007/s11829-009-9082-7

Ings, T. C., Montoya, J. M., Bascompte, J., Blüthgen, N., Brown, L., Dormann, C. F., Edwards, F., Figueroa, D., Jacob, U., Jones, J. I., Lauridsen, R. B., Ledger, M. E., Lewis, H. M., Olesen, J. M., Van Veen, F. J. F., Warren, P. H., & Woodward, G. (2009). Review: Ecological networks – beyond food webs. Journal of Animal Ecology, 78(1), 253–269. https://doi.org/10.1111/j.1365-2656.2008.01460.x

Ives, A. R., & Godfray, H. C. J. (2006). Phylogenetic Analysis of Trophic Associations. The American Naturalist, 168(1), E1–E14. https://doi.org/10.1086/505157

Joppa, L. N., Bascompte, J., Montoya, J. M., Solé, R. V., Sanderson, J., & Pimm, S. L. (2009). Reciprocal specialization in ecological networks. Ecology Letters, 12(9), 961–969. https://doi.org/10.1111/j.1461-0248.2009.01341.x

Joseph, M. B., Gentles, M., & Pearse, I. S. (2011). The parasitoid community of Andricus quercuscalifornicus and its association with gall size, phenology, and location. Biodiversity and Conservation, 20(1), 203–216. https://doi.org/10.1007/s10531-010-9956-0

Kaartinen, R., & Roslin, T. (2011). Shrinking by numbers: Landscape context affects the species composition but not the quantitative structure of local food webs: Landscape effects on food webs. Journal of Animal Ecology, 80(3), 622–631. https://doi.org/10.1111/j.1365-2656.2011.01811.x

Kembel, S. W., Cowan, P. D., Helmus, M. R., Cornwell, W. K., Morlon, H., Ackerly, D. D., Blomberg, S. P., & Webb, C. O. (2010). Picante: R tools for integrating phylogenies and ecology. Bioinformatics, 26(11), 1463–1464.

Kuzmanich, N., Giorgis, M. A., Bernaschini, L., Tavella, J., & Salvo, A. (2023). Galling insect communities mediate the effects of fire on their associated parasitoid communities. Insect Conservation and Diversity, 16(6), 817–827. https://doi.org/10.1111/icad.12674

Lampert, E. C., Dyer, L. A., & Bowers, M. D. (2010). Caterpillar Chemical Defense and Parasitoid Success: Cotesia congregata Parasitism of Ceratomia catalpae. Journal of Chemical Ecology, 36(9), 992–998. https://doi.org/10.1007/s10886-010-9840-0

Leppänen, S. A., Altenhofer, E., Liston, A. D., & Nyman, T. (2013). Ecological versus phylogenetic determinants of trophic associations in a plant–leafminer–parasitoid food web. Evolution, 67(5), 1493–1502.

Lev-Yadun, S. (2016). Gall Aposematism. In S. Lev-Yadun, Defensive (anti-herbivory) Coloration in Land Plants (pp. 213–223). Springer International Publishing. https://doi.org/10.1007/978-3-319-42096-7_43

Lill, J. T., Marquis, R. J., & Ricklefs, R. E. (2002). Host plants influence parasitism of forest caterpillars. Nature, 417(6885), 170–173.

López-Núñez, F. A., Ribeiro, S., Marchante, H., Heleno, R. H., & Marchante, E. (2019). Life inside a gall: Diversity, phenology and structure of Portuguese gall communities, their hosts, parasitoids and inquilines. Arthropod-Plant Interactions, 13(3), 477–488. https://doi.org/10.1007/s11829-018-9655-4

Lopez-vaamonde, C., Godfray, H. C. J., West, S. A., Hansson, C., & Cook, J. M. (2005). The evolution of host use and unusual reproductive strategies in Achrysocharoides parasitoid wasps. Journal of Evolutionary Biology, 18(4), 1029–1041.

Luz, F. A., Goetz, A. P. M., & Mendonça, M. D. S. (2021). What drives gallers and parasitoids interacting on a host plant? A network approach revealing morphological coupling as the main factor. Ecological Entomology, 46(2), 334–341. https://doi.org/10.1111/een.12967

MacArthur, R. H. (1972). Geografical ecology: Patterns in the distribution of species. Harper & Row.

Maldonado-López, Y., Cuevas-Reyes, P., & Oyama, K. (2016). Diversity of gall wasps (Hymenoptera: Cynipidae) associated with oak trees (Fagaceae: Quercus) in a fragmented landscape in Mexico. Arthropod-Plant Interactions, 10(1), 29–39. https://doi.org/10.1007/s11829-015-9404-x

Maldonado-López, Y., de Araújo, W. S., González-Rodríguez, A., Oyama, K., Fagundes, M., Diaz-Castelazo, C., Sánchez-Echeverría, K., Borges, M., Pérez-López, G., & Cuevas-Reyes, P. (2022). Quantitative trophic networks of insect gallers and their parasitoids in the hybrid oak complex Quercus magnoliifolia x Quercus resinosa. Arthropod-Plant Interactions, 16(6), 631–643. https://doi.org/10.1007/s11829-022-09927-8

Maldonado-Lopez, Y., Prieto-Dueñas, I. S., Cuevas-Reyes, P., Vaca-Sánchez, M. S., Oyama, K., de Araújo, W. S., Zazá Borges, M. A., Fagundes, M., & Fernandes, G. W. (2023). Tri-Trophic Networks of Gall-Inducing Insects and Their Parasitoids Associated to Oak Species in Mexican Avocado Agrosystems: Importance of Native Forest Coverage and Local Climatic Variables. SSRN Scholarly Paper 4392343. https://doi.org/10.2139/ssrn.4392343

Medianero, E., Barrios, H., & Nieves-Aldrey, J. L. (2014). Gall-Inducing Insects and Their Associated Parasitoid Assemblages in the Forests of Panama. In G. W. Fernandes & J. C. Santos (Eds.), Neotropical Insect Galls (pp. 465–496). Springer Netherlands. https://doi.org/10.1007/978-94-017-8783-3_22

Memmott, J., Godfray, H. C. J., & Gauld, I. D. (1994). The structure of a tropical host-parasitoid community. Journal of Animal Ecology, 521–540.

Miller III, D. G., & Raman, A. (2019). Host–plant relations of gall-inducing insects. Annals of the Entomological Society of America, 112(1), 1–19.

Moles, A. T., & Ollerton, J. (2016). Is the notion that species interactions are stronger and more specialized in the tropics a zombie idea? Biotropica, 48(2), 141–145.

Morris, R. J., Gripenberg, S., Lewis, O. T., & Roslin, T. (2014). Antagonistic interaction networks are structured independently of latitude and host guild. Ecology Letters, 17(3), 340–349. https://doi.org/10.1111/ele.12235

Morris, R. J., Lewis, O. T., & Godfray, H. C. J. (2004). Experimental evidence for apparent competition in a tropical forest food web. Nature, 428(6980), 310–313.

Morris, R. J., Lewis, O. T., & Godfray, H. C. J. (2005). Apparent competition and insect community structure: Towards a spatial perspective. Annales Zoologici Fennici, 449–462. https://www.jstor.org/stable/23735889

Muller, C. B., Adriaanse, I. C. T., Belshaw, R., & Godfray, H. C. J. (1999). The structure of an aphid–parasitoid community. Journal of Animal Ecology, 68(2), 346–370. https://doi.org/10.1046/j.1365-2656.1999.00288.x

Nieves-Aldrey, J. L., & Butterill, P. T. (2014). First evidence of cynipids from the Oceanian Region: The description of Lithonecrus papuanus a new genus and species of cynipid inquiline from Papua New Guinea (Hymenoptera: Cynipidae, Synergini). Zootaxa, 3846(2), 221–234.

Nyman, T., Bokma, F., & Kopelke, J.-P. (2007). Reciprocal diversification in a complex plant-herbivore-parasitoid food web. BMC Biology, 5(1), 49. https://doi.org/10.1186/1741-7007-5-49

Paniagua, M. R., Medianero, E., & Lewis, O. T. (2009). Structure and vertical stratification of plant galler–parasitoid food webs in two tropical forests. Ecological Entomology, 34(3), 310–320. https://doi.org/10.1111/j.1365-2311.2008.01079.x

Prauchner, C., & de Souza Mendonça Jr, M. (2024). What shapes galling insect–parasitoid interaction networks on closely related host plants? Ecological Entomology, 49(2), 285–294. https://doi.org/10.1111/een.13300

Poisot, T., Bever, J. D., Nemri, A., Thrall, P. H., & Hochberg, M. E. (2011). A conceptual framework for the evolution of ecological specialisation. Ecology Letters, 14(9), 841–851. https://doi.org/10.1111/j.1461-0248.2011.01645.x

Price, P. W., Fernandes, G. W., & Waring, G. L. (1987). Adaptive nature of insect galls. Environmental Entomology, 16(1), 15–24.

Redfern, M., Shirley, P., & Bloxham, M. (2002). British plant galls: Identification of galls on plants and fungi. FSC publications. https://www.cabdirect.org/cabdirect/abstract/20033023178

Ripley, B., Venables, B., Bates, D. M., Hornik, K., Gebhardt, A., Firth, D., & Ripley, M. B. (2013). Package 'mass.' Cran r, 538, 113–120.

Ronquist, F., Nieves-Aldrey, J.-L., Buffington, M. L., Liu, Z., Liljeblad, J., & Nylander, J. A. (2015). Phylogeny, evolution and classification of gall wasps: The plot thickens. PLoS One, 10(5), e0123301.

Rott, A. S., & Godfray, H. C. J. (2000). The structure of a leafminer-parasitoid community. Journal of Animal Ecology, 274–289.

Russo, R. (2006). Field guide to plant galls of California and other western states. University of California Press.

Schleuning, M., Fründ, J., Klein, A.-M., Abrahamczyk, S., Alarcón, R., Albrecht, M., Andersson, G. K., Bazarian, S., Böhning-Gaese, K., & Bommarco, R. (2012). Specialization of mutualistic interaction networks decreases toward tropical latitudes. Current Biology, 22(20), 1925–1931.

Schönrogge, K., Walker, P., & Crawley, M. J. (2000). Parasitoid and inquiline attack in the galls of four alien, cynipid gall wasps: Host switches and the effect on parasitoid sex ratios. Ecological Entomology, 25(2), 208–219. https://doi.org/10.1046/j.1365-2311.2000.00244.x

Stone, G. N., & Schönrogge, K. (2003). The adaptive significance of insect gall morphology. Trends in Ecology & Evolution, 18(10), 512–522.

Thompson, R. M., Brose, U., Dunne, J. A., Hall, R. O., Hladyz, S., Kitching, R. L., Martinez, N. D., Rantala, H., Romanuk, T. N., Stouffer, D. B., & Tylianakis, J. M. (2012). Food webs: Reconciling the structure and function of biodiversity. Trends in Ecology & Evolution, 27(12), 689–697. https://doi.org/10.1016/j.tree.2012.08.005

Tooker, J. F., Rohr, J. R., Abrahamson, W. G., & De Moraes, C. M. (2008). Gall insects can avoid and alter indirect plant defenses. New Phytologist, 178(3), 657–671. https://doi.org/10.1111/j.1469-8137.2008.02392.x

Tscharntke, T., Vidal, S., & Hawkins, B. A. (2001). Parasitoids of grass-feeding chalcid wasps: A comparison of German and British communities. Oecologia, 129(3), 445–451. https://doi.org/10.1007/s004420100733

Van Oudenhove, L., Mailleret, L., & Fauvergue, X. (2017). Infochemical use and dietary specialization in parasitoids: A meta-analysis. Ecology and Evolution, 7(13), 4804–4811. https://doi.org/10.1002/ece3.2888

Webb, C. O., Ackerly, D. D., & Kembel, S. W. (2008). Phylocom: Software for the analysis of phylogenetic community structure and trait evolution. Bioinformatics, 24(18). http://camwebb.info/files/pubs/webb2008_phylocom.pdf

Zuur, A. F., Ieno, E. N., Walker, N., Saveliev, A. A., & Smith, G. M. (2009). Mixed effects models and extensions in ecology with R. Springer New York. https://doi.org/10.1007/978-0-387-87458-

Supplementary material

Table S1. Taxonomic information for 32 plant species sampled, along with their associated gall-forming insect herbivores, listed in host plant phylogenetic order from basal to derived species. Gall trait categories are listed in columns *taxon*, *module*, *pubescence*, *apparency* & *open*.

No.	Host code	Clade	Plant family	Plant species	Gall code	Gall higher taxon	Gall family	Gall species	Taxon[3]	Module[4]	Pubescence[5]	Apparency[6]	Open[7]
1	GNE	Basal	Gnetaceae	*Gnetum gnemon*	GALL039	Diptera	Cecidomyiidae		CECI	LEAF	NOT	HI	F
					GALL040	Diptera	Cecidomyiidae		CECI	LEAF	NOT	LO	F
2	STG	Basal	Monimiaceae	*Kibara cf. coriacea*	GALL122	Diptera	Cecidomyiidae		CECI	LEAF	NOT	LO	F
3	MYL	Basal	Myristicaceae	*Paramyristica cf. sepicana*	GALL077	Diptera	Cecidomyiidae		CECI	LEAF	NOT	MID	F
					GALL078	Diptera	Cecidomyiidae		CECI	OTHER	NOT	HI	F
					GALL079	Diptera	Cecidomyiidae		CECI	COMP	NOT	MID	F
					GALL080	Insecta			UNKN	LEAF	NOT	LO	F
					GALL081	Hemiptera			HEMI	LEAF	NOT	LO	T
					GALL173	Thysanoptera			OTHE	LEAF	NOT	MID	T
4	DRA	Basal	Asparagaceae	*Dracaena angustifolia*	GALL038	Diptera	Cecidomyiidae		CECI	LEAF	NOT	LO	F
5	COR	Basal	Asparagaceae	*Cordyline fruticosa*	GALL031	Hemiptera			HEMI	COMP	NOT	MID	T
6	ARE	Basal	Arecaceae	*Hydriastele wendlandiana*	GALL161	Insecta			UNKN	LEAF	NOT	LO	T
7	HOR	Basal	Zingiberaceae	*Hornstedtia scottiana*									
8	MAA	Rosids I	Euphorbiaceae	*Macaranga aleuritoides*	GALL058	Diptera	Cecidomyiidae		CECI	LEAF	NOT	HI	F
					GALL059	Diptera	Cecidomyiidae	*Schizomyia novoguinensis* Kolesik[1]	CECI	OTHER	NOT	MID	F
					GALL062	Diptera	Cecidomyiidae		CECI	LEAF	HAIRY	MID	F
					GALL063	Diptera	Cecidomyiidae		CECI	LEAF	HAIRY	HI	F
9	MAQ	Rosids I	Euphorbiaceae	*Macaranga quadriglandulosa*	GALL069	Diptera	Cecidomyiidae		CECI	LEAF	NOT	LO	F

					GALL070	Diptera	Cecidomyiidae		CECI	OTHER	NOT	MID	F
10	MAU	Rosids I	Euphorbiaceae	*Macaranga novoguineensis*	GALL072	Diptera	Cecidomyiidae		CECI	LEAF	HAIRY	MID	T
					GALL075	Insecta			UNKN	LEAF	HAIRY	MID	F
					GALL170	Insecta			UNKN	LEAF	HAIRY	LO	F
					GALL171	Diptera	Cecidomyiidae		CECI	LEAF	NOT	LO	F
					GALL215	Diptera	Cecidomyiidae		CECI	LEAF	HAIRY	MID	F
					GALL216	Diptera	Cecidomyiidae		CECI	LEAF	HAIRY	HI	F
11	MAL	Rosids I	Euphorbiaceae	*Mallotus mollissimus*	GALL064	Diptera	Cecidomyiidae		CECI	LEAF	HAIRY	HI	F
12	HON	Rosids I	Euphorbiaceae	*Homalanthus novoguineensis*	GALL166	Insecta			UNKN	LEAF	NOT	LO	T
13	PTE	Rosids I	Fabaceae	*Pterocarpus indicus*	GALL008	Diptera	Cecidomyiidae		CECI	OTHER	NOT	LO	F
					GALL009	Diptera	Cecidomyiidae		CECI	COMP	NOT	MID	F
14	ART	Rosids I	Moraceae	*Artocarpus altilis*	GALL187	Insecta			UNKN	LEAF	HAIRY	LO	F
15	PUN	Rosids I	Moraceae	*Ficus pungens*	GALL002	Hemiptera			HEMI	COMP	HAIRY	LO	T
16	VAR	Rosids I	Moraceae	*Ficus variegata*	GALL003	Hemiptera	Triozidae	*Pauropsylla udei* Rübsaamen	HEMI	LEAF	NOT	HI	F
					GALL004	Diptera	Cecidomyiidae		CECI	LEAF	NOT	MID	F
					GALL005	Hemiptera			HEMI	LEAF	NOT	LO	F
					GALL006	Hemiptera			HEMI	OTHER	NOT	LO	T
17	COP	Rosids I	Moraceae	*Ficus copiosa*	GALL028	Diptera	Agromyzidae		OTHE	OTHER	NOT	MID	F
					GALL030	Insecta			UNKN	OTHER	NOT	MID	F
18	WAS	Rosids I	Moraceae	*Ficus wassa*	GALL028	Diptera	Agromyzidae		OTHE	OTHER	NOT	MID	F
					GALL145	Insecta			UNKN	OTHER	NOT	MID	F
					GALL147	Insecta			UNKN	LEAF	NOT	MID	F
					GALL148	Insecta			UNKN	LEAF	NOT	LO	F

19	CEL	Rosids I	Cannabaceae	*Celtis philippensis*	GALL015	Hemiptera	Triozidae	*Trioza incrustata* Percy[2]	HEMI	LEAF	NOT	MID	T
					GALL016	Hymenoptera	Eurytomidae	*Tenuipetiolus* n. sp. 5 (Gates)	OTHE	OTHER	NOT	MID	F
					GALL017	Hemiptera			HEMI	LEAF	NOT	LO	T
					GALL018	Hemiptera			HEMI	LEAF	NOT	LO	T
20	CLA	Rosids I	Cannabaceae	*Celtis latifolia*	GALL020	Diptera	Agromyzidae		OTHE	LEAF	NOT	LO	F
					GALL021	Diptera	Cecidomyiidae		CECI	LEAF	NOT	LO	F
					GALL024	Insecta			UNKN	OTHER	NOT	MID	F
					GALL025	Insecta			UNKN	LEAF	NOT	LO	T
					GALL163	Insecta			UNKN	OTHER	NOT	MID	F
					GALL164	Diptera	Agromyzidae		OTHE	OTHER	NOT	MID	F
21	SRS	Rosids II	Myrtaceae	*Syzygium malaccense*	GALL116	Hymenoptera	Eulophidae	*Euceratoneura* sp. 1 (Hansson)	OTHE	OTHER	NOT	MID	F
					GALL118	Hemiptera			HEMI	LEAF	NOT	LO	T
					GALL120	Insecta			UNKN	LEAF	NOT	LO	F
22	SRB	Rosids II	Myrtaceae	*Syzygium sp.*	GALL110	Hymenoptera	Eurytomidae	*Tenuipetiolus* n. sp. 8 (Gates)	OTHE	OTHER	NOT	HI	F
					GALL111	Diptera	Cecidomyiidae		CECI	LEAF	NOT	LO	F
					GALL112	Hemiptera	Aleyrodidae		HEMI	LEAF	NOT	LO	T
					GALL115	Insecta			UNKN	LEAF	NOT	MID	F
23	POM	Rosids II	Sapindaceae	*Pometia pinnata*	GALL085	Thysanoptera			OTHE	LEAF	NOT	HI	T
					GALL086	Lepidoptera			OTHE	COMP	HAIRY	MID	F
					GALL087	Hemiptera	Phacopteronidae	*Cornegenapsylla pometiae* Malenovský and Percy[2]	HEMI	LEAF	NOT	MID	T
24	KLE	Rosids II	Malvaceae	*Kleinhovia hospita*									
25	TRI	Rosids II	Malvaceae	*Trichospermum pleiostigma*									
26	PSL	Asterids	Rubiaceae	*Psychotria ramuensis*	GALL089	Diptera	Cecidomyiidae	*Rhopalomyia psychotriae* Kolesik[1]	CECI	COMP	NOT	MID	F

