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Community structure of caterpillar parasitoids from tropical rainforest

Ph.D. Thesis

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Annotation

The aim of this dissertation is to investigate the community structure of parasitoids and their interactions with their hosts – caterpillars in the rainforest of Papua New Guinea, focusing on beta diversity and altitudinal trends. We show that parasitoid beta diversity is higher than the beta diversity of their hosts, which was also projected in their interactions. The altitude plays important role in the diversity of parasitoids, where we show that Ichneumonidae are more diverse at high elevation than at low elevation, which was not observed for other large insect families. Further, we performed a manipulative experiment, exposing live caterpillars in different elevations of tropical rainforest. Caterpillars faced similar mortality from parasitoids between elevations, but higher mortality from predation at low elevation. In the last chapter, we point out how important is correct species identification because wrong identification can misinterpret observed interactions.

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.

České Budějovice, 28. 4. 2021

A handwritten signature in blue ink, reading "Martin Libra", is written over a horizontal dotted line.

Martin Libra

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List of papers and author's contribution

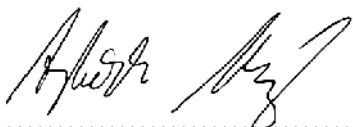
The thesis is based on the following papers (listed chronologically):

- I. Martin Libra*, Salape Tulai, Vojtech Novotný, and Jan Hrček. (2019) Elevational contrast in predation and parasitism risk to caterpillars in a tropical rainforest. *Entomologia Experimentalis et Applicata* 167: 922-931. DOI: 10.1111/eea.12851 (IF: 1.696)
ML conducted the experiment (100 %), formulated the hypotheses, did the statistical analysis of the data, and wrote the first draft of the manuscript (95 %).
- II. Martin Libra*, Vojtech Novotný, Scott E. Miller, Hiroshi Shima, Donald L.J. Quicke, James B. Whitfield, David Wahl, George D. Weiblen, Yves Basset, John Auga, Kenneth Molem, and Jan Hrček. „Beta diversity in a tropical rainforest is higher for parasitoids than their caterpillar hosts “. (Manuscript)
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Dr. Hiroshi Shima

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INTRODUCTION

Terrestrial food webs of plants, herbivores and their natural enemies harbor more than 75 % of global biodiversity (Price 2002). Despite the diversity of parasitoids (they comprise up to 10 % of all described insect species) a little is known about parasitoid community structure, especially in the tropical rainforests (Godfray & Shimada 1999; Sharanowski *et al.* 2021). Yet, understanding parasitoid community structure is important because parasitoids affect entire communities; they are part of third or fourth trophic level affecting the abundance of their hosts and thus play a role in the regulation of insect herbivores, including pest species (Janzen 1995; Rosenheim *et al.* 1995; Hrcek *et al.* 2013).

1.1. Diversity of species assemblages and their interactions

In 1960, R.H. Whittaker proposed a way to quantifying the dissimilarity of species composition among pair of sites – the beta diversity. Beta diversity was then defined as the ratio between gamma (regional) and alpha (local) diversities. The differences in beta diversity among communities can be caused by species replacement (species turnover), or nested loss of specimens (nestedness; Baselga 2010). Dissimilarity in species composition among communities changes the composition of food web interactions (beta diversity of interactions). The interaction turnover can be caused by the species turnover when the interactions are gained or lost with presence or absence of species, or by “rewiring” of the interactions, where the same species make different interactions within different assemblages (Poisot *et al.* 2012).

Revealing basic processes affecting the diversity within each trophic level is essential because the diversity of higher trophic levels is affected by lower trophic level (“bottom-up effect”). Lower trophic levels serve as a matrix for a diversity of higher trophic levels (Janzen 1970; Lewinsohn & Roslin 2008), but at the same time, higher trophic levels shape abundance and diversity of lower trophic levels in co-evolutionary feedback (“top-down effect”; Holt 1977; Frost *et al.* 2016). We know relatively a lot about the distribution and diversity of the lower trophic levels represented by plants and herbivorous insect (Novotny *et al.* 2007; Murakami *et al.* 2008; Raven *et al.* 2020), or about alpha diversity of higher (third) trophic levels, e.g., parasitoids (Hoffmeister 1992; Greeney *et al.* 2012; Hrcek *et al.* 2013).