					GALL090	Diptera	Cecidomyiidae		CECI	LEAF	NOT	LO	F
					GALL091	Diptera	Cecidomyiidae		CECI	LEAF	NOT	MID	F
					GALL096	Diptera	Cecidomyiidae		CECI	LEAF	NOT	MID	F
					GALL184	Lepidoptera			OTHE	OTHER	NOT	MID	F
27	PSM	Asterids	Rubiaceae	*Psychotria micralabastra*	GALL101	Hemiptera	Aleyrodidae		HEMI	LEAF	NOT	LO	T
					GALL102	Diptera	Cecidomyiidae		CECI	LEAF	NOT	MID	F
28	PSS	Asterids	Rubiaceae	*Psychotria micrococca*	GALL106	Diptera	Cecidomyiidae		CECI	LEAF	NOT	MID	F
					GALL109	Hymenoptera	Eulophidae	*Ophelimus* sp. (La Salle)	OTHE	OTHER	NOT	MID	F
					GALL194	Diptera	Cecidomyiidae		CECI	LEAF	NOT	HI	F
					GALL195	Diptera	Cecidomyiidae		CECI	LEAF	NOT	LO	F
					GALL214	Insecta			UNKN	LEAF	NOT	LO	F
29	PAV	Asterids	Rubiaceae	*Pavetta platyclada*	GALL082	Insecta			UNKN	LEAF	HAIRY	LO	T
					GALL211	Hemiptera	Aleyrodidae		HEMI	LEAF	NOT	LO	T
30	TAR	Asterids	Rubiaceae	*Tarenna buruensis*	GALL134	Thysanoptera			OTHE	LEAF	NOT	MID	T
					GALL135	Insecta			UNKN	OTHER	NOT	MID	F
					GALL137	Diptera	Cecidomyiidae		CECI	LEAF	NOT	MID	F
31	TAB	Asterids	Apocynaceae	*Tabernaemontana aurantiaca*	GALL129	Hemiptera			HEMI	LEAF	NOT	LO	T
					GALL130	Insecta			UNKN	LEAF	NOT	LO	F
32	TBM	Asterids	Apocynaceae	*Tabernaemontana panacaqui*									

---

[1] Kolesik, P. & Butterill, P.T. 2015. New gall midges (Diptera: Cecidomyiidae) from Papua New Guinea. *Austral Entomology,* 54, 79-86

[2] Percy, Diana M., Butterill, Philip T. & Malenovský, Igor (2016) Three new species of gall-forming psyllids (Hemiptera: Psylloidea) from Papua New Guinea, with new records and notes on related species. *Journal of Natural History*, **50**:17-18, 1073-1101, DOI:10.1080/00222933.2015.1104394

[3] CECI=Cecidomyiidae, HEMI=Hemiptera, UNKN=Unknown insect, OTHE=Known taxa other than Cecidomyiidae and Hemiptera

[4] LEAF=Leaf gall, COMP=complex of multiple modules, OTHER=Non-leaf galls

[5] HAIRY=Hairy, NOT=Not hairy

[6] LO=low apparency, MID=Medium apparency, HI=High apparency

[7] F=False, T=True

Table S2. Parasitoid (morpho)species (Hymenoptera).

sp_code	superfamily	family	genus	species	identifier
CERP001	Ceraphronoidea	Ceraphronidae		sp. 1	Butterill, P.T.
CERP003	Ceraphronoidea	Ceraphronidae		sp. 2	Gibson, G.
MEGA001	Ceraphronoidea	Megaspilidae		sp. 1	Butterill, P.T.
APHE001	Chalcidoidea	Aphelinidae		sp. 1	Butterill, P.T.
APHE002	Chalcidoidea	Aphelinidae		sp. 2	Butterill, P.T.
ENCY001	Chalcidoidea	Encyrtidae		sp. 1	Butterill, P.T.
EULO003	Chalcidoidea	Eulophidae		sp. 1	Butterill, P.T.
EULO005	Chalcidoidea	Eulophidae		sp. 2	Butterill, P.T.
EULO036	Chalcidoidea	Eulophidae		sp. 3	Butterill, P.T.
EULO058	Chalcidoidea	Eulophidae		sp. 5	Gibson, G.
EULO059	Chalcidoidea	Eulophidae		sp. 6	Gibson, G.
EULO047	Chalcidoidea	Eulophidae	Aprostocetus	s10	Hansson, C.
EULO002	Chalcidoidea	Eulophidae	Aprostocetus	sp1	Hansson, C.
EULO010	Chalcidoidea	Eulophidae	Aprostocetus	sp11	Hansson, C.
EULO055	Chalcidoidea	Eulophidae	Aprostocetus	sp12	Hansson, C.
EULO016	Chalcidoidea	Eulophidae	Aprostocetus	sp13	Hansson, C.
EULO015	Chalcidoidea	Eulophidae	Aprostocetus	sp14	Hansson, C.
EULO025	Chalcidoidea	Eulophidae	Aprostocetus	sp15	Hansson, C.
EULO009	Chalcidoidea	Eulophidae	Aprostocetus	sp16	Hansson, C.
EULO037	Chalcidoidea	Eulophidae	Aprostocetus	sp17	Hansson, C.
EULO045	Chalcidoidea	Eulophidae	Aprostocetus	sp2	Hansson, C.
EULO018	Chalcidoidea	Eulophidae	Aprostocetus	sp3	Hansson, C.
EULO020	Chalcidoidea	Eulophidae	Aprostocetus	sp4	Hansson, C.
EULO019	Chalcidoidea	Eulophidae	Aprostocetus	sp5	Hansson, C.
EULO032	Chalcidoidea	Eulophidae	Aprostocetus	sp6	Hansson, C.
EULO024	Chalcidoidea	Eulophidae	Aprostocetus	sp7	Hansson, C.
EULO056	Chalcidoidea	Eulophidae	Aprostocetus	sp8	Hansson, C.
EULO057	Chalcidoidea	Eulophidae	Aprostocetus	sp9	Hansson, C.
EULO035	Chalcidoidea	Eulophidae	Asecodes	sp1	Hansson, C.
EULO031	Chalcidoidea	Eulophidae	Asecodes	sp2	Hansson, C.
EULO051	Chalcidoidea	Eulophidae	Baryscapus	sp1	Hansson, C.
EULO044	Chalcidoidea	Eulophidae	Chrysonotomyia?	sp12	Hansson, C.
EULO001	Chalcidoidea	Eulophidae	Chrysonotomyia	sp1	Hansson, C.
EULO053	Chalcidoidea	Eulophidae	Chrysonotomyia	sp10	Hansson, C.
EULO054	Chalcidoidea	Eulophidae	Chrysonotomyia	sp11	Hansson, C.
EULO006	Chalcidoidea	Eulophidae	Chrysonotomyia	sp2	Hansson, C.
EULO034	Chalcidoidea	Eulophidae	Chrysonotomyia	sp3	Hansson, C.
EULO013	Chalcidoidea	Eulophidae	Chrysonotomyia	sp4	Hansson, C.
EULO008	Chalcidoidea	Eulophidae	Chrysonotomyia	sp5	Hansson, C.
EULO049	Chalcidoidea	Eulophidae	Chrysonotomyia	sp6	Hansson, C.
EULO033	Chalcidoidea	Eulophidae	Chrysonotomyia	sp7	Hansson, C.
EULO048	Chalcidoidea	Eulophidae	Chrysonotomyia	sp8	Hansson, C.
EULO029	Chalcidoidea	Eulophidae	Chrysonotomyia	sp9	Hansson, C.
EULO046	Chalcidoidea	Eulophidae	Closterocerus	sp1	Hansson, C.
EULO041	Chalcidoidea	Eulophidae	Closterocerus	sp2	Hansson, C.
EULO026	Chalcidoidea	Eulophidae	Closterocerus	sp3	Hansson, C.

ELAS001	Chalcidoidea	Eulophidae	Elasmus	sp1	Hansson, C.
EULO042	Chalcidoidea	Eulophidae	Omphalentedon	sp1	Hansson, C.
EULO023	Chalcidoidea	Eulophidae	Pediobius	sp1	Hansson, C.
EULO028	Chalcidoidea	Eulophidae	Piekna	nitens (Boucek, 1988)	Hansson, C.
EULO043	Chalcidoidea	Eulophidae	Zaommomyiella	sp1	Hansson, C.
EULO017	Chalcidoidea	Eulophidae	Zaommomyiella	sp2	Hansson, C.
EUPE005	Chalcidoidea	Eupelmidae	Eupelmus	sp1	Gibson, G.
EUPE001	Chalcidoidea	Eupelmidae	Eupelmus	sp1	Gibson, G.
EUPE004	Chalcidoidea	Eupelmidae	Eupelmus	sp2	Gibson, G.
EUPE010	Chalcidoidea	Eupelmidae	Neanastatus	sp2	Gibson, G.
EUPE002	Chalcidoidea	Eupelmidae	Neanastatus	sp3	Gibson, G.
EUPE006	Chalcidoidea	Eupelmidae	Reikosiella	n.sp1	Gibson, G.
EUPE008	Chalcidoidea	Eupelmidae	Reikosiella	n.sp2	Gibson, G.
EUPE007	Chalcidoidea	Eupelmidae	Reikosiella	n.sp3	Gibson, G.
EUPE003	Chalcidoidea	Eupelmidae	Reikosiella	n.sp4	Gibson, G.
EURY013	Chalcidoidea	Eurytomidae		sp. 1	Butterill, P.T.
EURY005	Chalcidoidea	Eurytomidae	Mangoma	n. sp. 1	Gates, M.
EURY014	Chalcidoidea	Eurytomidae	Phylloxeroxenus	n. sp. 1	Gates, M.
EURY015	Chalcidoidea	Eurytomidae	Phylloxeroxenus	n. sp. 2	Gates, M.
EURY007	Chalcidoidea	Eurytomidae	Phylloxeroxenus	n. sp. 3	Gates, M.
EURY021	Chalcidoidea	Eurytomidae	Phylloxeroxenus	n. sp. 4	Gates, M.
EURY011	Chalcidoidea	Eurytomidae	Phylloxeroxenus	n. sp. 4	Gates, M.
EURY001	Chalcidoidea	Eurytomidae	Tenuipetiolus	n. sp. 1	Gates, M.
EURY009	Chalcidoidea	Eurytomidae	Tenuipetiolus	n. sp. 3	Gates, M.
EURY020	Chalcidoidea	Eurytomidae	Tenuipetiolus	n. sp. 4	Gates, M.
EURY019	Chalcidoidea	Eurytomidae	Tenuipetiolus	n. sp. 7	Gates, M.
TORY001	Chalcidoidea	Megastigmidae	n. gen. 1 nr. Paramegastigmus	n. sp. 1	Jansta, P.
TORY002	Chalcidoidea	Megastigmidae	n. gen. 1 nr. Paramegastigmus	n. sp. 2	Jansta, P.
MYMA001	Chalcidoidea	Mymaridae		sp. 1	Butterill, P.T.
MYMA004	Chalcidoidea	Mymaridae		sp. 3	Butterill, P.T.
MYMA005	Chalcidoidea	Mymaridae		sp. 4	Butterill, P.T.
MYMA003	Chalcidoidea	Mymaridae		sp. 2	Butterill, P.T.
ORMY001	Chalcidoidea	Ormyridae	Ormyrus	n. sp. 1	Gates, M.
PERI001	Chalcidoidea	Perilampidae	Perilampus	sp. 1	Butterill, P.T.
PTRM015	Chalcidoidea	Pteromalidae		sp. 1	Hansson, C.
PTRM001	Chalcidoidea	Pteromalidae		sp. 2	Butterill, P.T.
PTRM010	Chalcidoidea	Pteromalidae	Cecidelllus	n.sp.	Gibson, G.
TETR002	Chalcidoidea	Tetracampidae	Tetracampe	sp1	Hansson, C.
TETR004	Chalcidoidea	Tetracampidae	Tetracampe	sp2	Hansson, C.
TETR001	Chalcidoidea	Tetracampidae	Tetracampe	sp3	Hansson, C.
TRIC001	Chalcidoidea	Trichogrammatidae		sp. 1	Butterill, P.T.
TRIC002	Chalcidoidea	Trichogrammatidae		sp. 2	Butterill, P.T.
BRAC009	Ichneumonoidea	Braconidae	Spathius (Doryctinae)	dq.2	Quicke, D.
BRAC002	Ichneumonoidea	Braconidae		sp. 4	Butterill, P.T.
BRAC007	Ichneumonoidea	Braconidae	Spathius (Doryctinae)	dq.1	Quicke, D.

BRAC008	Ichneumonoidea	Braconidae	Bracon (Braconinae)	dq.6	Quicke, D.
PLAT004	Platygastroidea	Platygastridae	Inostemma	butterilli (Buhl, 2013)	Buhl, P.N.
PLAT006	Platygastroidea	Platygastridae	Inostemma	convexifrons (Buhl, 2013)	Buhl, P.N.
PLAT003	Platygastroidea	Platygastridae	Inostemma	macarangae (Buhl, 2013)	Buhl, P.N.
PLAT005	Platygastroidea	Platygastridae	Inostemma	novoguineense (Buhl, 2013)	Buhl, P.N.
PLAT009	Platygastroidea	Platygastridae	Inostemma	paramyristicae (Buhl, 2013)	Buhl, P.N.
PLAT008	Platygastroidea	Platygastridae	Platygaster	psychotriae (Buhl, 2013)	Buhl, P.N.
PLAT010	Platygastroidea	Platygastridae	Synopeas	butterilli (Buhl, 2013)	Buhl, P.N.
PLAT002	Platygastroidea	Platygastridae	Synopeas	n. sp. 1	Buhl, P.N.
PLAT007	Platygastroidea	Platygastridae	Synopeas	psychotriae (Buhl, 2013)	Buhl, P.N.
PLAT001	Platygastroidea	Platygastridae	Synopeas	pterocarpi (Buhl, 2013)	Buhl, P.N.

Table S3. Results of Generalised Linear Models (GLMs) for the number of parasitoid species against minimum host gall abundance (host qty) and several galls traits: gall-forming taxon (taxon), gall physical apparency (apparency), gall openness (open), galled plant module (module), and gall pubescence (pubescence). *P* values from analysis of deviance tests report the significance of the dropping the parameter from the model. Significant parameters should, therefore, be retained in the model.

Model Parameters	Residual d.f.	Residual Deviance	Std. Err.	AIC	Explained Deviance (%)	*P*
Host Qty Model	76	79.1	0.38	261.4	29.2	
Log(Host qty)						< 0.001 ***
Taxon Model	73	78.0	0.73	251.3	43.5	
Taxon						< 0.001 ***
Log(Host qty)						< 0.001 ***
Apparency Model	74	80.6	0.80	250.7	42.6	
Apparency						< 0.001 ***
Log(Host qty)						< 0.001 ***
Open Model	75	77.0	0.43	257.6	34.6	
Open						< 0.05 *
Log(Host qty)						< 0.001 ***
Module Model	74	78.4	0.39	263.9	30.6	
Module						> 0.1
Log(Host qty)						< 0.001 ***
Pubescence Model	75	79.5	039	263.2	29.5	
Pubescence						> 0.1
Log(Host qty)						< 0.001 ***

Table S4. Results of Generalised Linear Models (GLMs) for the number of parasitoid species against minimum host gall abundance (host qty) and galls traits exclusively from galls in the family Cecidomyiidae, gall traits include: gall-forming taxon (taxon), gall physical apparency (apparency), gall openness (open), galled plant module (module), and gall pubescence (pubescence). *P* values from analysis of deviance tests report the significance of dropping each parameter from the model. Significant parameters should, therefore, be retained in the model.

Model Parameters	D.f.	Deviance	Std. Err.	AIC	Explained deviance (%)	*P*
Full model	24	35.7	2.01	146.5	47.4	
Apparency						0.002 **
Open						0.906
Module						0.022 *
Pubescence						0.970
Log(Host qty)						0.223
Drop Pubescence	25	35.7	2.01	144.6	47.4	
Apparency						< 0.001 ***
Open						0.890
Module						0.015 *
Log(Host qty)						0.221
Drop Open	26	35.6	2.00	142.6	47.4	
Apparency						< 0.001 ***
Module						0.011 *
Log(Host qty)						0.223
Drop Host qty (Final model)	27	37.8	2.32	253.8	48.1	
Apparency						< 0.001 ***
Module						0.003 **

Chapter 3

Vertical stratification and defensive traits of caterpillars against parasitoids in a lowland tropical forest in Cameroon

Sam Finnie, Philip Butterill, Vojtech Novotn, Conor Redmond, Leonardo Ré Jorge, Tomokazu Abe, Greg P.A. Lamarre, Vincent Maicher, and Katerina Sam

Oecologia (2024): 1-16.

DOI: 10.1007/s00442-024-05542-x

(IF = 2.7)

Vertical stratification and defensive traits of caterpillars against parasitoids in a lowland tropical forest in Cameroon

Sam Finnie[1,2], Philip Butterill[1,2], Vojtech Novotny[1,2], Conor Redmond[1,2], Leonardo Ré Jorge,[1,2], Tomokazu Abe[3], Greg P.A. Lamarre[1,4], Vincent Maicher [1,2,5], Katerina Sam[1,2]

[1] Biology Centre of the Czech Academy of Sciences, Institute of Entomology, České Budějovice, Czech Republic

[2] Faculty of Science, University of South Bohemia, České Budějovice, Czech Republic

[3] Department of Biology, Faculty of Science, Chiba University, Chiba, 263-8522 Japan

[4] Smithsonian Tropical Research Institute, Apartado 0843-03092, Balboa, Ancon, Panamá

[5] The Nature Conservancy (TNC), Libreville, Gabon

Abstract

Insect herbivores and their parasitoids play a crucial role in terrestrial trophic interactions in tropical forests. These interactions occur across the entire vertical gradient of the forest. This study compares how caterpillar communities, and their parasitism rates, vary across vertical strata and between caterpillar defensive strategies in a semi deciduous tropical forest in Nditam, Cameroon. Within a 0.1 ha plot, all trees with a diameter at breast height (DBH) ≥5 cm were felled and systematically searched for caterpillars. We divided the entire vertical gradient of the forest into eight, five-meter strata. All caterpillars were assigned to a stratum based on their collection height, reared, identified, and classified into one of three defensive traits: aposematic, cryptic and shelter-building. Caterpillar species richness and diversity showed a midstory peak, whereas density followed the opposite pattern, decreasing in the midstory and then increasing towards the highest strata. This trend was driven by some highly dense shelter-building caterpillars in the upper canopy. Specialisation indices indicated decreasing levels of caterpillar generality with increasing height, a midstory peak in vulnerability, and increasing connectance towards the upper canopy, although the latter was likely driven by decreasing network size. Both aposematic and shelter-building caterpillars had significantly higher parasitism rates than cryptic caterpillars. Our results highlight nuanced changes in caterpillar communities across forest strata and provide evidence that defences strategies are important indicators of parasitism rates in caterpillars and that both aposematic and shelter-building caterpillars could be considered a "*safe haven"* for parasitoids.

Introduction

Approximately 75% of all terrestrial trophic relationships involve insect herbivores, host plants, and parasitoids (Slinn et al. 2018) with the vast majority of these interactions occurring in tropical forests (Amorim et al. 2022). When studying these interactions, it is imperative to consider the entire vertical gradient of the forest, as many of these interactions happen high up in the canopy (Schowalter and Chao 2021). Due to the inaccessibility of the canopy, the majority of studies that focus on assemblages of insects in tropical forests are limited to saplings in the understory or focus on communities occurring solely on focal tree species or individuals. The few studies on caterpillar communities and their parasitism rates that include the entire vertical gradient of the forest, generally divide the forest into a maximum of three strata, understory, midstory and canopy (Šigut et al. 2018; Seifert et al. 2020a). Segregating the forest in this way gives us an oversimplified view of the vertical changes in insect communities and only allows direct comparisons between broadly defined strata, masking potential patterns that occur across the entire vertical gradient of the forest. Tropical forests are multi-layered ecosystems where the spatial dynamics of different tree species across vertical strata create a mosaic of microhabitats that alter the community structure of insect inhabitants at a nuanced level and they should be viewed as such (Moffett 2013). Here, we divide the forest into multiple, equally sized strata which allows us to uncover incremental patterns and changes whilst still providing an objective, standardised method of investigating the stratification of insect communities.

Lepidopteran caterpillars are the ideal study group when comparing communities of herbivorous insects across vertical gradients. This highly diverse order has an estimated 255,000 extant species, with over 150,000 already described, and is one the largest radiations of phytophagous insects (Menken et al. 2010; Mitter et al. 2017). Being apterous and having limited mobility allows caterpillars to be sampled with relative ease and ensures that they are likely to be found on their associated host plant within the vertical stratum that they occur at naturally.

In tropical forests, it is likely that the midstory, with its abundant young foliage, favourable climatic conditions and and increased overlap of host plant species and microclimates (Basset 2001; Hirao et al. 2009) leads to an increase in caterpillar species richness, diversity, and density, although this has never been explicitly studied. In contrast, the harsh weather conditions (Ulyshen 2011; Nakamura et al. 2017), and reduced foliage quality (Coley and Barone 1996; Murakami et al. 2005) in the upper canopy, lead to a decline in caterpillars populations. Additionally, in tropical adult lepidopteran assemblages, neighbouring strata have the highest similarity likely due to the overlap in biotic (e.g. plant species composition and leaf quality) and abiotic (e.g. light penetration, temperature humidity and wind speed) conditions (Intachat and Holloway 2000; Schulze et al. 2001). Changes in caterpillar composition at higher taxonomic levels also shape the vertical stratification of caterpillar assemblages due to family-specific height preferences (Brehm 2007; Smedt et al. 2019). Larger caterpillars are more prevalent in the understory and smaller species are more prevalent in the canopy (Seifert et al. 2020a). This pattern is frequently observed in insects from tropical systems (Wardhaugh 2014). Specialisation in host plant caterpillar networks has been observed to increase towards the upper canopy (Seifert et al. 2020a). This is likely due to the fewer plant species and more specialised caterpillar species present in these upper strata. The trend is driven by the dominant feeding guild of shelter-building caterpillars (i.e. caterpillars that construct protective structures for themselves), which are generally more specialised than exposed feeders (caterpillars feeding openly on their host plants) (Le Corff and Marquis 1999; Seifert et al. 2020a). Despite these findings, there remains a scarcity of studies on the vertical stratification of larval Lepidoptera, with the majority focusing solely on adult communities (e.g. Schulze et al. 2001; Stork and Grimbacher 2006; Ashton et al. 2016; Amorim et al. 2022) or temperate forests (e.g. Šigut et al. 2018; Seifert et al. 2020a). Addressing this gap, our study aims to provide a novel insight into the subtle variations within caterpillar communities to uncover fine-scale changes across a vertical tropical forest gradient.

Previous studies of parasitism rates on caterpillars are often derived as a by-product from large-scale caterpillar rearing experiments in which parasitoids would often emerge from reared caterpillars. These experiments were primarily designed to assess herbivore communities, and to compare between herbivore feeding guilds. As a direct outcome of this focus, caterpillar hosts were classified into either exposed or concealed feeders. These studies have shed light on how these guilds can vary in their susceptibility to parasitoids and other predators, thereby shaping their distributions across vertical strata (e.g. Hrcek et al. 2013; Šigut et al. 2018). However, feeding guilds are not the sole determinants of parasitism rates in caterpillars. Defensive traits, such as aposematism, crypticity, and shelter-building also play a significant role.

Aposematic caterpillars use visual and chemical signals to advertise their unpalatability, often sequestering toxins from their host plants to deter predators (Aslam et al. 2020). However, this chemical sequestration may compromise the caterpillar's immune response to parasitoids rendering them more susceptible to parasitism. Interestingly, it is this unique combination of a compromised immune system and enhanced predator protection that can make aposematic caterpillars ideal hosts for parasitoids, a phenomenon known as the 'safe haven' hypothesis (Dyer and Gentry 1999; Gentry and Dyer 2002; Smilanich et al. 2009).

Cryptic caterpillars employ specific coloration or mimicry to camouflage themselves from predators. Cryptic caterpillars, like aposematic caterpillars, are exposed feeders. However, they lack the chemical or visual defences that aposematic caterpillars possess, making them more vulnerable to predation, particularly from visually oriented predators such as birds (Tvardikova and Novotny 2012).

This vulnerability might render them less suitable hosts for parasitoids. Parasitoids themselves are susceptible to intraguild predation (Frago 2016) and have been shown to prolong the developmental time of their hosts (Chen et al. 2017). This extended exposure increases the likelihood of the caterpillars being preyed upon, further reducing their suitability as hosts for parasitoids

Shelter-building caterpillars construct physical shelters around themselves by rolling or tying leaves, creating a defensive barrier against predators and parasitoids. This defensive trait has been associated with increased parasitism rates compared to exposed caterpillars (Hawkins 1994; Hrcek et al. 2013; Šigut et al. 2018). Similar to aposematic caterpillars, shelter-building caterpillars may also provide a “safe haven” for parasitoids due to their enhanced protection from predators (Covarrubias-Camarillo et al. 2016). Furthermore, shelter-building caterpillars are more specialised on their host plants than exposed feeders (Menken et al. 2010), which has been linked to increased parasitism rates (Hrcek et al. 2013) Finally, shelter-builders are easier for parasitoids to locate than exposed feeders due to their sessile nature, in contrast, exposed feeders will often leave their feeding sites as they search for new leaves, making it more difficult for parasitoids to rely on olfactory cues to locate them.

Here we investigate various aspects of how a caterpillar community in a tropical forest in Cameroon was vertically structured as well as how their defensive traits effect parasitism rates, by testing four hypotheses:

1. We expect caterpillar species richness, diversity and density will be highest in the midstory due to higher amounts of foliage combined with favourable biotic and abiotic conditions (Basset 2001; Hirao et al. 2009), and lowest in the upper strata where there is expected to be reduced foliage quality and harsher abiotic conditions, that can only be exploited by specialist species (Coley and Barone 1996; Basset et al. 2003; Murakami et al. 2005; Ulyshen 2011).

2. We expect compositional turnover in caterpillar communities to increase between neighbouring strata towards the uppermost canopy, where changes in biotic and abiotic conditions become more drastic. We also expect to see increased dissimilarity between caterpillar communities with increased

distance between the upper and lower strata, where conditions become more distinct. (Intachat and Holloway 2000; Schulze et al. 2001).

3. Network specialisation will increase towards the upper strata due to increased specialisation in the caterpillar community. We expect that this pattern will arise due to the greater abundance of specialised shelter-building caterpillars observed in previous studies (Le Corff and Marquis 1999; Seifert et al. 2020a).

4. Parasitism rates are expected to be highest in aposematic and shelter-building caterpillars that provide a “safe haven” for parasitoids as hosts and parasitism rates will decrease with increased canopy height where conditions become less favourable for both parasitoids and their hosts (Chaij et al. 2016; Vosteen et al. 2020)

Materials and Methods

Study site

We conducted our sampling in a 0.1-ha plot of semi-deciduous tropical forest in the village of Nditam (province of Mbam et Kim), Cameroon in West Africa (5° 22' N, 11° 13' E and 709 m a.s.l.). Our forest plot was marked in a mosaic of late-secondary and primary forest and savannahs. We chose this patch as it was the least disturbed patch of forest in the nearby area destined for logging. Sampled tree height within the plot ranged from 4 to 42 meters. There was a mean annual temperature of 29°C, annual precipitation 2383 mm, and 72% mean annual humidity (measured by the local weather station). Sampling took place between the 1st of April and the 26th of June 2019, which corresponds with the “*light*” rainy season. This area of Cameroon is characterised by four seasons: a light rainy season from May to June, a short dry season from July to October, a heavy rainy season from October to November, and a long dry season from December to May.

Sampling design

The plot was marked out, ensuring there were no forest edges, gaps or roads within a 150m radius of the chosen area. All trees with a diameter at breast height (DBH) ≥ 5 cm were then felled one at a time.

The trees were felled in a specific order to minimize disturbance to the surrounding trees, starting from the smallest and progressing to the largest. Immediately after felling, trees were thoroughly and systematically searched by 5-15 assistants for caterpillars, ensuring all individuals were collected including any that were displaced from the tree as it fell. All felled trees were identified to species, except for some species in the *Drypetes* genus, one *Ficus* and one *Chytranthus* that could only be identified to morphospecies (Appendix 2). This plot-based approach has proven successful in the assessment of communities of apterous arthropod herbivores (see Volf et al. 2019).

We recorded the exact height (measured from the base of the tree) where each caterpillar individual was located. Each caterpillar was photographed **(**Canon EOS 700D; 60 mm macro lens**)** and measured (total length in mm). Caterpillars were then placed individually in aerated rearing containers and given leaves from the host plant on which they were found. Rearing continued until either an adult Lepidoptera emerged, or the individual died. In some cases, parasitoids would emerge from caterpillars during rearing. Parasitoids and caterpillars were stored in 96% DNA grade ethanol and adult Lepidoptera were pinned for future identification. This method of no-choice rearing allows for successful associations between host plants, caterpillars, and parasitoids to be determined (Lill et al. 2002). Caterpillars were categorized into one of three groups: aposematic, cryptic, and shelter-building. A caterpillar was deemed aposematic if it was exposed, had bright or contrasting colours or if it had prominent hairs, spines, or bristles which although not always strikingly coloured, are still considered aposematic (Caro and Ruxton 2019). Cryptic caterpillars were, by default, any exposed caterpillars that were not considered aposematic due to their plain colouration or benign morphology. Shelter-building caterpillars were any concealed caterpillars that were found within a leaf tie, roll or a self-constructed case. All caterpillars were assigned exclusively to one of these categories, in rare cases, a caterpillar would exhibit aposematic characteristics but still be a shelter-builder, in these instances, they were always classified as shelter-building as their visual characteristics are redundant whilst they are concealed within a shelter. These three categories encompassed all caterpillars collected in this study.

For every sampled tree, the total tree height, trunk height, crown height and maximum crown width were measured. All leaves were stripped from each tree and categorized into mature or young leaves which were then placed into separate bags. A subset of leaves was taken randomly from each bag and were then spread over a white leaf frame (50 x 50 cm board), photographed and then weighed. The specific leaf area (SLA) for young and mature leaves was then calculated by dividing the total surface area (calculated using the software (ImageJ v1.48) of the leaves on the leaf frame(s) by their total dry mass. For trees with larger leaves, multiple leaf frames were used, and their dry mass was combined to calculate SLA. The bags containing all the leaves were then weighed and total surface areas were calculated by multiplying the SLA by the total try mass. The total surface area per tree crown was calculated by combining the total surface area for all the young leaves and all the mature leaves. In some cases on very large trees, an estimated 25% or 50% of total leaves were weighed and the total was quadrupled or doubled respectively, to approximate the total weight of all leaves for that tree. Similar methods are often used to calculate total leaf area (e.g. Sam et al. 2020; Houska Tahadlova et al. 2023)

Vertical stratification

The vertical gradient of the forest plot was divided into 8 equally sized strata of 5 meters: 0–5 m, 5–10 m, 10–15 m, 15–20 m, 20–25 m, 25–30 m, 30–35 m and 35-40 m. The vertical strata of the forest are grouped into three categories: the lower strata (0-10m), the midstory strata (10-30m), and the upper strata (30-40m). Within the midstory strata, we further identify the low midstory (10-20m), the central midstory (15-25m), and the upper midstory (20-30 m) for more specific observations. This terminology enables us to articulate general patterns across the forest's vertical gradient without the need to reference individual strata. There were three trees that marginally exceeded 40 meters in height, but this additional stratum was not included in our analyses as it contained no caterpillars, and the total surface area was deemed too small. To date there is no unified method of segmenting vertical forest layers, however previous studies adhere to keeping each layer the same size (Parker and Brown 2000; Seifert et al. 2020a; Amorim et al. 2022). Each caterpillar was assigned to a given stratum dependent on the height at which it was found on the tree. Most tree crowns in our plot were spread across multiple strata so total leaf area of the crown was divided proportionally for each stratum. This division enabled separate estimation of caterpillar densities for each stratum. To facilitate this, the volume of a spheroid was used to approximate crown volume (V_{crown}) using the equation:

$$V_{crown} = \frac{4}{3}\pi a^2 c,$$

Here, *a* is the horizontal radius of the crown (0.5 × maximum crown width) and *c* is the vertical radius (0.5 × crown height). When one of the upper or lower caps of the crown occurred within a stratum the total volume was calculated using the equation:

$$V_{cap} = \frac{\pi a^2}{3c^2} h^2 (3c - h),$$

Here, *h* is the height of the crown cap, and *a* and *c* represent the same as for crown volumes. When a stratum occurred within the centre of the crown (the top and bottom parts of the crown were not present within the stratum) then the volume was calculated by subtracting the volume of the cap above and below and subtracting their combined total volume from the total volume of the spheroid.

Additionally, we calculated the surface area of each tree trunk within a given stratum assuming each tree trunk to be cylindrical using the equation:

$$A_{trunk} = 2\pi \mathrm{rh},$$

This equation calculates the lateral surface area of the trunk where r is the radius of the trunk (0.5 x tree diameter) and *h* is the length of trunk within a given stratum. For this study, the trunk and crown were considered two distinct parts of the tree. The total height of the trunk ended where the bottom of the tree crown began. In cases where a stratum contained sections of both crown and tree trunk, leaf and trunk area were combined. This provided us with a standardised method of calculating the total amount of occupiable area, hereafter referred to as total surface area, for a caterpillar within a given stratum for each tree individual in our plot. The use of spheroids is commonplace when analysing the structure of forests (e.g. Chen et al. 2005; Walcroft et al. 2005; Seifert et al. 2020a). However, the addition of trunk surface area is a novel concept. This approach provides a comprehensive representation of the entire tree, acknowledging that branches and foliage can occur along the trunk, even before the 'defined' tree crown. In our study, 13% of caterpillars were found below the crown, emphasising the importance of including these areas for an accurate reflection of ecological reality and caterpillar distribution. Additionally, this method accounts for species- and age-specific trunk-to-crown ratios, ensuring a realistic representation of tree structure within our study..

Insect identification

All Lepidoptera and parasitoid specimens were identified as far as possible taxonomically and assigned to a morphotype where appropriate based on their physical characteristics. At least one specimen from each morphotype was then barcoded at the Canadian Centre for DNA Barcoding (CCDB; Guelph, Canada) using standard Sanger sequencing protocols (Wilson 2012). In instances where a definitive identification was not possible, the 'Barcode Index Number System' (BIN system; Ratnasingham and Hebert 2013) was used. This method allowed us to distinguish between putative species and has been adopted in many ecological studies on Lepidoptera in recent years (e.g. Delabye et al. 2019; Hausmann et al. 2020). All preserved Lepidoptera and parasitoid specimens are deposited at the Institute of Entomology in České Budějovice (Czech Republic).

Statistical analyses

All statistical analyses were performed using the statistical software R version 4.2.2 (R Development Core Team 2022).

Based on sample sizes, we opted not to include parasitoids in any density-related analyses or network analyses. The low number of parasitoid individuals after dividing the data into eight strata was insufficient for any robust analyses. Parasitoids were only included in analyses that specifically focused on the percentage of individuals within a given population that were successfully parasitised, hereafter referred to as parasitism rate.

All linear models used in our analyses were developed using the '*lme4´* package in R (Bates et al. 2014). For each model, tree individual (N = 142) nested within tree species (N = 44) were included as random factor. Both variables used as a random factor have been shown to alter caterpillar-parasitoid communities (e.g. Šigut et al. 2018). All best-fitting linear models in our analyses were tested against the null model using both Akaike information criterion (AIC) using the '*bbmle*' package (Bolker 2017) and Analysis of Variance (ANOVA) using the '*lmerTest*' package (Kuznetsova et al. 2017) to estimate the P value. When comparing the effects of the different caterpillar defensive traits, we did pairwise comparisons using estimated marginal means (EMMs) using the '*emmeans*' package (Lenth 2023) to calculate P values between each group.

Species richness, diversity, and community composition

Caterpillar species richness (SR) was calculated as the total number of caterpillar species per stratum. To compare diversity between strata we calculated Shannon diversity indices (H') for each strata using the 'diversity' function in the '*vegan*' package (Oksanen 2010). We generated 1000 bootstrap replicates of each diversity index using the '*boot*' package for each stratum, we then calculated the standard error of these replicates to estimate the standard error for each index.

To compare proportional composition of the most common caterpillar families (min. total abundance ≥ 100) among strata we used Chi squared contingency tests. For these, we adjusted the P values using the Bonferroni correction to account for multiple comparisons and reduce the risk of type I error. To compare overlap, we calculated pairwise Morisita-Horn (D_{MH}) dissimilarity index (Morisita 1959; Horn 1966) values between strata of caterpillar assemblages using the '*vegdist*' function in the '*vegan*' package. This index is based on the abundance of species and was chosen because of its robustness to variations in sample sizes and diversities as it is less affected by the presence of rare species (Beck et al. 2013).

After the removal of singletons, to increase the robustness of richness estimates (Lim et al. 2012), individual-based rarefaction and extrapolation curves for species richness, were calculated for each stratum and between defensive traits using the '*iNEXT*' package (Hsieh et al. 2016). Species richness estimates (SChao) were calculated for each strata based on asymptotic diversity (Chao and Jost 2015). Additionally, confidence intervals (CI) were calculated and plotted; nonoverlapping CI indicate significant differences between strata (Colwell et al. 2004).

Caterpillar density

To ensure all comparisons between strata were standardised, caterpillar densities (individuals per m² of total leaf + trunk area) were calculated for a given tree species in each stratum. Caterpillars that were found within a stratum containing a total surface area of less than 1m² of foliage for a given tree species were excluded from the dataset (3.4%). To meet the assumption of normality, we log10-transformed the density values prior to further analyses. When comparing density patterns across the entire vertical gradient, median height values of each stratum were substituted so that height could be treated as a continuous variable within our models. Two linear mixed models (LMMs) were developed to test the density distribution of all caterpillars across strata and caterpillar defensive traits across strata. For both LMMs, a second-degree polynomial distribution was used to approximate the expected density pattern across the vertical gradient.

Network specialisation

Quantitative, density-based interaction matrices were created for each stratum and analysed using the R package "*bipartite"* (Dormann et al. 2009). Density values were preferred over raw abundances when comparing networks to account for differences in vegetation between strata. To compare specialisation, we used three quantitative network indices that account for interaction frequencies. These indices are less affected by differences in sample size and sampling effort than qualitative indices and thus reflect the network structure more realistically (Banašek-Richter et al. 2004; Blüthgen et al. 2006). They are derived using Shannon diversity indices. We calculated weighted connectance, weighted generality, and weighted vulnerability using the 'networklevel' function implemented in the R package '*bipartite*' to characterize the interactions networks for each stratum. Weighted connectance is the proportion of realized interactions measured as the proportion of links weighted by interaction frequency. Weighted generality and vulnerability are two indices that describe the feeding relationships between caterpillar species and host plants. Weighted generality indicates the average number of host plants that a caterpillar species feeds on, while vulnerability indicates the average number of caterpillar species that feed on a plant species. They are both weighted by interaction strength. Generality and vulnerability indicate the specialisation of a certain trophic level (resource level: vulnerability; consumer level: generality). Only plant species interacting with at least one caterpillar species were considered for all calculated network metrics.

To interpret index values for connectance, weighted generality, and weighted vulnerability, we used null model simulations of the interaction networks for each stratum. To generate null models, we used the 'vaznull' function available in the '*bipartite*' package to randomize the interaction network matrix 999 times within each stratum. These null models were constrained by connectance, with marginal totals proportional to the observed ones (Vázquez et al. 2007), and we measured all network metrics in these random networks, creating a null distribution for each index. The use of null models allows us to gain a better understanding of network properties beyond what we can observe from the index values alone (Dormann et al. 2009).

To account for network size, we calculated standardized effect sizes (Z-scores) and corresponding P values for each specialisation index. This allowed us to compare the interaction networks and determine the degree of specialisation in each. An increase in Z-scores indicates an increase in specialisation between the networks, while a decrease in Z-scores indicates a decrease in specialisation.

Parasitism rates

Parasitism rates were calculated for each stratum for all caterpillars and separately for caterpillars from each defensive strategy. The effect of caterpillar defensive traits on parasitism was tested by a Generalized Linear Model (GLM). An additional GLM was designed to determine whether parasitism was affected by vertical strata (i.e. median strata height).

Results

We sampled caterpillars on a total of 142 trees (DBH ≥ 5 cm) from 44 species and 19 families growing within the 0.1 ha plot, only 1 tree species represented by a single individual could not be identified. The trees provided more than 5600 m² of surface area on which the caterpillars were collected (850 m² of trunk area and 4750 m² of leaf area). In total, we sampled 1675 caterpillars from 248 species and 17 families. In total, 1554 caterpillars (92.8%) were successfully assigned to a stratum, and a host plant species. This included 379 aposematic caterpillars from 66 species, 543 cryptic caterpillars from 109 species, and 632 shelter-building caterpillars from 72 species (Table S1). In total, 121 individuals (7.2%) could not be categorized into a stratum and were therefore excluded from density-related analyses. Caterpillar abundance varied across strata increasing from 87 individuals in the lowest stratum (0-5 m) to 331 individuals at 10-15 m. Total surface area varied from 1241.11 m² at 5-10 m to 271.20 m² in the highest stratum (35-40 m) (Table 1). Among tree species, *Hylodrendron gabunense* harboured the highest diversity of caterpillars (69 spp.) and there were 11 tree species on which no caterpillars occurred. The most abundant caterpillar species was a Crambid, likely from the genus *Coachena* (BOLD:AEE1691) with 189 individuals.

Table 1. Total surface area (trunk + leaf area on which caterpillars were collected), and the abundance and diversity of caterpillars and plants within each vertical stratum and for all strata combined (Total).