The diversity of caterpillars is driven by a diversity of their host plants, mainly in tropical areas (Lewinsohn *et al.* 2005; Novotny *et al.* 2006). In general, the alpha diversity of tropical host plants is high, therefore the alpha diversity of caterpillars is also high (Lewinsohn & Roslin 2008). However, at a larger spatial scale, caterpillar beta diversity is relatively low (Dyer *et al.* 2007; Novotny *et al.* 2007). In contrast, we have lack of information about the components of beta diversity of the third trophic level, represented mainly by parasitoids (Cuevas-Reyes *et al.* 2007; Maunsell *et al.* 2015; Pereira Martins *et al.* 2019).

1.2. Parasitoids

Parasitoids are insects that lay their eggs on or in the bodies of their hosts, primarily other insects (or rarely other arthropods). The term parasitoid was first used by Reuter (1913) to make a distinction between parasites (host is harmed but generally not killed) and predators (multiple preys are killed). Parasitoid's larvae are feeding like true parasites, but the single host is eventually killed unless the host successfully defends itself with its immune system. Adult parasitoids are free-living (Godfray *et al.* 1994; Quicke 1997).

The majority of parasitoids belongs to the order Hymenoptera (75 % of species), mainly to suborder Apocrita (so-called "Parasitica"). The most prominent Hymenoptera parasitoids are Ichneumonidae, Braconidae, Chalcidoidea and Proctotrupoidea. A substantial diversity of parasitoids is contained in the Diptera order, mainly the family Tachinidae. The remaining groups of parasitoids are representatives of Coleoptera, Lepidoptera, Neuroptera or Trichoptera (Eggleton & Belshaw 1992; Shaw 1994; Godfray & Shimada 1999).

Parasitoids attach to the specific developmental stage of the host - eggs, larvae, pupa and, more rarely, adults (Price 1972; Waage & Hassell 1982) and emerge either from the same stage or host is not killed until it enters a later developmental stage (Soller & Lanzrein 1996). Larvae are the most commonly attacked stage (Hawkins *et al.* 1997). The parasitism rate in exposed hosts is usually 10 – 20 % (Singer & Stireman 2003; Barbosa & Caldas 2004).

Obtaining good quality data about parasitoids and their hosts is difficult (Shaw 1997). Morphological identification of parasitoids, and also their hosts, is problematic due to their high diversity and impossibility to reveal cryptic species (Pfenninger & Schwenk 2007; Smith *et al.* 2008) and must be combined with

molecular identification (Sigut *et al.* 2017). A potential possible source of the interaction data is mass rearing campaigns of likely hosts, like those of Daniel H. Janzen in Costa Rica (Janzen 2000), Lee A. Dyer across North and Central America (Gentry & Dyer 2002), or Vojtech Novotný in Papua New Guinea (of which this thesis is part of), and several others (Stireman *et al.* 2005).

1.3. Parasitoid specificity

Parasitoids are typically divided into two major groups depending on the effect on host development and the intimacy of contact between parasitoid larvae and host individuals (Askew & Shaw 1986). Parasitoids allowing their host to continue in development after oviposition are termed koinobionts; those that kill or paralyze their hosts permanently at the time of oviposition are termed idiobionts. Most koinobionts are endoparasitoids, whose larvae live and feed inside of the host body. In contrast, idiobionts are typically ectoparasitoids and feed on the outside surface of their hosts and usually attack older host larvae or pupae to obtain enough food supply for the development of the parasitoid. Idiobionts usually attack concealed larvae of mining insect (miners) and folivorous insect protected by plant tissue (borers or gallers). On the other hand, koinobionts usually attack the younger larval stages of exposed hosts. Host larvae are not completely paralyzed or killed and larvae of koinobionts are growing together with their host. Parasitized host are therefore exposed to potential predation of the host, and koinobionts therefore sometimes affect the behaviour of their host (Holt & Lawton 1993; Grosman *et al.* 2008).