Stratum	**Total surface area (m²)**	**Caterpillar**				**Plant**		
		Abundance	**Species richness**	**Family richness**	**Shannon diversity**	**Abundance**	**Species richness**	**Family richness**
0-5m	416.73	87	37	11	2.37	131	44	17
5-10m	1241.11	243	82	20	3.38	130	44	17
10-15m	903.70	331	87	16	3.23	80	36	14
15-20m	913.97	304	92	18	3.31	46	23	13
20-25m	789.08	230	73	16	3.94	33	18	10
25-30m	457.56	172	64	16	2.83	18	11	5
30-35m	609.85	108	44	11	2.52	10	10	5
35-40m	271.20	67	25	7	2.30	6	6	3
Total	5603.20	1554	248	17	5.12	142	55	19

Species richness, diversity and community composition

The most abundant families (>100 caterpillar individuals) across the entire vertical gradient were Erebidae (405 indiv.), Geometridae (222 indiv.), Crambidae (214 indiv.), Totricidae (137 indiv.) and Pyralidae (120 indiv.). Over 73% of all caterpillars belonged to one of these five families. Each of these families were present in every stratum, with the exception of the highest stratum (35–40 m) from which there were no caterpillars from Erebidae and Crambidae (**Fig. S1**). The most speciose families were Erebidae (54 spp.) and Geometridae (47 spp.) which collectively accounted for 40% of all species in this study.

Overall caterpillar species richness increased from 37 species in the lowest stratum (0-5 m) to 92 species in the at 15-20 m, where it peaked, and then declined towards the highest stratum (35-40 m, 35 species). Shannon diversity indices (H') indicate that diversity was highest (H' = 3.94) at 20-25 m where there was also the lowest SE, and lowest (H' = 2.30) in the highest stratum (35-40 m) (**Fig. 1**).

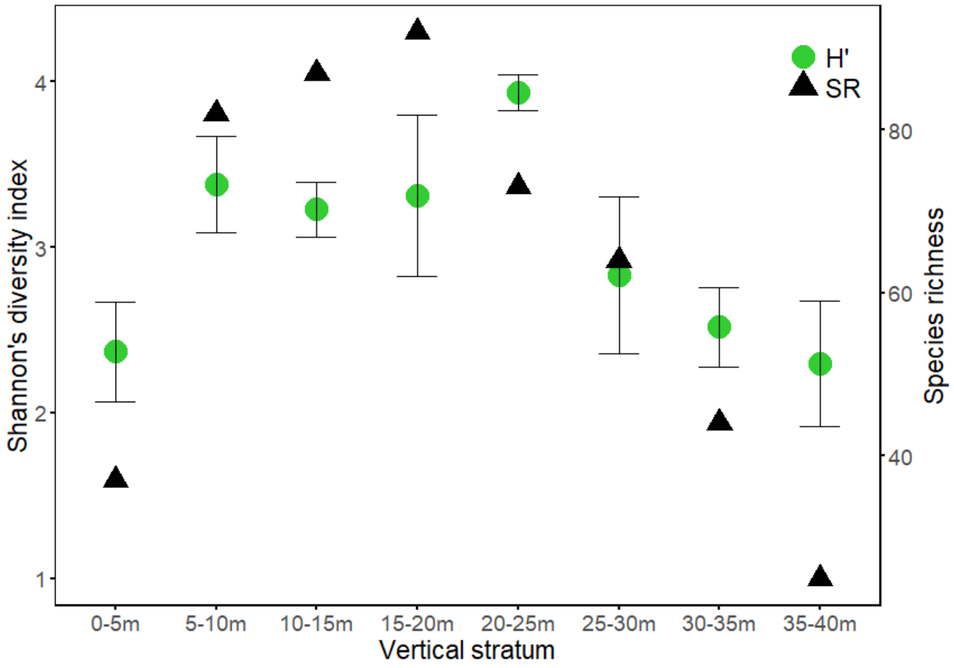

Fig. 1 Shannon diversity index (H') and its standard error (green dot and whiskers, left Y axis) and observed species richness (SR) (black triangles, right Y axis) of caterpillars along vertical forest strata in a tropical forest in Cameroon.

Proportional abundance of the 5 most common families was significantly different among strata across the entire gradient ($X^2 = 257.9$, df = 28, $P<0.001$). Only four of the pairwise comparisons did not significantly differ, all of which were between neighbouring strata. Compositional turnover was highest between 10-15 m and 30-35 m . There was no visible trend in turnover between neighbouring strata, with increasing height (**Fig. 2a**). Based on Morisita-Horn dissimilarity indices, the highest compositional turnover was between 10-15 m and 30-35 m (DMH = 0.417). There was no visible trend in dissimilarity with increasing distance between strata (**Fig. 2b**).

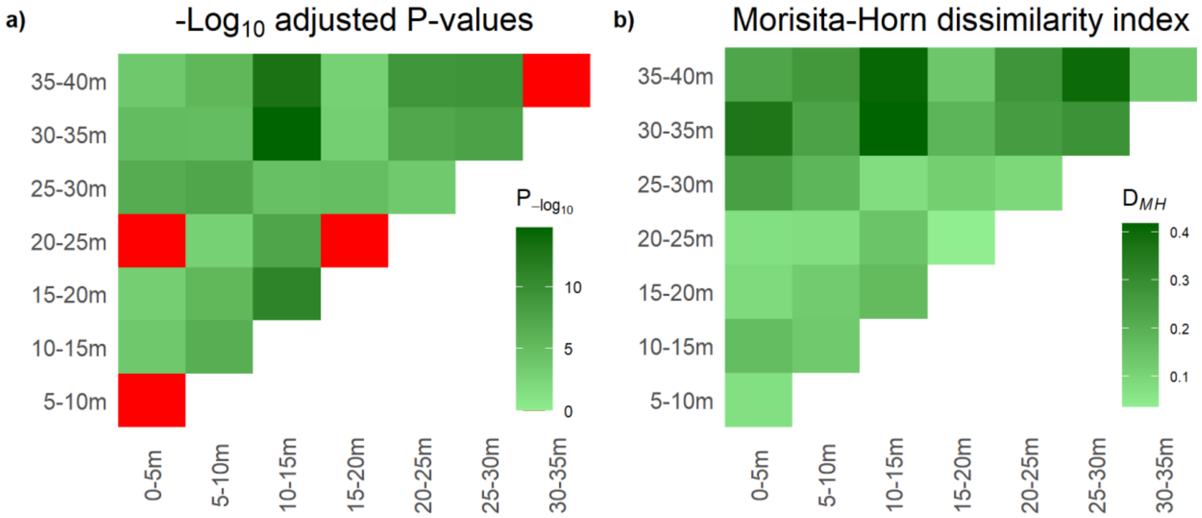

Fig. 2 Heatmaps showing pairwise comparisons of caterpillar communities between strata across a vertical forest gradient in a tropical forest in Cameroon. Values in a) show $-\log_{10}$ adjusted P-values from pairwise, Bonferroni corrected Chi Squared contingency tests, where darker shades indicate larger significant differences in caterpillar family proportions between strata and red boxes indicate no significant difference and b) Morisita-Horn dissimilarity indices (D_{MH}) where darker shades indicate a higher compositional turnover of caterpillar species between strata and lighter shades indicate higher overlap between caterpillar communities.

The species richness curves reveal two distinct strata groups, with an intermediate stratum between them. All strata between 5-10 m and 20-25 m are the most species-rich, with significantly more species than the other four strata. The least species-rich are the lowest (0-5 m) and two highest strata (30-35 m and 35-40 m). The third highest stratum (25-30 m) serves as an intermediate, with its species richness significantly lower than the most species-rich group and higher than the least. The asymptotic nature of all curves suggests comprehensive species sampling in each stratum (**Fig. 3**).

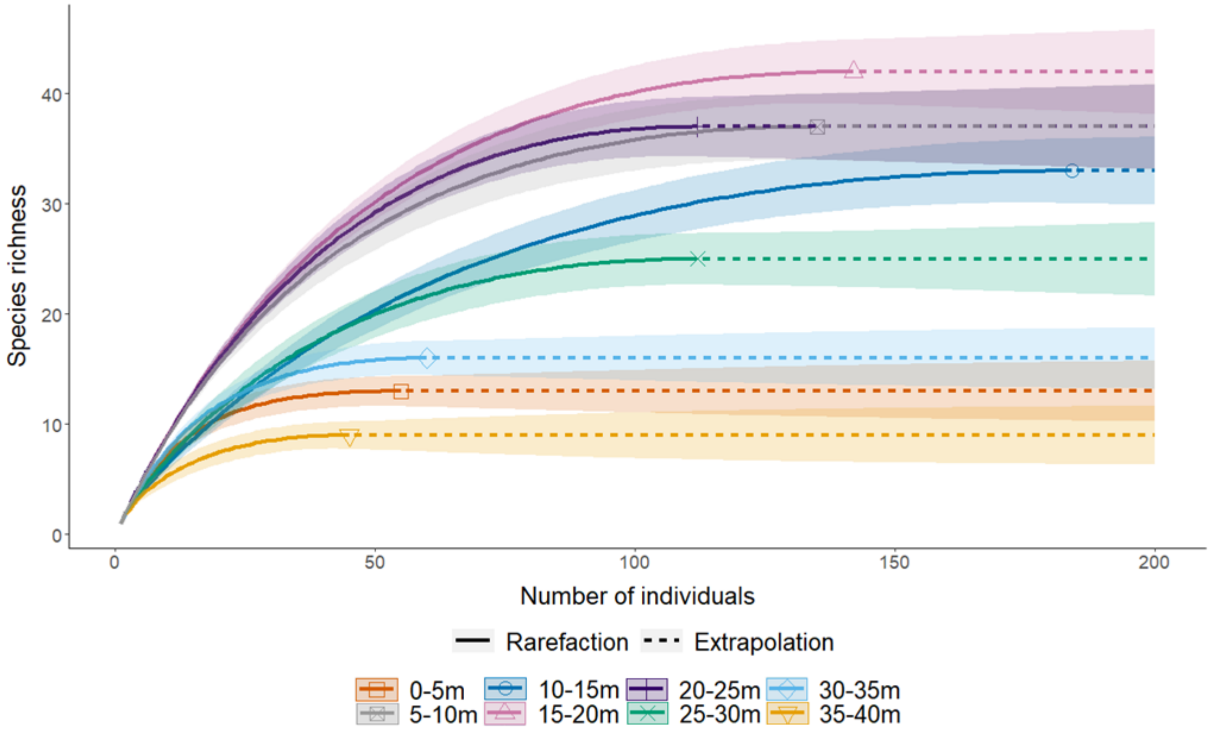

Fig. 3 Individual-based rarefaction curves (solid lines) and short-range extrapolation (dashed lines) for the species richness of each stratum after singletons were removed. Shaded areas represent ±95% confidence intervals, non-overlapping confidence intervals indicate significant difference.

Caterpillar density

Overall caterpillar density had a significant 2nd degree polynomial distribution (df = 262.38, t = 4.67 P<0.0001) where density decreased towards the central midstory and then increased towards the top of the canopy (**Fig. 4a**). Caterpillar density distributions between defensive traits showed no significant difference between aposematic and cryptic (P=0.24), concealed and cryptic (P=0.66) and aposematic and concealed (P=0.06) (**Fig. 4b**).

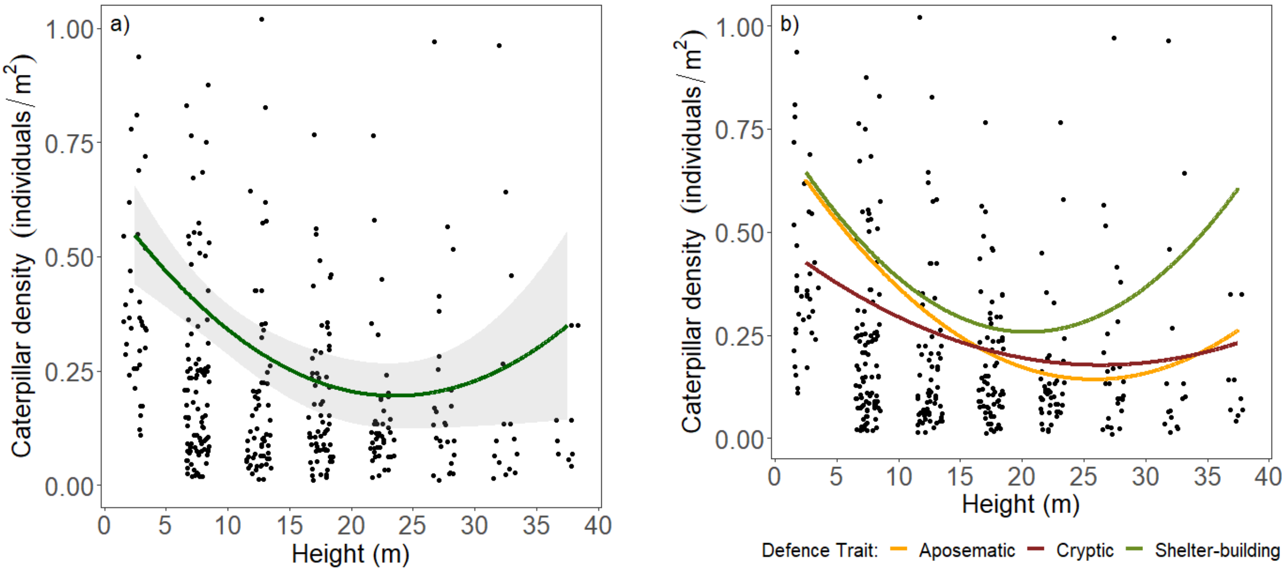

Fig. 4 Caterpillar density across a vertical gradient in a tropical forest in Cameroon. Graph a) shows overall caterpillar density (individual per m^2) fitted by 2nd degree polynomial distribution ± s.e. and b) shows caterpillar density partitioned into three defensive traits. Individual points represent the density of caterpillars for a particular tree species within a stratum. Median height values are used to approximate the height range for each stratum. In graph a) the grey area represents the standard error across the entire vertical gradient. The y-axis limits were set to 1 for ease of visualisation which led to the visual exclusion of 26 points, but all data points, including those above the limit, were included in the analysis.

Network specialisation

Observed weighted specialisation network values showed a general, gradual decrease in generality with increasing height after an initial increase between the two lowest strata (0-5 m and 5-10 m) (**Fig. 5a**) Vulnerability showed a strong, midstory peak at 20-25 m (**Fig. 5b**). Connectance showed a broad tendency to increase with increasing height, although there is also a secondary, midstory peak at 20-25 m (**Fig. 5c**). Z-scores for all indices indicate increased specialisation toward the midstory and decreased specialisation towards the upper canopy (**Table S2**).

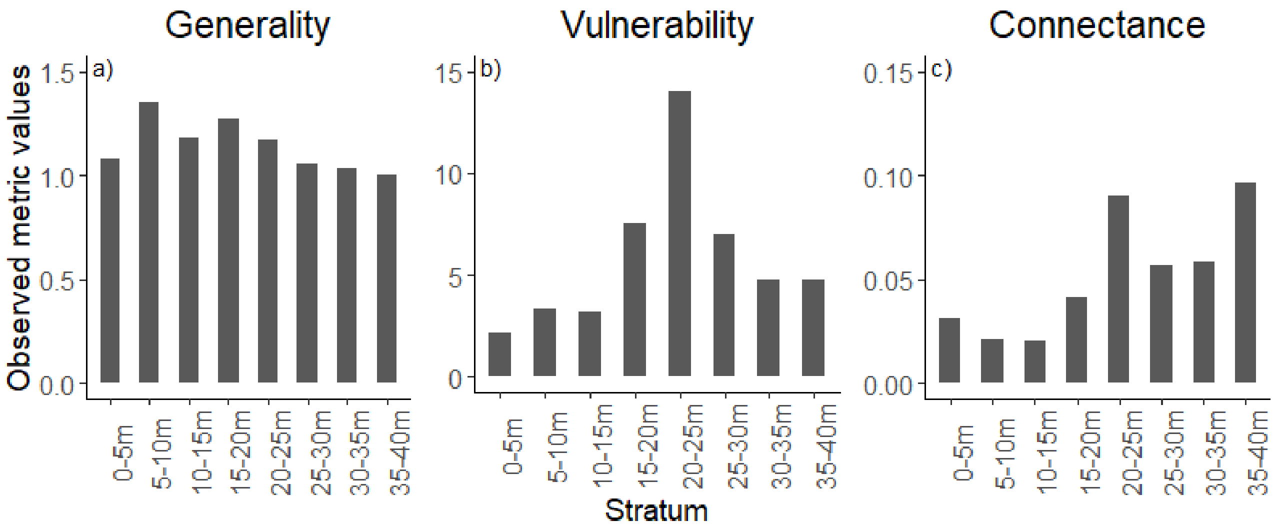

Fig. 5 Observed metric values for generality (number of host plants per caterpillar), vulnerability (specialisation in interactions), and connectance (proportion of realised interactions) for each vertical forest stratum.

Parasitism rate

The mean parasitism rate across all caterpillars was 9.5%. Mean parasitism rates were significantly higher in aposematic (11.1%, SE = 0.024) and shelter-building (10.7%, SE = 0.019) caterpillar than in cryptic (6.1%, SE = 0.013) caterpillars (odds ratio = 1.91, P<0.05 and odds ratio = 1.83, P<0.05 respectively). There was no significant difference between aposematic and shelter-building caterpillar (odds ratio = 1.05, P>0.05) (**Fig. 6**). Parasitism rates showed no significant pattern across strata (P>0.05) for all caterpillars (P>0.05) and between defensive traits (P>0.05) although parasitism was generally higher in the lower and midstory strataand lower in the upperr strata (**Table 2**).

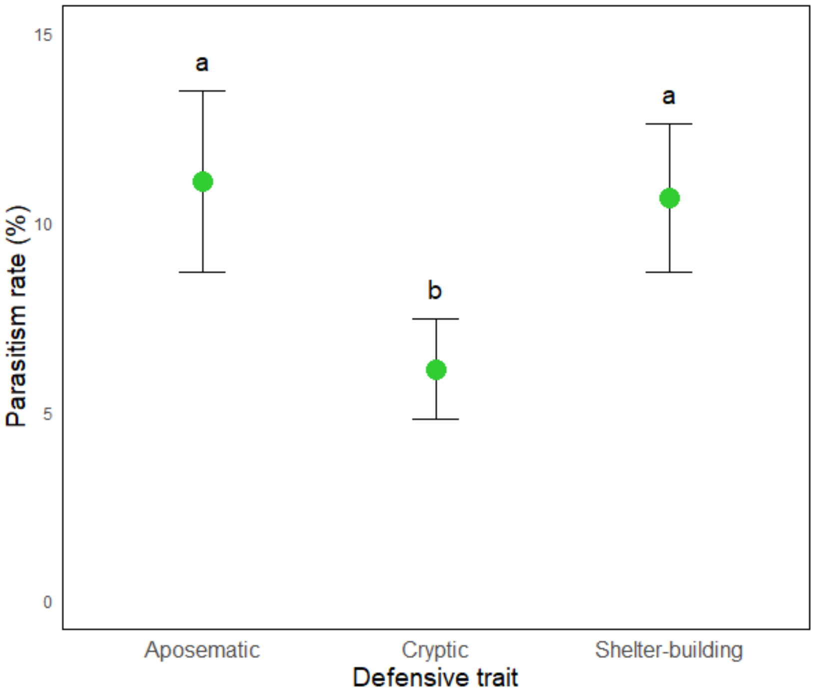

Fig. 6 Mean parasitism rates (in %, ±SE) of caterpillars grouped by their defensive traits. The dots represent the mean parasitism rate for each group: aposematic, cryptic, and shelter-building. Whiskers represent the standard error from the mean. Different letters above the whiskers indicate significant differences between the defensive traits.

Table 2. Parasitism rates (%) defined as the percentage of parasitised caterpillars across strata for all caterpillars and between caterpillar defensive traits.

Stratum	***Parasitism rate (%)***			
	All caterpillars	***Defensive trait***		
		Aposematic	***Cryptic***	***Shelter-building***
0-5 m	7.3	12.0	3.7	8.8
5-10 m	14.5	8.5	15.2	16.1
10-15 m	8.8	10.6	6.1	9.6
15-20 m	6.5	7.8	7.0	5.3
20-25 m	15.2	17.2	10.0	19.7
25-30 m	7.7	15.2	3.1	8.2
30-35 m	5.5	4.3	5.6	6.0
35-40 m	2.9	0	5.5	3.2

Discussion

Species richness, diversity, and community composition

By dividing the forest into multiple strata, we observed nuanced, incremental changes in caterpillar species richness and diversity across a vertical gradient. To our knowledge, this is the first study comparing the species richness and diversity of larval lepidoptera across a vertical gradient in the tropics, with previous studies focusing exclusively on adults (e.g. Schulze et al. 2001; Amorim et al. 2022). We found a clear increase in both the species richness and diversity of caterpillars toward the central midstory strata and then a distinct decrease towards the upper strata. This pattern is consistent with our hypothesis (H1); however, it was not entirely driven by the ecological parameters we expected. Our initial hypothesis was that increasing foliage availability, favourable climatic conditions, and a higher overlap of host plant species would drive this pattern. However, this was not completely reflected in our results. In our study, the increase in caterpillar species appears to be mainly driven by their abundance, as the strata containing the highest number of caterpillar individuals generally have more species. Curiously, caterpillar abundance and richness were not entirely driven by available foliage, as the stratum containing the most foliage (5-10 m) had fewer species and individuals than the two midstory strata directly above. This suggests that the environmental conditions in the midstory are more favourable to caterpillars, increasing their overall abundance and diversity. Our results partially align with other studies on the vertical stratification of caterpillars from temperate forests, which found higher midstory diversity compared to the canopy, but similar or greater diversity in the understory (Hirao et al. 2009; Seifert et al. 2020a). The variation between the biotic and abiotic factors in tropical and temperate forests across vertical strata may explain this distinction. In tropical forests, the midstory has significantly higher plant diversity than the emergent canopy and higher structural complexity than the understory, thus providing more resources and niches for caterpillars (Basset 2001). In contrast to the dense canopies of tropical forests, temperate canopies exhibit a more open structure, permitting greater light penetration to the understory. This results in a less favourable environment for certain host plant species that serve as critical resources for many caterpillar species and decreasing caterpillar diversity in the understory. It is also likely that many exposed caterpillar species avoid the upper, emergent canopy where they are more susceptible to desiccation. Within the midstory, the more stable, intermediate levels of light, temperature, and humidity may be more favourable for the developmental success of caterpillars which facilitates the high diversity of caterpillars within the midstory strata in our study. It is also important to note that these results could be, in part, driven by our experimental design. Smaller saplings (≤5 cm DBH) were not sampled within our plot, so it is likely that some of the caterpillar community and therefore diversity in the lower strata were not fully accounted for. Surprisingly, there were no visible trends in the increase of species turnover across the vertical gradient of our forest (H2) although there was a significant difference in the proportional abundance of caterpillar species. We hypothesized that there would be a greater turnover of species across the midstory strata compared to other neighbouring strata, driven by the larger diversity of foliage, structural complexity and distinct microhabitats conducive to different caterpillar species. Additionally, we thought that the intermediate conditions of the midstory would encourage spillover from both understory and upper canopy species, which would drive an increase in diversity. Instead, species turnover was consistently high across all strata. Large amounts of species turnover are often a result of high diversity (Coelho et al. 2018), which is certainly apparent in our study. Seifert et al. (2020a) also found that species turnover changed significantly among strata in temperate forest. It may be that although the strata in our study are hosts to distinct communities of caterpillars, the high diversity in all strata are overshadowing any underlying trends in species turnover across the vertical gradient in our plot. Furthermore, we expected to find increased dissimilarity between caterpillar communities with increasing distance between strata. Although there was no clearly identifiable trend, there was a tendency for comparisons between the upper strata to have higher dissimilarity values in comparison to the strata below. This is probably due to the fact that fewer species were found in these upper strata and the species that did occur in these strata were highly specialised and therefore not found in the lower strata.

Caterpillar density

Our results reveal an intriguing, complex distribution of caterpillar density across strata, revealing novel patterns and emphasising how certain defensive traits may play an important role in shaping them. Studies comparing caterpillar density across vertical gradients in both tropical and temperate forests typically find changes across vertical strata (e.g. Basset 2001; Pontes Ribeiro and Basset 2007). The nature of these changes, however, are inconsistent between studies (Ulyshen 2011). Here we provide a novel approach by uncovering patterns in caterpillar density across multiple strata spanning an entire vertical gradient instead of comparing density in the understory, midstory and canopy (e.g. Šigut et al. 2018; Seifert et al. 2020a). Based on the literature, we expected to find the lowest caterpillar density at the top of the canopy. This is due to several factors: increased visibility and susceptibility to predation (Posa et al. 2007), higher risk of desiccation (Greeney et al. 2012), exposure to harsh weather (Basset et al. 2003), and lower leaf quality as canopy leaves are typically smaller, tougher, and have higher phenolic contents, making them less appealing to caterpillars (Coley and Barone 1996). Curiously, we found that caterpillar density actually increased towards the upper strata in our forest plot. This follows the opposite pattern to caterpillar abundance which increased towards the midstory and decreased towards the upper strata. It is possible that whilst the high availability of edible foliage in the midstory increases the overall abundance of caterpillars, it also decreases the competition between individuals feeding on the same plant, allowing them to co-exist in high abundances whilst also being at relatively low densities within this layer of the forest. In our study in particular, the pattern in density appears to be driven by a small number of shelter-building caterpillars occurring at high densities in the uppermost strata. Both Le Corff and Marquis (1999) and Seifert et al. (2020a) found that shelter-building caterpillars occur at higher densities than exposed feeders in the canopy. The natural history of shelter-building caterpillars makes them less susceptible to the aforementioned conditions at the top of the canopy and it is possible that certain species of shelter-building caterpillars have evolved to exploit the generally less favourable conditions in the emergent canopy and thrive where there is significantly less competition from other insect herbivores and a reduced risk from predators and parasitoids, allowing them to occur at a higher density. Predation is also an important determinant of insect distributions and has been shown to reduce caterpillar density by over 60% in a temperate forest (Singer et al. 2017) and caterpillars may preferentially locate themselves in enemy-free space (Šigut et al. 2018). Interian-Aguiñaga et al. (2022) found higher predation rates on midstory model caterpillars compared to the lower canopy and found the lowest abundance of insectivorous birds in the upper canopy. Predation rates have also been shown to increase with increasing plant diversity in tropical and temperate forests at small spatial scales (Leles et al. 2017) and the midstory contains the largest diversity of foliage. It is therefore possible that increased predation rates in the midstory strata are driving the density patterns observed in our study.

It is also important to mention that caterpillars within the upper strata are more likely to occur within a '*spheroid cap'* (see Methods), where the crown model we used assigns less volume and therefore less leaf area. It is therefore possible that the observed increase in density toward the upper strata may be a product of our analytical design. Indeed, the use of spheroids relies on certain assumptions about the geometry of tree crowns that may not always hold true in nature. However, it is common for the volume of tree crowns to decrease towards the top of the crown where the branches and foliage of trees become smaller and less dense. Additionally, these '*spheroid caps'* were present across all strata as tree height varied within our plot making it unlikely that density patterns were driven by this aspect of our methodology. Equally, the addition of trunk surface areas, which were generally lower than total leaf area within a stratum, may have affected the higher densities in the lower strata. Although, as trunk heights varied to slightly below thirty metres in our study and caterpillars were found below the crown across all strata below this height, these effects should be inconsequential.

Network specialisation

To our knowledge, this is the first study addressing specialisation patterns in host plant-caterpillar networks along a vertical forest gradient in the tropics. Our results concur with both our hypothesis (H3) and Seifert et al. (2020a), that used similar metrics in a temperate north American forest, and found that generality was highest in the understory, vulnerability was highest in the midstory and connectance was highest in the canopy, although concluded that the latter result was likely a product of small sample size. The gradual decrease of generality towards the higher strata indicates that caterpillars occupying the higher strata are more specialised. Proportionally, shelter-building caterpillars were the most abundant caterpillars in the upper canopy strata (**Fig. S2**), which is consistent with other studies (Le Corff and Marquis 1999; Seifert et al. 2020a). Furthermore, shelter-builders were also the most specialised of our three caterpillar groups with 58% of species being specialists (only being found on a single host plant species) within our forest plot (**Table S3**). These findings align with previous studies (Seifert et al. 2020b; Molleman et al. 2022). It is therefore likely that the natural distribution of shelter-building caterpillars has, in part, driven the overall trend in generality across the vertical gradient of the forest. However, it is worth noting that while there is a significant presence of shelter-building caterpillars in the lower strata, the high diversity of other generalist caterpillar species in these areas likely mitigates their impact on overall generality.

The strong, midstory peak in vulnerability, indicates that a significant proportion of caterpillar species are confined to a few host plants within this forest layer. This is exemplified in our study, where the majority of caterpillars were found on the two plant species that exhibited the highest caterpillar abundance and diversity within our plot: *Celtis philippensis* and *Hylodendron gabunense*. Together, these two species were host to 65% of all caterpillar species and 80% of individual caterpillars within the stratum with the highest vulnerability (20-25 m) (Appendix 3). These species also explain the anomalous increase in connectivity within this stratum as they are responsible for so many interactions within the network. The general trend in connectedness is likely due to decreasing network size rather than increased redundance and stability in the uppermost strata as smaller networks tend to have higher connectance due to sampling effects (Pellissier et al. 2018) which is also consistent with Seifert et al. (2020a).

Parasitism rates

In line with our hypothesis (H4), the parasitism rates of aposematic caterpillars are higher than in cryptic caterpillars and comparable to the parasitism rates of the shelter-building caterpillars within our forest plot. Aposematic caterpillars often sequester toxins from their host plants, which along with their warning colouration and morphology enhances their ability to deter predators. Parasitoids can be much more tolerant to the defensive compounds of aposematic caterpillars than generalist predators (Lampert et al. 2010). This is because the chemical sequestration of aposematic caterpillars can increase the probability of experiencing an impaired immune response, making them more susceptible to parasitoids and a safe haven for oviposition and the subsequent development of their larvae. The "*safe haven*" hypothesis (Dyer and Gentry 1999; Gentry and Dyer 2002; Smilanich et al. 2009) refers exclusively to chemically defended caterpillars. However, we argue that shelter-building caterpillars also fit into this category. Their shelters not only protect them from predators and hinder their ability to escape parasitoid oviposition, but they also create favourable environmental conditions by reducing water loss, blocking direct sunlight and wind, and reducing the chance of desiccation (Abarca and Boege 2011; Greeney et al. 2012). As hosts, shelter-building caterpillars provide ideal conditions for parasitoid larvae to develop within a pre-built refugium which could explain their relatively high parasitism rates in our study and others (e.g. Hrcek et al. 2013; Šigut et al. 2018). Conversely, cryptic caterpillars rely on camouflage and behavioural adaptations to evade detection by predators. However, they lack the chemical defences or protective structures necessary to avoid predation by vertebrate insectivores, such as birds, which can be the primary mediators of caterpillar populations in tropical regions (Mäntylä et al. 2011). This vulnerability makes cryptic caterpillars less suitable hosts for parasitoids, as they are more likely to be consumed after being parasitised, which may explain their reduced parasitism rate. Previous studies grouping caterpillars based on their feeding guilds concluded that semi-concealed feeders (shelter-building) have higher parasitism rates than exposed feeders (aposematic and cryptic) (e.g. Hrcek et al. 2013; Šigut et al. 2018). Our study reveals a more refined perspective on parasitism rates in caterpillars, highlighting that the presence of defensive traits in caterpillars may exert a more significant influence on parasitism rates than their feeding-guild.

Parasitism rates were extremely variable across strata for all caterpillars and between caterpillar defensive traits, which is likely due to the reduced incidence of parasitism when divided across all the forest strata. However, there is an apparent decrease in parasitism rates towards the upper canopy within all the defensive traits and across all caterpillars. In temperate forests, Chaij et al. (2016) found parasitism rates to be lowest in the upper canopy in concealed hosts and Šigut et al. (2018) found a similar pattern in leaf-chewing insects. One suggestion is that the increased structural complexity of adult tree crowns may lead to reduced foraging success for parasitoids (Godfray 1994; Yamazaki

2010). It is also probable that, similarly to their hosts, parasitoids avoid the upper canopy where the climatic conditions are less favourable. Adverse weather conditions such as increased wind speed and temperatures, which are more prevalent at the top of the canopy, have been shown to reduce the likelihood of parasitoids finding their hosts (Vosteen et al. 2020). Furthermore, Caterpillars feeding on the less nutritious leaves in the upper canopy may have reduced fitness which would make them less suitable hosts for parasitoids. Another possibility is predation avoidance, parasitoids are susceptible to intraguild predation and Chmel et al. (2016) found that sallying, insectivorous birds were more abundant in higher vertical strata in a Cameroonian rainforest. As suggested by Šigut et al. (2018), parasitoids are more likely to aggregate in patches where they can minimise predator avoidance whilst increasing their likelihood of encountering a host. Additionally, this may explain why shelter-building caterpillars appear to occur at higher densities in the upper canopy but had the lowest parasitism rates, as they were occupying a more parasitoid-free space.

The higher peak is within the central midstory which is also where we found the highest diversity of caterpillars. Murdoch and Stewart-Oaten (1989) suggested that parasitoids may aggregate in patches with more potential hosts. For generalist parasitoids (those with multiple host species) especially, occupying the stratum with the highest diversity of parasitoids would maximise the likelihood of encountering a suitable host species. Additionally, this central midstory stratum could potentially represent the optimal combination of biotic (e.g. more caterpillar species and lower risk of predation) and abiotic (e.g. better visibility and wind protection) creating ideal foraging conditions for parasitoids, which would in turn, increase overall parasitism rates. Alternatively, these conditions might be more conducive for the hosts themselves, thereby explaining the observed high caterpillar diversity. In this scenario, the increased parasitoid activity could simply be a reflection of the conditions preferred by their hosts. Without more targeted studies, we can only speculate whether the observed patterns are primarily driven by the preferences and behaviours of the parasitoids, the hosts, or a complex interplay of both. Future research should aim to disentangle these possibilities to enhance our understanding of these intricate ecological dynamics. Curiously, cryptic and shelter-building caterpillars followed similar patterns in parasitism rates across strata, albeit with cryptic caterpillars being parasitised less frequently. This similarity may be attributed to their passive defence strategies of concealment and camouflage which both rely on avoiding detection to avoid predators and parasitoids. It is therefore possible that whilst shelter-building caterpillars are parasitised more frequently, the relative detection by parasitoids remains the same, leading to a similar pattern across the vertical forest gradient (Baer and Marquis 2020). For aposematic caterpillars, parasitism rates were notably higher in the upper midstory. Aposematic caterpillars rely on their warning signals and conspicuity to avoid predation, and previous studies have established that insectivores can identify and actively avoid aposematic insects (Exnerová et al. 2015; Aslam et al. 2020). Their conspicuity, and therefore predator protection, is likely enhanced in the upper midstory of the forest, where there is enhanced visibility from greater light penetration. However, this increased visibility also increases the likelihood of being detected by parasitoids that have been posited to be the most active in the midstory (Šigut et al. 2018). Therefore, it is possible that aposematic caterpillars occupying these strata are both easy to locate and less vulnerable to predation, making them ideal hosts for parasitoids. To our knowledge, this is the first study to compare parasitism rates in caterpillars across vertical forest strata in a tropical environment. Our findings suggest that the behaviour, morphology, and vertical distribution of caterpillars significantly impact their interactions with parasitoids. These results underscore the need for future research to further investigate the role of defensive traits, and vertical gradients in shaping caterpillar-parasitoid interaction in tropical forests.

Conclusion

This study reveals the high diversity and complexity of caterpillar communities across a complete vertical gradient in a tropical forest in Cameroon. By dividing the forest into multiple vertical strata, we uncovered nuanced patterns of caterpillar diversity, density, specialisation, and parasitism rates that are obscured when comparing only the understory and canopy. We argue that categorizing caterpillars by their defensive traits is a more intuitive approach than by their feeding guild when focusing on caterpillar-parasitoid interactions. The aposematic and shelter-building caterpillars had comparably high parasitism rates and should both be considered a "*safe haven*" for parasitoids. These results highlight the importance of the vertical dimensions of the forest and the natural history of caterpillars when studying their ecology in tropical forests and emphasises the importance of further research for unravelling the intricate and diverse factors that shape caterpillar communities and their interactions with parasitoids across entire vertical forest gradients.

Acknowledgements

We are thankful to the Tikar king Gandji, his queen, prince Aliou and all the volunteers from the Village of Nditam for their crucial support during the project. We are also thankful to Dr Philippe Le Gall (IRD), John Fogoh Muafor, Roger Louis Kamgang and Prof. Joseph Tamesse (University of Yaounde) for their help during the project. We also thank the anonymous reviewer and the editors for their valuable comments helping to improve the manuscript. This study was funded by the European Research Council (Project No. 669609 to VN) and by the Grant agency of Czech Republic (GACR 19-28126X).

References

Abarca M, Boege K (2011) Fitness costs and benefits of shelter building and leaf trenching behaviour in a pyralid caterpillar. Ecological Entomology 36:564–573

Ashton LA, Nakamura A, Basset Y, et al (2016) Vertical stratification of moths across elevation and latitude. J Biogeogr 43:59–69. https://doi.org/10.1111/jbi.12616

Aslam M, Nedvěd O, Sam K (2020) Attacks by predators on artificial cryptic and aposematic insect larvae. Entomologia Exp Applicata 168:184–190. https://doi.org/10.1111/eea.12877

Baer CS, Marquis RJ (2020) Between predators and parasitoids: Complex interactions among shelter traits, predation and parasitism in a shelter-building caterpillar community. Functional Ecology 34:2186–2198. https://doi.org/10.1111/1365-2435.13641

Banašek-Richter C, Cattin M-F, Bersier L-F (2004) Sampling effects and the robustness of quantitative and qualitative food-web descriptors. Journal of Theoretical Biology 226:23–32

Basset Y (2001) Invertebrates in the canopy of tropical rain forests How much do we really know? In: Linsenmair KE, Davis AJ, Fiala B, Speight MR (eds) Tropical Forest Canopies: Ecology and Management. Springer Netherlands, Dordrecht, pp 87–107

Basset Y, Hammond PM, Barrios H, et al (2003) Vertical stratification of arthropod assemblages. Arthropods of tropical forests 17–27

Bates D, Mächler M, Bolker B, Walker S (2014) Fitting Linear Mixed-Effects Models using lme4

Beck J, Holloway JD, Schwanghart W (2013) Undersampling and the measurement of beta diversity. Methods Ecol Evol 4:370–382. https://doi.org/10.1111/2041-210x.12023

Blüthgen N, Menzel F, Blüthgen N (2006) Measuring specialization in species interaction networks. BMC Ecol 6:9. https://doi.org/10.1186/1472-6785-6-9

Bolker B (2017) Package ‘bbmle’. Tools for General Maximum Likelihood Estimation. Tools for General Maximum Likelihood Estimation 641:

Brehm G (2007) Contrasting patterns of vertical stratification in two moth families in a Costa Rican lowland rain forest. Basic and Applied Ecology 8:44–54

Caro T, Ruxton G (2019) Aposematism: unpacking the defences. Trends in Ecology & Evolution 34:595–604

Chaij J, Devoto M, Oleiro M, et al (2016) Complexity of leaf miner–parasitoid food webs declines with canopy height in P atagonian beech forests. Ecological Entomology 41:599–610. https://doi.org/10.1111/een.12332

Chao A, Jost L (2015) Estimating diversity and entropy profiles via discovery rates of new species. Methods Ecol Evol 6:873–882. https://doi.org/10.1111/2041-210X.12349

Chen JM, Menges CH, Leblanc SG (2005) Global mapping of foliage clumping index using multi-angular satellite data. Remote Sensing of Environment 97:447–457

Chen W, Vasseur L, You M, et al (2017) Parasitised caterpillars suffer reduced predation: potential implications for intra-guild predation. Sci Rep 7:42636. https://doi.org/10.1038/srep42636

Chmel K, Riegert J, Paul L, Novotný V (2016) Vertical stratification of an avian community in New Guinean tropical rainforest. Population Ecology 58:535–547. https://doi.org/10.1007/s10144-016-0561-2

Coelho MS, Carneiro MAA, Branco CA, et al (2018) Species turnover drives β-diversity patterns across multiple spatial scales of plant-galling interactions in mountaintop grasslands. PLOS ONE 13:e0195565. https://doi.org/10.1371/journal.pone.0195565

Coley PD, Barone JA (1996) Herbivory and Plant Defenses in Tropical Forests. Annual Review of Ecology and Systematics 27:305–335. https://doi.org/10.1146/annurev.ecolsys.27.1.305

Colwell R, Mao CX, Chang J (2004) Interpolating, extrapolating, and comparing incidence-based species accumulation curves. Ecology 85:2717–2727. https://doi.org/10.1890/03-0557

Corff JLe, Marquis RJ (1999) Differences between understorey and canopy in herbivore community composition and leaf quality for two oak species in Missouri. Ecological Entomology 24:46–58. https://doi.org/10.1046/j.1365-2311.1999.00174.x

Covarrubias-Camarillo T, Osorio-Beristain M, Legal L, Contreras-Garduño J (2016) Baronia brevicornis caterpillars build shelters to avoid predation. Journal of Natural History 50:2299–2310. https://doi.org/10.1080/00222933.2016.1193640

de Souza Amorim D, Brown BV, Boscolo D, et al (2022) Vertical stratification of insect abundance and species richness in an Amazonian tropical forest. Scientific reports 12:1734

Delabye S, Rougerie R, Bayendi S, et al (2019) Characterization and comparison of poorly known moth communities through DNA barcoding in two Afrotropical environments in Gabon. Genome 62:96–107. https://doi.org/10.1139/gen-2018-0063

Dormann CF, Fründ J, Blüthgen N, Gruber B (2009) Indices, graphs and null models: analyzing bipartite ecological networks