Hawkins (1994) identified the degree of host concealment as a key parameter for parasitoid communities. Parasitoid species richness and parasitism rate increase with the degree of concealment. The most attacked are leaf-miners and gallers, followed by case bearers and web-tiers. The lowest parasitism rate is recorded for exposed feeders and leaf-rollers. Endophagous hosts are preferred by hymenopteran parasitoids, and ectophagous hosts by dipteran parasitoids (Stireman & Singer 2003; Hreck *et al.* 2013). This is not caused just by morphological adaptation but also by their host specificity. Host specificity is an important factor affecting food web interactions and obtaining information about the parasitoid host specificity requires a study on a broad range of potential hosts (Mills 1992; Connahs *et al.* 2009). Host specificity varies among the parasitoid

groups and feeding mode of the host. Hrcek *et al.* (2013) analyzed a diverse community caterpillar and their parasitoids in lowland forest in Papua New Guinea. He found that far more species and a higher proportion of species of Braconidae were host species-specific. Also, Tachinidae, unexpectedly showed higher host specificity than Ichneumonidae. It could mark that the Ichneumonidae family showing a rather low species richness on external feeding tropical hosts. Stireman *et al.* (2017) conducted a research on the host specificity of tachinid flies. Tachinids were often called as generalists among parasitoids, however, according to it seems, that truly generalized tachinids may be rare, most species known as generalists are complexes of closely related taxa (Smith *et al.* 2007).

1.4. Diversity of parasitoids – latitudinal gradient

One of the clearest patterns in ecology is a latitudinal pattern of diversity, with higher species richness at low latitudes than at high latitudes (Gaston & Blackburn 2000). Among insects, we observe some exceptions which do not follow the latitudinal pattern of diversity. Well-known are aphids, bumblebees or sawflies which have their centers of diversity in the temperate zone (Dixon *et al.* 1987). Among parasitoids, there could be one important exception – a family Ichneumonidae – parasitoid wasps, possibly the world largest animal family (Gauld *et al.* 2002). Ichneumonids are often mentioned as an example of an anomalous diversity pattern with the highest diversity at temperate zones (Owen & Owen 1974; Janzen 1981).

Owen & Owen (1974) found out that Ichneumonids are more abundant at the temperate than at the tropical localities. This unusual pattern soon became known as an anomalous diversity gradient (Rathcke & Price 1976) and has been replicated by several other studies (Janzen & Pond 1975; Timms *et al.* 2016). This unexpected pattern initiated further work and many hypotheses were put forward to explain the anomalous diversity.

In 1975, Janzen and Pond have introduced a fragmentation hypothesis which postulates that biomass of tropical host species is divided into many dissimilar species, each one often being too rare to sustain a species of parasitoids and parasitoids in a community, therefore, increase non-linearly with host diversity.

Rathke and Price (1976) have proposed a predation hypothesis. They suggested that predation is more severe in the tropics and parasitized host tend to be particularly susceptible to predation. Parasitoids might prolong host development, force the host to act abnormally (e.g., become sluggish) and it causes a greater exposure to predation. The third hypothesis suggested by Gauld *et al.* (1992) is called a nasty host hypothesis (NHH). The NHH says that possible herbivorous hosts in tropics are more toxic than equivalent possible hosts in temperate forests. The toxicity is a good defense mechanism against bird predation and parasitoids as well (Greeney *et al.* 2012). Sime and Brower (1998) tested the NHH on a toxic group of butterflies and their specialist ichneumonid parasitoids. They found out that the species richness of toxic caterpillars increases dramatically towards to tropics whereas their parasitoids do not. They discussed that possible trend might be affected by the host specificity of parasitoids. In temperate regions, parasitoids tend to be more polyphagous than in a tropical forest.