Dyer LA, Gentry G (1999) Predicting natural-enemy responses to herbivores in natural and managed systems. Ecological Applications 9:402–408. https://doi.org/10.1890/1051-0761(1999)009[0402:PNERTH]2.0.CO;2

Exnerová A, Ježová D, Štys P, et al (2015) Different reactions to aposematic prey in 2 geographically distant populations of great tits. Behavioral Ecology 26:1361–1370. https://doi.org/10.1093/beheco/arv086

Frago E (2016) Interactions between parasitoids and higher order natural enemies: intraguild predation and hyperparasitoids. Current Opinion in Insect Science 14:81–86

Gentry GL, Dyer LA (2002) On the conditional nature of neotropical caterpillar defenses against their natural enemies. Ecology 83:3108–3119. https://doi.org/10.1890/0012-9658(2002)083[3108:OTCNON]2.0.CO;2

Godfray HCJ (1994) Parasitoids: Behavioral and Evolutionary Ecology. Princeton University Press

Greeney HF, Dyer LA, Smilanich AM (2012) Feeding by lepidopteran larvae is dangerous: a review of caterpillars' chemical, physiological, morphological, and behavioral defenses against natural enemies. Invertebrate Survival Journal 9:7–34

Hausmann A, Diller J, Moriniere J, et al (2020) DNA barcoding of fogged caterpillars in Peru: A novel approach for unveiling host-plant relationships of tropical moths (Insecta, Lepidoptera). Plos one 15:e0224188

Hawkins BA (1994) Pattern and process in host-parasitoid interactions. Cambridge University Press

Hirao T, Murakami M, Kashizaki A (2009) Importance of the understory stratum to entomofaunal diversity in a temperate deciduous forest. Ecological Research 24:263–272

Horn HS (1966) Measurement of “Overlap” in Comparative Ecological Studies. The American Naturalist 100:419–424. https://doi.org/10.1086/282436

Houska Tahadlova M, Mottl O, Jorge LR, et al (2023) Trophic cascades in tropical rainforests: Effects of vertebrate predator exclusion on arthropods and plants in Papua New Guinea. Biotropica 55:70–80. https://doi.org/10.1111/btp.13160

Hrcek J, Miller SE, Whitfield JB, et al (2013) Parasitism rate, parasitoid community composition and host specificity on exposed and semi-concealed caterpillars from a tropical rainforest. Oecologia 173:521–532

Hsieh TC, Ma KH, Chao A, Hsieh MT (2016) Package ‘iNEXT’. Interpolation and Extrapolation for Species Diversity. URL http://chao stat nthu edu tw/blog/software-download/(accessed 228 2017)

Intachat J, Holloway JD (2000) Is there stratification in diversity or preferred flight height of geometroid moths in Malaysian lowland tropical forest? Biodiversity & Conservation 9:1417–1439

Interian-Aguiñaga J, Parra-Tabla V, Abdala-Roberts L (2022) Effects of topical tree diversity and prey spatial distribution on predation by birds and arthropods. Arthropod-Plant Interactions 16:449–457. https://doi.org/10.1007/s11829-022-09908-x

Kuznetsova A, Brockhoff PB, Christensen RH (2017) lmerTest package: tests in linear mixed effects models. Journal of statistical software 82:1–26

Lampert EC, Dyer LA, Bowers MD (2010) Caterpillar Chemical Defense and Parasitoid Success: Cotesia congregata Parasitism of Ceratomia catalpae. J Chem Ecol 36:992–998. https://doi.org/10.1007/s10886-010-9840-0

Leles B, Xiao X, Pasion BO, et al (2017) Does plant diversity increase top–down control of herbivorous insects in tropical forest? Oikos 126:1142–1149. https://doi.org/10.1111/oik.03562

Lenth RV (2023) emmeans: Estimated Marginal Means, aka Least-Squares Means

Lill JT, Marquis RJ, Ricklefs RE (2002) Host plants influence parasitism of forest caterpillars. Nature 417:170–173

Lim GS, Balke M, Meier R (2012) Determining species boundaries in a world full of rarity: singletons, species delimitation methods. Systematic biology 61:165–169

Mäntylä E, Klemola T, Laaksonen T (2011) Birds help plants: a meta-analysis of top-down trophic cascades caused by avian predators. Oecologia 165:143–151

Menken SB, Boomsma JJ, Van Nieukerken EJ (2010) Large-scale evolutionary patterns of host plant associations in the Lepidoptera. Evolution 64:1098–1119

Mitter C, Davis DR, Cummings MP (2017) Phylogeny and Evolution of Lepidoptera. Annu Rev Entomol 62:265–283. https://doi.org/10.1146/annurev-ento-031616-035125

Moffett MW (2013) Comparative Canopy Biology and the Structure of Ecosystems. In: Lowman M, Devy S, Ganesh T (eds) Treetops at Risk. Springer New York, New York, NY, pp 13–54

Molleman F, Walczak U, Melosik I, et al (2022) What Drives Caterpillar Guilds on a Tree: Enemy Pressure, Leaf or Tree Growth, Genetic Traits, or Phylogenetic Neighbourhood? InSects 13:367

Morisita M (1959) Measuring of interspecific association and similarity between assemblages. Mem Fac Sci Kyushu Univ Ser E Biol 3:65–80

Murakami M, Yoshida K, Hara H, Toda MJ (2005) Spatio-temporal variation in Lepidopteran larval assemblages associated with oak, Quercus crispula: the importance of leaf quality. Ecol Entomol 30:521–531. https://doi.org/10.1111/j.0307-6946.2005.00724.x

Murdoch WW, Stewart-Oaten A (1989) Aggregation by Parasitoids and Predators: Effects on Equilibrium and Stability. The American Naturalist 134:288–310. https://doi.org/10.1086/284981

Nakamura A, Kitching RL, Cao M, et al (2017) Forests and their canopies: achievements and horizons in canopy science. Trends in ecology & evolution 32:438–451

Oksanen J (2010) Vegan: community ecology package. http://vegan r-forge r-project org/

Parker GG, Brown MJ (2000) Forest Canopy Stratification—Is It Useful? The American Naturalist 155:473–484. https://doi.org/10.1086/303340

Pellissier L, Albouy C, Bascompte J, et al (2018) Comparing species interaction networks along environmental gradients. Biological Reviews 93:785–800. https://doi.org/10.1111/brv.12366

Pontes Ribeiro S, Basset Y (2007) Gall-forming and free-feeding herbivory along vertical gradients in a lowland tropical rainforest: the importance of leaf sclerophylly. Ecography 30:663–672. https://doi.org/10.1111/j.2007.0906-7590.05083.x

Posa MRC, Sodhi NS, Koh LP (2007) Predation on artificial nests and caterpillar models across a disturbance gradient in Subic Bay, Philippines. Journal of Tropical Ecology 23:27–33

R Development Core Team (2022) R: A language and environment for statistical computing. Vienna, Austria

Ratnasingham S, Hebert PD (2013) A DNA-based registry for all animal species: the Barcode Index Number (BIN) system. PloS one 8:e66213

Sam K, Koane B, Sam L, et al (2020) Insect herbivory and herbivores of Ficus species along a rain forest elevational gradient in Papua New Guinea. Biotropica 52:263–276. https://doi.org/10.1111/btp.12741

Schowalter T, Chao J-T (2021) Canopy Insect Sampling. In: Santos JC, Fernandes GW (eds) Measuring Arthropod Biodiversity. Springer International Publishing, Cham, pp 467–493

Schulze CH, Linsenmair KE, Fiedler K (2001) Understorey versus canopy: patterns of vertical stratification and diversity among Lepidoptera in a Bornean rain forest. In: Linsenmair KE, Davis AJ, Fiala B, Speight MR (eds) Tropical Forest Canopies: Ecology and Management. Springer Netherlands, Dordrecht, pp 133–152

Seifert CL, Lamarre GPA, Volf M, et al (2020a) Vertical stratification of a temperate forest caterpillar community in eastern North America. Oecologia 192:501–514. https://doi.org/10.1007/s00442-019-04584-w

Seifert CL, Volf M, Jorge LR, et al (2020b) Plant phylogeny drives arboreal caterpillar assemblages across the Holarctic. Ecology and Evolution 10:14137–14151. https://doi.org/10.1002/ece3.7005

Šigut M, Šigutová H, Šipoš J, et al (2018) Vertical canopy gradient shaping the stratification of leaf-chewer–parasitoid interactions in a temperate forest. Ecology and Evolution 8:7297–7311. https://doi.org/10.1002/ece3.4194

Singer MS, Clark RE, Lichter-Marck IH, et al (2017) Predatory birds and ants partition caterpillar prey by body size and diet breadth. Journal of Animal Ecology 86:1363–1371. https://doi.org/10.1111/1365-2656.12727

Slinn HL, Richards LA, Dyer LA, et al (2018) Across Multiple Species, Phytochemical Diversity and Herbivore Diet Breadth Have Cascading Effects on Herbivore Immunity and Parasitism in a Tropical Model System. Frontiers in Plant Science 9:

Smedt P de, Vangansbeke P, Bracke R, et al (2019) Vertical stratification of moth communities in a deciduous forest in Belgium. Insect conservation and diversity 12:121–130

Smilanich AM, Dyer LA, Chambers JQ, Bowers MD (2009) Immunological cost of chemical defence and the evolution of herbivore diet breadth. Ecology Letters 12:612–621. https://doi.org/10.1111/j.1461-0248.2009.01309.x

Stork NE, Grimbacher PS (2006) Beetle assemblages from an Australian tropical rainforest show that the canopy and the ground strata contribute equally to biodiversity. Proc R Soc B 273:1969–1975. https://doi.org/10.1098/rspb.2006.3521

Tvardikova K, Novotny V (2012) Predation on exposed and leaf-rolling artificial caterpillars in tropical forests of Papua New Guinea. Journal of Tropical Ecology 28:331–341. https://doi.org/10.1017/S0266467412000235

Ulyshen M (2011) Arthropod vertical stratification in temperate deciduous forests: Implications for conservation-oriented management. Fuel and Energy Abstracts 261:1479–1489. https://doi.org/10.1016/j.foreco.2011.01.033

Vázquez DP, Melián CJ, Williams NM, et al (2007) Species abundance and asymmetric interaction strength in ecological networks. Oikos 116:1120–1127. https://doi.org/10.1111/j.0030-1299.2007.15828.x

Volf M, Klimeš P, Lamarre GP, et al (2019) Quantitative assessment of plant-arthropod interactions in forest canopies: A plot-based approach. PLoS One 14:e0222119

Vosteen I, Bianchi FJJA, Poelman EH (2020) Adverse weather conditions impede odor-guided foraging of parasitoids and reduce their host-finding success. Agriculture, Ecosystems & Environment 301:107066. https://doi.org/10.1016/j.agee.2020.107066

Walcroft AS, Brown KJ, Schuster WSF, et al (2005) Radiative transfer and carbon assimilation in relation to canopy architecture, foliage area distribution and clumping in a mature temperate rainforest canopy in New Zealand. Agricultural and Forest Meteorology 135:326–339. https://doi.org/10.1016/j.agrformet.2005.12.010

CW (2014) The spatial and temporal distributions of arthropods in forest canopies: uniting disparate patterns with hypotheses for specialisation: Drivers of insect distribution patterns. Biol Rev 89:1021–1041. https://doi.org/10.1111/brv.12094

Wilson JJ (2012) DNA Barcodes for Insects. In: Kress WJ, Erickson DL (eds) DNA Barcodes. Humana Press, Totowa, NJ, pp 17–46

WS R (2011) Imagej, us national institutes of health, bethesda, maryland, usa. http://imagej nih gov/ij/

Yamazaki K (2010) Leaf mines as visual defensive signals to herbivores. Oikos. https://doi.org/10.1111/j.1600-0706.2010.18300.x

Supplementary material

Table S1 Caterpillar abundance and species richness for each defensive trait (aposematic, cryptic, and shelter-building) for each stratum and across the entire vertical gradient (total).

Stratum	***Defensive traits***					
	Aposematic		***Cryptic***		***Shelter-building***	
	Abundance	***Sp. Richness***	***Abundance***	***Sp. Richness***	***Abundance***	***Sp. Richness***
0-5m	25	12	27	16	34	10
5-10m	47	21	79	39	99	23
10-15m	65	23	98	39	155	25
15-20m	90	30	100	42	101	21
20-25m	64	23	100	38	66	13
25-30m	33	20	65	30	85	18
30-35m	23	14	35	19	51	14
35-40m	18	7	18	10	31	10
Total	365	66	522	109	622	73

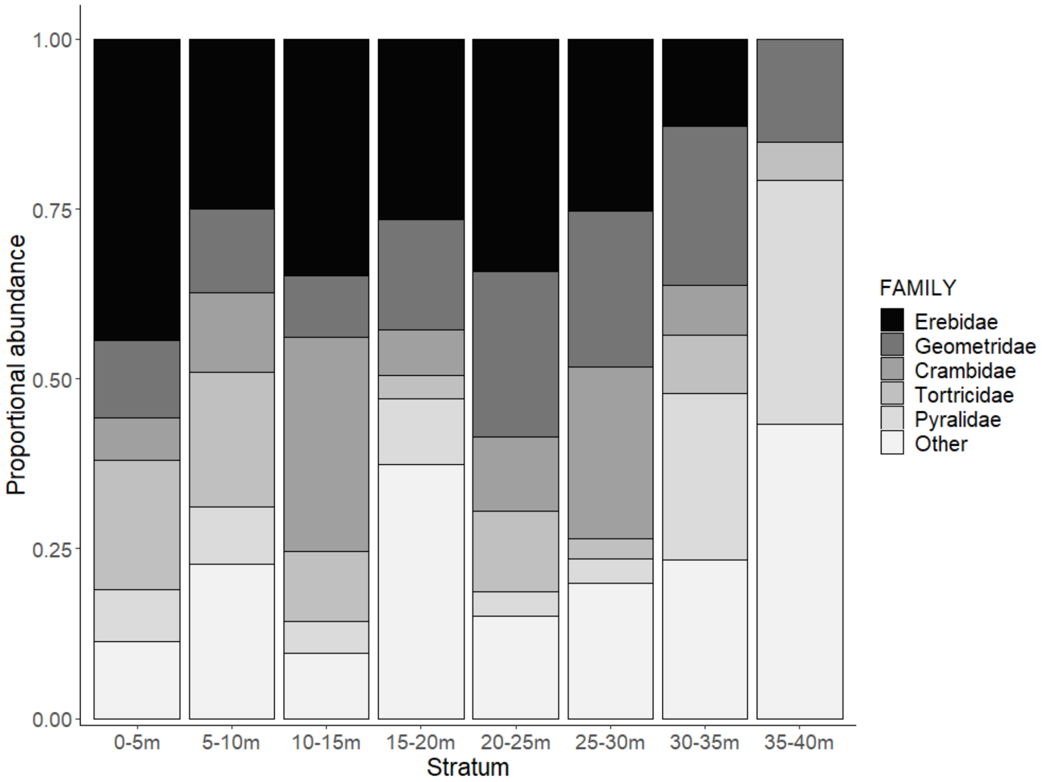

Fig. S1 The proportional abundance of the five most common caterpillar families (>100 individuals) and the remaining families combined (Other) for each forest stratum.

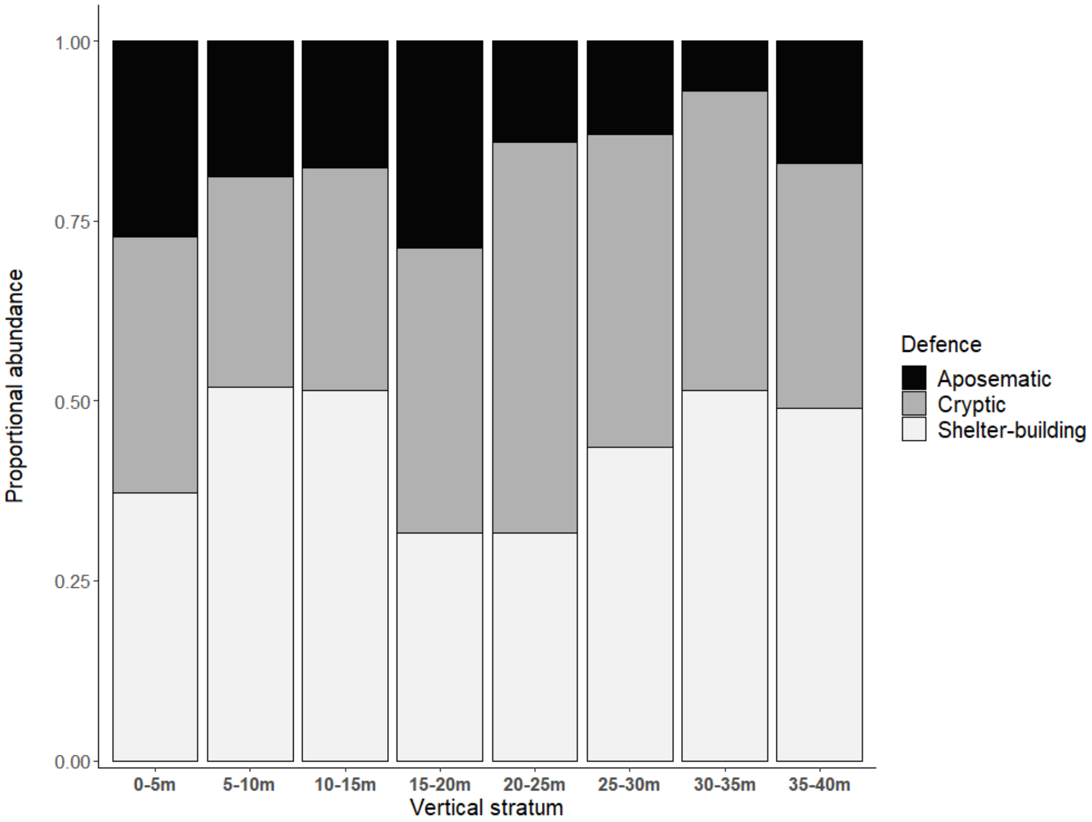

Fig. S2 The proportional abundance of each of the caterpillar defensive traits for each forest stratum.

Table S2 Total number of caterpillar species (and the percentage of the total species) found feeding on only one host plant species (i.e. specialist) and two or more species (i.e. generalist) for each defensive trait after the removal of singletons.

Defensive trait	**Specialist**	**Generalist**
Aposematic	21 (45%)	26 (55%)
Cryptic	37 (46%)	44 (54%)
Shelter-building	31 (58%)	20 (42%)

Table S3 Metrics for caterpillar–host plant interaction networks of individual vertical strata. Based on comparisons with null models, Z-scores and respective P values are reported for weighted generality, weighted vulnerability, and weighted connectance.

	Weighted specialisation networks metrics								
	Generality			***Vulnerability***			***Connectance***		
Stratum	***Obs.***	***Z-score***	***P value***	***Obs.***	***Z-score***	***P value***	***Obs.***	***Z-score***	***P value***
0-5m	1.08	2.55	<0.005	2.19	2.76	<0.003	0.031	3.14	<0.001
5-10m	1.35	2.83	<0.002	3.32	4.02	<0.001	0.021	4.52	<0.001
10-15m	1.18	4.47	<0.001	3.22	6.15	<0.001	0.020	6.55	<0.001
15-20m	1.27	4.06	<0.001	7.51	6.52	<0.001	0.041	6.83	<0.001
20-25m	1.173	4.39	<0.001	13.99	1.56	<0.05	0.090	1.65	<0.05
25-30m	1.06	2.67	<0.003	7.02	5.62	<0.001	0.057	5.70	<0.001
30-35m	1.03	4.15	<0.001	4.76	5.81	<0.001	0.058	5.90	<0.001
35-40m	1	1.58	0.056	4.74	4.32	<0.001	0.096	4.34	<0.001

Table S4 Taxonomic checklist of caterpillar species included in this study as well as the total number of individuals for each species for each vertical stratum (0-5m, 5-10m, 10-15m, 15-20m, 20-25m, 25-30m, 30-35m, 35-40, and non-assigned, which are represented by 1, 2, 3, 4, 5, 6, 7, 8, and NA respectively) and across the entire vertical gradient (Total). The assigned defensive trait of each species: aposematic (A), cryptic (C), and Shelter-building (SB), and taxonomic BIN code is also given.

Taxonomy	*Stratum*									*Total*	*Defence*	*BIN*
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*NA*			
Crambidae												
Crambidae Sp1	4	11	77	17	18	39	6		17	189	SB	BOLD:AEE1691
Crambidae Sp2						2			1	3	SB	BOLD:AEE2153
Crambidae Sp3	1	7	9	1						18	SB	BOLD:AEE7272
Crambidae Sp4				1					3	4	SB	BOLD:AEI6464
Crambidae Sp5		3	1							4	SB	BOLD:AEI6657
Crambidae Sp7							1			1	SB	BOLD:AAZ8695
Crambidae Sp8			7						4	11	SB	BOLD:ACW7425
Crambidae Sp9					3	1				4	SB	BOLD:ADZ0948
Pardomima Sp1		4								4	SB	BOLD:ABZ4988
Parotis Sp1			1							1	SB	BOLD:AAL8871
Pyraustinae Sp1									1	1	SB	BOLD:ACN8440
Depressariidae												
Depressariidae Sp2		1								1	C	BOLD:AEI7040
Depressariidae Sp3				1	1					2	C	BOLD:ADZ1034
Depressariidae Sp4	1			7	2				2	12	C	BOLD:AEN0449
Ennominae												
Cleora Sp1		1								1	C	BOLD:AAF4532
Erebidae												
Achaea catocaloides		3	4	1	2	2				12	C	BOLD:AAJ6790
Achaea Sp1	5	3							1	9	C	BOLD:ACN5980
Amerila luteibarba	2	10	5	13	4	4		2	2	42	A	BOLD:AAC9900
Amerila brunnea		1	3		1	1				6	A	BOLD:AAH6190
Amerila niveivitrea	1	4	3	2	1	1				12	A	BOLD:AAD8431
Amerila Sp1	1				1					2	A	BOLD:ACF2883
Anoba Sp1	1			1			3	3	1	9	C	BOLD:AEG2598
Anomis leona				1					1	2	C	BOLD:AAG6096
Anomis Sp1								2		2	C	BOLD:AEE4441
Arctiinae Sp1				3					2	5	A	BOLD:AEN3282
Arctiinae Sp2		1								1	A	BOLD:AEI5993
Arctiinae Sp3		1								1	A	BOLD:AEI8572
Argyrostagma niobe						1				1	A	BOLD:AAP1161
Balacra elegans								2		2	A	BOLD:AAZ8679
Balacra flavimacula	1		12	2	1					16	A	BOLD:AEE3340
Balacra haemalea	7	2	9	5	2		1	1	1	28	A	BOLD:ACE6803
Balacra herona	5		3	2		2	1	1		14	A	BOLD:AAZ8680

Cautatha drepanodes				1						1	C	BOLD:AAL8493
Cerynea Sp1		5	4	3	9	3				24	A	BOLD:AEE5739
Erebidae Sp1			3							3	C	BOLD:AEE0461
Erebidae Sp10					1		1			2	C	BOLD:AEI9658
Erebidae Sp11				2						2	A	BOLD:ABW8960
Erebidae Sp15	3	2								5	C	BOLD:AEI6009
Erebidae Sp2	3	1			1					5	C	BOLD:AEE0597
Erebidae Sp3			1							1	C	BOLD:AEE7367
Erebidae Sp4		1			2	3				6	C	BOLD:ABW3624
Erebidae Sp5							1	2	4	7	C	BOLD:ACN7658
Erebidae Sp6		4								4	A	BOLD:ACR1238
Erebidae Sp7			1					1		2	C	BOLD:ACR1238
Erebidae Sp8		1	1		1	2				5	C	BOLD:AEE9494
Erebidae Sp9			3	1	1	2			1	8	A	BOLD:AEI4753
Erebidae Sp12		1	2							3	C	BOLD:AEE8644
Erebidae Sp13	1									1	C	BOLD:AEI4420
Erebidae Sp14									1	1	C	BOLD:AEI5880
Erebidae Sp16							1			1	C	BOLD:AEI3886
Eublemma Sp1					1	2				3	SB	BOLD:AEE8923
Euproctis Sp1		1		1		2		1		5	A	BOLD:AAL8759
Euproctis Sp2			1							1	A	BOLD:AAZ8438
Hippurarctia Sp1	1									1	C	BOLD:AEH2268
Lymantriinae Sp2					1	1				2	A	BOLD:AEE2466
Lymantriinae Sp3			1	3		1	1			6	A	BOLD:AEI6191
Lymantriinae Sp1		1		2	1					4	A	BOLD:AAH6293
Lymantriinae Sp4			1					1		2	A	BOLD:ACS1265
Manulea Sp1	2		1				1	1	2	7	A	BOLD:AAG6346
Marcipa Sp1			1				1		1	3	A	BOLD:AEE9713
Melisa diptera				1						1	A	BOLD:AAZ8922
Oruza Sp1						1			1	2	C	BOLD:AEE4870
Paremonia Sp1			1	1	1					3	A	BOLD:AEE8835
Plecoptera Sp1		6	40	1	1	1				49	C	BOLD:ACN8511
Rhipidarctia Sp1		1					1			2	A	BOLD:ACN4127
Rougeotiana Sp1	1	1	3	16	31	11			1	64	C	BOLD:AAL8616
Rougeotiana Sp2	1	1	2	3	3	2				12	C	BOLD:AAH6227
Soloe trigutta		2								2	C	BOLD:AAC6175
Tumicla Sp1				10						10	A	BOLD:ACW5918
Euteliidae												
Eutelia leucodelta					1					1	C	BOLD:AEJ3716
Euteliidae Sp1				3	5	5				13	A	BOLD:AEN2119
Gelechiidae												
Gelechiidae Sp1							1	1	1	3	SB	BOLD:ADG9652
Gelechiidae Sp10		2								2	SB	BOLD:AEI9210
Gelechiidae Sp12						1		1		2	SB	BOLD:ADH3603

Gelechiidae Sp3						3	3			6	SB	BOLD:AEH9388
Gelechiidae Sp4					1	6	2	1	3	13	SB	BOLD:AEE6047
Gelechiidae Sp5		1								1	SB	BOLD:AEE6345
Gelechiidae Sp6			1	1		1				3	SB	BOLD:AEI0060
Gelechiidae Sp7							1			1	SB	BOLD:AEI3356
Gelechiidae Sp8	1	2								3	SB	BOLD:AEI8015
Gelechiidae Sp9						1				1	SB	BOLD:AEI8048
Gelechiidae Sp11								1		1	SB	BOLD:AEI8085
Gelechiidae Sp13				1		1			1	3	SB	BOLD:ADJ2439
Gelechiidae Sp14				1					1	2	SB	BOLD:ADY9887
Gelechiidae Sp15		3	2						1	6	SB	BOLD:AEE9495
Gelechiidae Sp16		1				1				2	SB	BOLD:AEH9345
Gelechiidae Sp17								1	1	2	SB	BOLD:AEI0814
Geometridae												
Antharmostes Sp1									1	1	C	BOLD:AAL8485
Antharmostes Sp2			1		1					2	C	BOLD:AEI8982
Buzura Sp1		1								1	C	BOLD:AEI8982
Chelotephrina Sp1					1					1	C	BOLD:AEF0041
Cleora dargei	1	5	2	3						11	C	BOLD:AAI4131
Cleora lamottei		2	2	1	2	2	1		2	12	C	BOLD:ABY6002
Cleora oculata				1			1	1		3	C	BOLD:AAD7512
Colocleora linearis		1	2	1	2				1	7	C	BOLD:AAH6357
Colocleora divisaria			2	3	3	7	2		1	18	C	BOLD:AAF9551
Colocleora indivisa					1					1	C	BOLD:AAF9539
Cyclophora diplosticta	1					6	1		1	9	C	BOLD:AAF9525
Cyclophora sp2			2							2	C	BOLD:AAV7876
Dasymacaria nr. plebeia			1		1	1				3	C	BOLD:ACN8806
Dasymacaria plebeia							1		1	2	C	BOLD:ABA8685
Dioptrochasma specularia			1						1	2	C	BOLD:ACM8628
Dorsifulcrum canui				1	1					2	C	BOLD:AAL8852
Dorsifulcrum Sp1		1		1						2	C	BOLD:AAH6418
Ennominae Sp1			1	6	2				1	10	C	BOLD:AEN4459
Ennominae Sp2			1							1	C	BOLD:AAP1549
Ennominae Sp3		3	1	1						5	C	BOLD:ADH4832
Eulycia Sp1		1			3	3	7	4	6	24	C	BOLD:AAQ1712
Eupithecia Sp1						1				1	C	BOLD:AEI0703
Gelasmodes fasciata									1	1	C	BOLD:AAL8839
Geolyces Geolyces nr. contenta									2	2	C	BOLD:AAH6123
Geolyces smithi	1	2	2	3	2					10	C	BOLD:AAV3418
Geolyces Sp2	2		2	1		1				6	C	BOLD:AAF9540
Geolyces Sp3						1				1	C	BOLD:ACN5336
Geolyces Sp4				1	1	2				4	C	BOLD:ACN7692
Geometridae Sp1		2	2						1	5	C	BOLD:AEE2500
Geometridae Sp2						1				1	C	BOLD:ACN3388
Megadrepana cinerea				2						2	C	BOLD:AAH6217

Metallochlora misera			2	3	1					6	C	BOLD:AAU3523
Metallochlora Sp1					5	1				6	C	BOLD:AAU0078
Metallospora catori							1	1		2	C	BOLD:AAP2330
Miantochora sp1					1	1				2	C	BOLD:AAF9523
Miantochora sp2		1	1							2	C	BOLD:ABZ0934
Miantochora venerata					1					1	C	BOLD:AAH6366
Omphalucha Sp1				1						1	C	BOLD:ACE9415
Prasinocyma Sp1					1	1				2	C	BOLD:AAF8220
Prasinocyma Sp2						1	1		1	3	C	BOLD:ADB3411
Pycnostega stilbia				1	2	1	1			5	C	BOLD:AAP3132
Racotis squalida	3	1								4	C	BOLD:AAL0856
Racotis zebrina		1	1			4				6	C	BOLD:AAF9530
Thalassodes immissaria	1			2	4	2	5	2	3	19	C	BOLD:AAH6372
Thenopa diversa		3		2	1					6	C	BOLD:ACR1171
Xylopteryx Sp1		2								2	C	BOLD:ACG8725
Zamarada emaciata			1	12	11	2	1		2	29	C	BOLD:AAL9687
Gracillariidae												
Caloptilia sapporella					1	1				2	SB	BOLD:AEF5553
Gracillariidae sp1					1					1	SB	BOLD:AEO4471
Lamprolectica Sp1						4				4	SB	BOLD:AAG7423
Hesperiidae												
Fresna nyassae				1						1	C	BOLD:ADJ8751
Immidae												
Immidae Sp1						1			1	2	C	BOLD:AEI0061
Lasiocampidae												
Gonobombyx angulata	1	1	1							3	A	BOLD:AAH6137
Lasiocampidae Sp1	1									1	A	BOLD:AAW0452
Leipoxais proboscifera				1						1	A	BOLD:AAV7792
Pachymetana Sp1				1	1					2	A	BOLD:AEE9328
Pachymetana Sp2									1	1	A	BOLD:AEE2135
Pachytrina Sp1		1								1	A	BOLD:AEN3023
Ptyssophlebia discocellularis							2			2	A	BOLD:AAL8695
Stoermeriana Sp1		1				1	1			3	A	BOLD:AAL9924
Limacodidae												
Anilina Sp1			1	1			1			3	A	BOLD:AEE4541
Delorhachis Sp1						1				1	A	BOLD:AEI2806
Latoia Sp1		1					4			5	A	BOLD:AEK7653
Limacodidae Sp1				1	1		1			3	A	BOLD:AEH8965
Limacodidae Sp2					10		1	10		21	A	BOLD:AEI7405
Limacodidae Sp3						1				1	A	BOLD:AEE9784
Limacodidae Sp4		1	1		2	1	1			6	A	BOLD:ABV2905

Limacodidae Sp5						1				1	A	BOLD:AEE3799
Limacodidae Sp6			1	1	1				1	4	A	BOLD:AAE9783
Limacodidae Sp7				1						1	A	BOLD:AEI6891
Limacodidae Sp8			3		11	1			1	16	A	BOLD:AEI7009
Limacodinae Sp1							1			1	A	BOLD:ACR1185
Trachyptena Sp1									1	1	A	BOLD:AEE8473
Lycaenidae												
Aphnaeus argyrocyclus						1				1	C	BOLD:AAQ3171
Epitolina dispar		1								1	A	BOLD:AAI5315
Lycaenidae Sp1					1			1		2	C	BOLD:AEE1211
Lycaenidae Sp2	1			1						2	A	BOLD:AEN8829
Ornipholidotos Sp1	1									1	A	BOLD:ABY9895
Syrmoptera Sp2	1		1		1					3	A	BOLD:AAH7454
Metarbelidae												
Metarbelidae Sp1	1									1	SB	BOLD:ABV0335
Noctuidae												
Amphipyrinae Sp1				1						1	C	BOLD:AAH6007
Lophoptera Sp1				1	3	1			1	6	A	BOLD:AED4227
Noctuidae Sp1		1								1	C	BOLD:AEI4967
Noctuidae Sp2		3								3	C	BOLD:AEI6104
Noctuidae Sp3						1				1	A	BOLD:AEO2444
Noctuidae Sp10			1				1			2	C	BOLD:ACN6535
Noctuidae Sp11					1					1	C	BOLD:AEI3801
Noctuidae Sp4			1							1	C	BOLD:AAH6400
Noctuidae Sp5							3		1	4	C	BOLD:AAL8519
Noctuidae Sp6			1							1	C	BOLD:AAL8660
Noctuidae Sp7				1						1	C	BOLD:AAY7143
Noctuidae Sp8		1								1	C	BOLD:ACN6341
Noctuidae Sp9			6	13	3					22	A	BOLD:ACQ1007
Nolidae												
Lophocrama phoennicochlora							2			2	C	BOLD:AAD9056
Meganola Sp1			1	2					1	4	A	BOLD:AAP3232
Negeta approximans		2		2						4	C	BOLD:ACR3594
Nolidae Sp1				26						26	C	BOLD:AEE4029
Nolidae Sp2			1	1						2	A	BOLD:AEO3954
Notodontidae												
Anaphe venata				10						10	A	BOLD:AEI3868
Daulopaectes Sp1				1						1	C	BOLD:AEF0256
Desmeocraera Sp1				1						1	C	BOLD:ACN6989
Janthinisca Sp1				1		1				2	A	BOLD:AAL8882
Notodontidae Sp1			1	1						2	C	BOLD:AEE9010

Notodontidae Sp2		1	2	6					1	10	C	BOLD:AEI4093
Notodontidae Sp3				1						1	C	BOLD:AEI8602
Notodontidae Sp4			2	1					1	4	C	BOLD:AAH6298
Notodontidae Sp5		2	1							3	C	BOLD:AAH6387
Notodontidae Sp6			1							1	C	BOLD:AAL8512
Peratodonta Sp1					1	1				2	C	BOLD:AAL8504
Nymphalidae												
Bebearia Sp1		1								1	A	BOLD:AAC0918
Catuna Sp1				10						10	A	BOLD:AEE5128
Charaxes Sp1	1	1	1	2	1	1			3	10	C	BOLD:AAA6009
Charaxes Sp2		1	1	3	1					6	C	BOLD:AAC1064
Charaxes Sp3					1					1	C	BOLD:AAC1745
Euphaedra Sp1				1						1	A	BOLD:AAB0368
Euptera neptunoides				1						1	A	BOLD:ACJ5855
Euryphura Sp1					1					1	A	BOLD:AAA7865
Libythea labdaca		3	1							4	C	BOLD:AAY7212
Papilionidae												
Graphium policenes		3							1	4	A	BOLD:AAF7125
Pieridae												
Pieridae Sp1				1						1	SB	BOLD:ACN8780
Pterophoridae												
Stenoptilia Sp1		2	1							3	SB	BOLD:AEE9782
Pyralidae												
Mussidia Sp1			1							1	SB	BOLD:AAM7055
Pyralidae Sp1			1							1	SB	BOLD:AEI5889
Pyralidae Sp2		9	1	1	3	2	1		2	19	SB	BOLD:AAH5937
Pyralidae Sp5				7						7	SB	BOLD:AEE6695
Pyralidae Sp6	6	7	1							14	SB	BOLD:AAE9011
Pyralidae Sp7				1						1	SB	BOLD:AEI6192
Pyralidae Sp3				3		3	22	19	3	50	SB	BOLD:AAL8720
Pyralidae Sp4		2	10	15	4	1			1	33	SB	BOLD:ADJ2942
Saturniidae												
Carnegia mirabilis					2					2	A	BOLD:AAA7819
Carnegia Sp1						1	6			7	A	BOLD:AEE2751
Pselaphelia neglecta		5	1							6	A	BOLD:AAB1026
Sphingidae												
Nephele rosae		1	1							2	C	BOLD:ACE9121
Polyptychus carteri		2		1						3	C	BOLD:AAA9585

Tineidae												
Tineidae Sp1		3								3	SB	BOLD:AEE2426
Tineidae Sp2		1								1	SB	BOLD:AEE9452
Tortricidae	+											
Afroploce Sp1			2	2	12	5	1		3	25	SB	BOLD:AAJ2244
Choristoneura dinota			2		4					6	SB	BOLD:AAI0649
Eccopsis Sp1			2							2	SB	BOLD:ACN7373
Eccopsis Sp2							1	2		3	SB	BOLD:ABV2389
Tortricidae Sp1							1			1	SB	BOLD:AEF0103
Tortricidae Sp10		1								1	SB	BOLD:AEE9625
Tortricidae Sp11				2						2	SB	BOLD:AEI0984
Tortricidae Sp12			1						1	2	SB	BOLD:AEI1685
Tortricidae Sp13							1			1	SB	BOLD:AEM7284
Tortricidae Sp15				1						1	SB	BOLD:AEN3931
Tortricidae Sp16									1	1	SB	BOLD:AEI4990
Tortricidae Sp17			1							1	SB	BOLD:AEI5133
Tortricidae Sp18				2						2	SB	BOLD:AEM6824
Tortricidae Sp2					3		4	1		8	SB	BOLD:AEF0154
Tortricidae Sp20	7	17								24	SB	BOLD:AEO0854
Tortricidae Sp3			2		1					3	SB	BOLD:AEH0539
Tortricidae Sp4		1								1	SB	BOLD:AEH1664
Tortricidae Sp5		4	1							5	SB	BOLD:AEH9321
Tortricidae Sp6			3							3	SB	BOLD:AEM5086
Tortricidae Sp7	6	13							1	20	SB	BOLD:AEE0880
Tortricidae Sp8	1	2								3	SB	BOLD:AEE6490
Tortricidae Sp21			2	3	3				1	9	SB	BOLD:AEE3548
Tortricidae Sp22		4	15						3	22	SB	BOLD:AEH8919

Table S5 Tree species and the **a**) caterpillar abundance and **b**) caterpillar richness across the whole vertical forest gradient (total abun./total richness) and for each vertical stratum (0-5m, 5-10m, 10-15m, 15-20m, 20-25m, 25-30m, 30-35m, 35-40, and non-assigned (NA) within the 0.1 ha plot.

a)

Species	**Total abun.**	**Stratum**								
		0-5m	**5-10m**	**10-15m**	**15-20m**	**20-25m**	**25-30m**	**30-35m**	**35-40m**	**NA**
Antiaris africana	0	0	0	0	0	0	0	0	0	0
Celtis adolfi-friderici	9	6	1	0	0	2	0	0	0	0
Celtis mildbraedii	28	4	2	1	1	10	8	2	0	2
Celtis philippensis	284	0	15	100	26	52	77	14	0	27
Chrysophyllum boukokoensis	80	3	6	15	54	2	0	0	0	1
Chrysophyllum perpulchrum	73	8	2	11	46	6	0	0	0	5
Chytranthus sp.1	2	1	1	0	0	0	0	0	0	0
Cola gigantea	24	0	10	0	14	0	0	0	0	3
Copaifera mildbraedii	1	1	0	0	0	0	0	0	0	0
Drypetes aframensis	5	1	2	2	0	0	0	0	0	0
Drypetes aylmeri	71	3	0	22	39	7	0	0	0	3
Drypetes capillipes	20	10	10	0	0	0	0	0	0	0
Drypetes gossweileri	19	0	4	8	7	0	0	0	0	2
Drypetes molunduana	12	0	3	9	0	0	0	0	0	0
Drypetes sp. 5	8	1	7	0	0	0	0	0	0	0
Drypetes sp.2	7	0	6	1	0	0	0	0	0	0
Entandrophragma candollei	4	0	3	1	0	0	0	0	0	0
Ficus sp.1	11	0	0	1	6	3	1	0	0	1
Garcinia ovalifolia	3	0	0	3	0	0	0	0	0	0
Greenwayodendron suaveolens	8	1	1	6	0	0	0	0	0	0
Hylodendron gabunense	276	3	3	12	70	124	49	13	2	18
Mallotus oppositifolius	3	1	2	0	0	0	0	0	0	0
Mansonia altissima	0	0	0	0	0	0	0	0	0	0
Massularia acuminata	0	0	0	0	0	0	0	0	0	0
Nesogordonia papaverifera	5	0	1	0	4	0	0	0	0	1
Olax subscorpioidea	30	6	12	12	0	0	0	0	0	0
Pancovia pedicellaris	6	1	5	0	0	0	0	0	0	0
Pausinystalia macroceras	9	0	2	7	0	0	0	0	0	0
Pentaclethra macrophylla	6	0	0	6	0	0	0	0	0	0
Petersianthus macrocarpus	1	0	1	0	0	0	0	0	0	0
Pterocarpus soyauxii	38	0	0	0	0	0	3	21	14	10
Rothmannia lateriflora	44	0	12	12	20	0	0	0	0	3
Scottelia klainea	3	0	0	2	1	0	0	0	0	0
Staudtia kamerunensis	13	1	5	1	4	2	0	0	0	0
Sterculia rhinopetala	46	0	2	0	0	2	3	16	23	5
Strombosia grandifolia	85	0	11	74	0	0	0	0	0	9
Strombosia pustulata	8	0	2	2	4	0	0	0	0	0
Terminalia superba	77	0	2	0	0	10	28	25	12	16
Tricalysia pangoli	6	0	6	0	0	0	0	0	0	0

Trichilia prieuria	87	7	49	23	8	0	0	0	0	8
Trilepisium madagascariense	0	0	0	0	0	0	0	0	0	0
Uvariastrum pierreanum	80	27	53	0	0	0	0	0	0	4
Xylopia acutiflora	3	0	3	0	0	0	0	0	0	0

b)

Species	**Total richness**	**Stratum**								
		0-5m	**5-10m**	**10-15m**	**15-20m**	**20-25m**	**25-30m**	**30-35m**	**35-40m**	**NA**
Antiaris africana	0	0	0	0	0	0	0	0	0	0
Celtis adolfi-friderici	4	3	1	0	0	2	0	0	0	0
Celtis mildbraedii	19	3	2	1	1	7	7	2	0	2
Celtis philippensis	38	0	5	17	12	18	24	7	0	11
Chrysophyllum boukokoensis	23	3	5	11	13	2	0	0	0	1
Chrysophyllum perpulchrum	19	2	1	2	18	3	0	0	0	5
Chytranthus sp.1	2	1	1	0	0	0	0	0	0	0
Cola gigantea	6	0	2	0	4	0	0	0	0	2
Copaifera mildbraedii	1	1	0	0	0	0	0	0	0	0
Drypetes aframensis	1	0	1	1	0	0	0	0	0	0
Drypetes aylmeri	16	3	0	2	15	5	0	0	0	3
Drypetes capillipes	6	3	5	0	0	0	0	0	0	0
Drypetes gossweileri	11	0	4	7	4	0	0	0	0	2
Drypetes molundua	5	0	1	4	0	0	0	0	0	0
Drypetes sp. 5	6	1	5	0	0	0	0	0	0	0
Drypetes sp. 2	5	0	5	1	0	0	0	0	0	0
Entandrophragma candollei	2	0	1	1	0	0	0	0	0	0
Ficus sp. 1	3	0	0	1	1	2	1	0	0	1
Garcinia ovalifolia	2	0	0	2	0	0	0	0	0	0
Greenwayodendron suaveolens	6	1	1	5	0	0	0	0	0	0
Hylodendron gabunense	64	3	3	8	28	39	26	7	2	17
Mallotus oppositifolius	1	1	1	0	0	0	0	0	0	0
Mansonia altissima	0	0	0	0	0	0	0	0	0	0
Massularia acuminata	0	0	0	0	0	0	0	0	0	0
Nesogordonia papaverifera	4	0	1	0	4	0	0	0	0	1
Olax subscorpioidea	17	6	11	7	0	0	0	0	0	0
Pancovia pedicellaris	4	1	4	0	0	0	0	0	0	0
Pausinystalia macroceras	3	0	1	3	0	0	0	0	0	0
Pentaclethra macrophylla	3	0	0	3	0	0	0	0	0	0
Petersianthus macrocarpus	1	0	1	0	0	0	0	0	0	0
Pterocarpus soyauxii	21	0	0	0	0	0	3	13	10	7
Rothmannia lateriflora	22	0	5	7	14	0	0	0	0	2
Scottelia klainea	3	0	0	2	1	0	0	0	0	0

Staudtia kamerunensis	9	1	3	1	4	2	0	0	0	0
Sterculia rhinopetala	21	0	2	0	0	2	3	9	9	4
Strombosia grandifolia	12	0	3	12	0	0	0	0	0	4
Strombosia pustulata	4	0	1	2	3	0	0	0	0	0
Terminalia superba	18	0	2	0	0	6	9	12	6	5
Tricalysia pangoli	3	0	3	0	0	0	0	0	0	0
Trichilia prieuria	35	4	23	15	7	0	0	0	0	6
Uvariastrum pierreanum	15	9	12	0	0	0	0	0	0	4
Trilepisium madagascariense	0	0	0	0	0	0	0	0	0	0
Xylopia acutiflora	3	0	3	0	0	0	0	0	0	0

Table S6 Total number of individuals per tree species (abundance), total surface area (trunk + leaf surface area [m^2]), total number of caterpillar individuals (abundance) and caterpillar species (species richness) for each tree species across the entire vertical forest gradient.

Trees within 0.1 ha plot				Caterpillars	
Family	**Species**	**Abundance**	**Surface area (m^2)**	**Abundance**	**Species richness**
Moraceae	*Antiaris africana*	1	37.38	0	0
Cannabaceae	*Celtis adolfi-friderici*	2	48.35	9	4
Cannabaceae	*Celtis mildbraedii*	4	175.54	28	19
Cannabaceae	*Celtis philippensis*	5	506.04	284	38
Sapotaceae	*Chrysophyllum boukokoensis*	5	261.97	80	23
Sapotaceae	*Chrysophyllum perpulchrum*	4	128.00	73	19
Sapindaceae	*Chytranthus sp.1*	2	32.08	2	2
Malvaceae	*Cola gigantea*	4	46.78	24	6
Fabaceae	*Copaifera mildbraedii*	2	5.81	1	1
Putranjivaceae	*Drypetes aframensis*	1	108.06	5	2
Putranjivaceae	*Drypetes aylmeri*	2	87.66	71	16
Putranjivaceae	*Drypetes capillipes*	5	123.85	20	6
Putranjivaceae	*Drypetes gossweileri*	2	100.58	19	11
Putranjivaceae	*Drypetes molunduana*	1	18.36	12	5
Putranjivaceae	*Drypetes sp. 2*	1	19.36	8	6
Putranjivaceae	*Drypetes sp. 5*	5	66.83	7	5
Meliaceae	*Entandrophragma candollei*	1	5.82	4	2
Moraceae	*Ficus sp. 1*	1	184.80	11	3
Clusiaceae	*Garcinia ovalifolia*	1	63.93	3	2
Annonaceae	*Greenwayodendron suaveolens*	2	35.14	8	6
Fabaceae	*Hylodendron gabunense*	9	691.81	276	64
Euphorbiaceae	*Mallotus oppositifolius*	1	1.98	3	1
Malvaceae	*Mansonia altissima*	1	183.47	0	0
Rubiaceae	*Massularia acuminata*	1	4.78	0	0
Malvaceae	*Nesogordonia papaverifera*	2	37.83	5	4
Olacaceae	*Olax subscorpioidea*	10	314.21	30	17

Sapindaceae	*Pancovia pedicellaris*	6	58.70	6	4
Rubiaceae	*Pausinystalia macroceras*	1	26.62	9	3
Fabaceae	*Pentaclethra macrophylla*	1	18.46	6	3
Lecythidaceae	*Petersianthus macrocarpus*	1	3.83	1	1
Fabaceae	*Pterocarpus soyauxii*	1	343.01	38	21
Rubiaceae	*Rothmannia lateriflora*	5	108.42	44	22
Achariaceae	*Scottelia klaineana*	3	78.25	3	3
Myristicaceae	*Staudtia kamerunensis*	7	277.53	13	9
Malvaceae	*Sterculia rhinopetala*	1	206.98	46	21
Olacaceae	*Strombosia grandifolia*	1	65.69	85	12
Olacaceae	*Strombosia pustulata*	2	21.46	8	4
Combretaceae	*Terminalia superba*	1	303.89	77	18
Rubiaceae	*Tricalysia pangolina*	2	28.09	6	3
Meliaceae	*Trichilia prieuriana*	10	442.90	87	35
Moraceae	*Trilepisium madagascariense*	1	8.44	0	0
Annonaceae	*Uvariastrum pierreanum*	11	112.72	80	15
Annonaceae	*Xylopia acutiflora*	1	12.36	3	3

3. Conclusion and future prospects

The thesis aimed to explore various biotic and abiotic factors that shape insect communities, particularly those involving parasitoids and their hosts.

In **Chapter 1**, we conducted a comprehensive survey of fruit fly species along an elevational gradient on Mt Wilhelm in Papua New Guinea, a region known for its remarkable diversity of dacine fruit flies. Our study collected a total of 10,700 fruit flies, representing 77 different species. We observed that both the total abundance and species richness of fruit flies decreased with increasing elevation. The species richness was notably high at lower elevations and mid-elevations, but it dropped significantly at 1700 m with no species found at sites above this elevation. We conclude that the upper limit of fruit distribution in PNG likely occurs between 1700 and 2200 m a.s.l., and that the center of diversity is foun between 0 and 700 m a.s.l. Our study also revealed that the fruit fly assemblages were significantly influenced by both the type of lure used and the elevation. This research underscores the importance of understanding the geographical and elevational distribution of these significant agricultural pests and raises important ecological questions for further exploration.

In **Chapter 2**, we delved into the complex interactions within a highly specialised guild of gall forming insects and their parasitoids. We found that both parasitoids and gallers were dominated by specialist species, leading to high network specialisation. The potential for apparent competition was therefore minimal, with one-third of host gall species not sharing any parasitoids. Additionally, we discovered that gall apparency and gall-forming taxon significantly predicted parasitoid assemblage size with more conspicuous galls hosting larger parasitoid assemblages. This research highlights the high network specialisation in a diverse plant-gall-parasitoid network, where specialist gall formers were attacked by specialist parasitoids. This is the largest such network reported from tropical rainforests to date, contributing significantly to our understanding of these complex ecological interactions.

In **Chapter 3**, we examined the complex interactions between insect herbivores and their parasitoids in a tropical forest in Nditam, Cameroon. We systematically searched for caterpillars across the entire vertical gradient of the forest, dividing it into eight, five-metre vertical strata. Our study revealed that caterpillar species richness and diversity peaked in the midstory, while density decreased in the midstory before increasing towards the highest strata. This trend was driven by highly dense shelter-building caterpillars in the upper canopy. We found that both aposematic and shelter-building caterpillars had significantly higher parasitism rates than cryptic caterpillars. Our results highlight the nuanced changes in caterpillar communities across forest strata and underscore the importance of defensive strategies as indicators of parasitism rates in caterpillars.

The research conducted in this thesis has several important implications. Firstly, it underscores the significance of geographical and elevational distribution in shaping insect communities, as demonstrated by the study on fruit flies in Papua New Guinea, the first chapter of this thesis. The documented elevational pattern of fruit fly assemblages has since been referenced in numerous studies comparing various insect groups across elevational gradients (de Dieu Uwizelimana, 2022; Lai et al., 2021; Santos, 2023; Sivault et al., 2023; Sommaggio et al., 2022; Weiss et al., 2024). Moreover, the observed decrease in species richness and abundance, coupled with the defined altitudinal limit of these agricultural pests, offers crucial insights for agriculture in regions where Dacine pests are prevalent. Considering that temperature tolerance is likely one of the most significant drivers of the altitudinal limit, this research implies that the range of certain pest species could expand due to rising global temperatures. This is particularly significant considering the known propensity of tephritid pests to rapidly invade non-native areas (Goergen et al., 2011).

Building upon the work of Butterill & Novotny, (2015), the second chapter of this thesis introduces an additional trophic level to the study of gall-forming insects and their parasitoids. The predominance of specialist parasitoids in this food web is a unique and remarkable finding, underscoring the high degree of network specialisation within this diverse plant-gall-parasitoid network. Notably, this research represents the largest tropical gall-parasitoid network ever analysed to date. This research is the first to suggest and provide evidence for a latitudinal gradient in gall parasitoid specialisation. Furthermore, it highlights the role of host conspicuity in driving increased parasitoid richness, pointing to the significance of visual cues in shaping parasitoid assemblages in gall-parasitoid networks. The insights gained from this study offer potential directions for future research to further investigate the theories suggested in this work.

The final chapter of this thesis presents an in-depth examination of the complex interactions between caterpillars and their parasitoids in a tropical forest in Nditam, Cameroon. This research reveals a highly diverse and stratified caterpillar community, marking a significant contribution to the study of insect communities across ecological gradients in tropical regions. The unique and innovative inclusion of trunk area, in addition to crown volume, has proven to be an improvement on previous methods that used volumetric equations and tree dimensions to calculate leaf area per strata. Furthermore, by categorising externally feeding caterpillars based on their defensive traits, this study provides valuable insights into their interactions with parasitoids. It also supports the extension of the 'safe haven' hypothesis (Lampert et al., 2010) for parasitoids to other forms of enemy-free space, such as caterpillar-built shelters. Given the anthropocentric value of caterpillar-parasitoid interactions in terms of agricultural pest control, this research suggests that certain defensive traits of caterpillars may influence their susceptibility to parasitoids, a factor that could be crucial in determining optimal pest control strategies. This study underscores the need for future research on insect communities across ecological gradients in the tropics to better understand the factors shaping the assemblages of diverse insect groups, thereby aiding in their future conservation.

References

Butterill, P. T., & Novotny, V. (2015). Gall-forming insects in a lowland tropical rainforest: Low species diversity in an extremely specialised guild. Ecological Entomology, 40(4), 409–419. https://doi.org/10.1111/een.12198

de Dieu Uwizelimana, J. (2022). Spatial an temporal distribution of butterflies across an elevation gardient in Nyungwe National Park; Rwanda [PhD Thesis, Universität Koblenz-Landau]. https://kola.opus.hbz-nrw.de/files/2383/Dissertation_Uwizelimana+Jean+de+Dieu+2022.pdf

Goergen, G., Vayssières, J.-F., Gnanvossou, D., & Tindo, M. (2011). Bactrocera invadens (Diptera: Tephritidae), a new invasive fruit fly pest for the Afrotropical region: host plant range and distribution in West and Central Africa. Environmental Entomology, 40(4), 844–854.

Lai, Y., Liu, Y., & Liu, X. (2021). Elevational diversity patterns of green lacewings (Neuroptera: Chrysopidae) uncovered with DNA barcoding in a biodiversity hotspot of southwest China. Frontiers in Ecology and Evolution, 9, 778686.

Lampert, E. C., Dyer, L. A., & Bowers, M. D. (2010). Caterpillar Chemical Defense and Parasitoid Success: Cotesia congregata Parasitism of Ceratomia catalpae. Journal of Chemical Ecology, 36(9), 992–998. https://doi.org/10.1007/s10886-010-9840-0

Santos, M. dos. (2023). Moscas-das-frutas do gênero Anastrepha Schiner, 1868 (Diptera, Tephritidae) na Reserva Particular do Patrimônio Natural Serra Bonita, Camacã, Bahia [PhD Thesis, Universidade de São Paulo]. https://www.teses.usp.br/teses/disponiveis/11/11146/tde-03102023-114704/en.php

Sivault, E., Amick, P. K., Armstrong, K. N., Novotny, V., & Sam, K. (2023). Species richness and assemblages of bats along a forest elevational transect in Papua New Guinea. Biotropica, 55(1), 81–94. https://doi.org/10.1111/btp.13161

Sommaggio, D., Zanotelli, L., Vettorazzo, E., Burgio, G., & Fontana, P. (2022). Different distribution patterns of hoverflies (Diptera: Syrphidae) and bees (Hymenoptera: Anthophila) along altitudinal gradients in Dolomiti Bellunesi National Park (Italy). Insects, 13(3), 293.

Weiss, M., Zimová, K., Mogia, M., & Novotny, V. (2024). Bark and ambrosia beetles on native and transplanted dead wood along an altitudinal tropical forest gradient. Biotropica, e13316. https://doi.org/10.1111/btp.13316

4. Curriculum vitae

Sam Finnie

https://multitrophicinteractions.blog
https://www.researchgate.net/profile/Sam-Finnie-2

28/11/1994
British
+420721947463
sam.c.finnie@gmail.com

SUMMARY

As an imminent PhD graduate based in the Czech Republic, I am seeking a postdoctoral researcher position to further explore my passion for tropical ecology and entomology. My expertise lies in host-parasitoid interactions, ecological gradients, trophic cascades, and the defense mechanisms of herbivorous insects. My doctoral journey has taken me to diverse tropical and temperate locations for fieldwork, enriching my research perspective. I offer advanced statistical and writing skills, a track record of publications, and an unwavering enthusiasm for academic research.

EDUCATION AND WORK EXPERIENCE

Entomology | PhD | University of South Bohemia **02/2019 – Present (Defending 06/24)**

Supervisor: Dr. Katerina Sam

Thesis: The role of ecological gradients and host-parasitoid interaction in tropical insect communities.

- Successfully managed and supervised high-intensity, large-scale fieldwork projects in both temperate and tropical locations in forest understories and in canopy cranes (Daintree Rainforest - Australia, Bubeng Rainforest - China, Tomakomai Experimental Forest - Japan, Auenwald Forest, Germany).
- Demonstrated strong leadership in coordinating teams of researchers under time-sensitive conditions.
- Gained a wide array of lab work techniques including PCR, electrophoryses and advanced insect dissection
- Edited numerous scientific manuscripts and PhD theses as a native English speaker to publication standard
- Efficiently handled large datasets, statistical analysis, and data visualization with R and Python.

Entomology | MSc | Harper Adams University **09/2018 – 02/2019**

Supervisor: Dr. Heather Campbell

Thesis: Parasitoid communities in forested and hedged field margins: an agricultural perspective

- Developing and applying a scientifically novel concept of my own design and critically and creatively evaluating it with reference to current literature
- Learned entomological skills such as identification, pinning, trapping and sampling techniques
- Gained a broad knowledge of insect ecology in both agricultural settings and in their natural ecosystems.
- Advanced identification skills of European species of both solitary and parasitoid wasps

Wildlife Biology | BSc | Manchester Metropolitan University **09/2014 – 09/2017**

EMPLOYMENT

02/2019 – Present

Biology Centre of the Czech Academy of Sciences, Institute of Entomology, Ph.D. candidate.

PUBLICATIONS

Finnie, S., Sam, K., Leponce, M., Basset, Y., Drew, D., Schutze, M.K., Dahl, C., Damag, M., Dilu, M., Gewa, B. and Kaupa, B., 2021. Assemblages of fruit flies (Diptera: Tephritidae) along an elevational gradient in the rainforests of Papua New Guinea. Insect Conservation and Diversity, 14(3), pp.348-355.

Finnie, S., Butterill, P., Novotny, V., Redmond, C., Jorge, L.R., Abe, T., Lamarre, G.P., Maicher, V. and Sam, K., 2023. Vertical stratification and defensive traits of caterpillars against parasitoids in a lowland tropical forest in Cameroon. Oecologia. (accepted 3/2024)

Sivault, E., Kollross, J., Jorge, L.R., **Finnie, S.**, Mendez, D.D., Garzon, S.F., Maraia, H., Lenc, J., Libra, M., Murakami, M. and Nakaji, T., 2023. Insectivorous birds and bats outperform ants in the top-down regulation of arthropods across strata of a Japanese temperate forest. bioRxiv, pp.2023-11.

Xiao, X., Jorge, L.R., Volf, M., Moos, M., Gélin, U., **Finnie, S.**, Freiberga, I., Jancuchova-Laskova, J., Weiss, M., Novotny, V., Sam, K., 2024. The effect of drought-induced leaf traits on Ficus leaf palatability is species specific. Ecosphere, (accepted with minor revisions).

Butterill, P., **Finnie, S**., Sam, K., Fayle, T., Novotny, V., 2024. Gall apparency drives parasitoid in a highly specialised gall-parasitoid food web from a tropical rainforest. (presubmission).

INTERNATIONAL CONFERENCES

19-23 June 2023: **European Conference of Tropical Ecology**, Vertical stratification and defensive traits of caterpillars against parasitoids in a lowland tropical forest in Cameroon. Ceske Budejovice, Czech Republic. Oral presentation.

7-9 June 2022**: European Conference of Tropical Ecology,** The effects of aposematism and feeding on the parasitsm rates of caterpillars across forst strata in Cameroonian rainforest. Montpellier, France. Poster.

OTHER SCIENTIFIC EXPERIENCE

Responsible for liaising with the authors of published papers to aquire and format datasets on parasitoid-host interactions for the ongoing, collaborative and meta-anayltic project LIFEWEBS.

In charge of maintaing malaise traps and collecting samples in the Czech Republic for the global biodiversity monitoring program LIFEPLAN.

Assistant curator of Hymenoptera in the Entomological department of the Manchester Museum for 2 years.

AWARDS

Royal Entomological Society (RES) Student Award 02/2018

Writing a scientific article about an invasive species of scorpion in the UK which was published in the annual RES journal Antenna

LANGUAGES

English
Native speaker

Spanish
B1