Quicke (2012) tested a relationship between life strategy (idiobionts vs koinobionts) and both latitude and body length of Ichneumonidae and Braconidae. For both families, the proportion of described koinobionts significantly increases with latitude. Life history was also significantly correlated with the body size of both families, the proportion of koinobionts decreases with increased body size latitude.

All previous hypotheses trying to explain the anomalous diversity phenomenon are based on reduced accessibility of hosts to parasitoids in tropical rainforests. However, a few recent studies show that this pattern could also be caused by a lack of knowledge and by biases in the data (Quicke 2012; Veijalainen *et al.* 2014). The bias is caused mainly by missing data across the world, missing studies from higher elevation at tropics or by the big number of cryptic species which have not been revealed.

Timms *et al.* (2016) published a study where the species richness of Ichneumonidae along the latitudinal gradient was tested. In the study, they have also included data from arctic regions which have not been often used in previous comparisons. They demonstrated a wide variety of latitudinal patterns, reflecting ecological variation between subfamilies. Besides, they showed models where host taxon and subfamily identities were better predictors of the shape of the relationship between subfamily abundance and latitude than other characteristics

previously hypothesized to be important (life history of parasitoids or body size). They also noticed that only for of the Ichneumonidae subfamilies show the anomalous diversity gradient. A good way to improve the current data might be more accurate studies in tropical ecosystems, mainly at higher elevation where parasitoids are still not well documented.

1.5. Diversity of parasitoids – elevational gradient

Ecological studies focused on diversity patterns across spatial environmental gradients are becoming more popular, mainly for possible distribution shifts caused by global warming (Burrows *et al.* 2014; Maunsell *et al.* 2015; Sam *et al.* 2020). However, information about parasitoid community and interactions within food webs in higher elevation sites are still not well documented (Peck *et al.* 2008; Hall *et al.* 2015; Jeffs *et al.* 2021). The study of parasitoid communities at higher elevations in tropical regions may thus be one of the possible ways of resolving the anomalous diversity pattern in parasitoids on an analogous temperature gradient. Timms *et al.* (2016) found that only a few subfamilies of the Ichneumonidae family are following the anomalous diversity trend and the remaining are increasing with latitude. The anomalous subfamilies can be affected by missing information from higher elevation at lower latitudes. Studies of tropical Ichneumonidae show different diversity trends along with altitude. Connahs *et al.* (2009) recorded a high proportion of Ichneumonids at higher altitudes and far more braconids in the lowlands of Ecuador. The high proportion of braconids over ichneumonids was found also by studies focused on lowlands only (Janzen & Gauld 1997; Hrcek *et al.* 2013). On the other hand, Veijalainen *et al.* (2014) argue that Ichneumonidae at the tropics are more species-rich at middle elevations (also known as mid-elevation peak). Parasitoids were collected and compared with different places with different elevation (Ecuador, Peru, Costa Rica and Georgia (USA)). Finally, van Noort (2004) published a taxonomic study where Ichneumonidae were more species-rich at lower elevations in the Gabon mountains.

Different trends of parasitoid diversity may be connected with the feeding strategy of parasitoids as well as their hosts, which could be essential for the understanding of changes in host-parasitoid relationship along an altitudinal gradient (Péré *et al.* 2013). For example, the parasitism rate of ectoparasitoids

feeding on ectophagous hosts decrease with increasing elevation and the same pattern was observed also for parasitoid species richness (Péré *et al.* 2013). Morris *et al.* (2015) studied food-web interactions of cavity-nesting Hymenoptera along an altitude gradient in Australia. They found a general decrease in parasitism rate across elevation sites. More studies interested in food web changes along altitudinal gradient mainly focused on leaf miner communities (Cagnolo *et al.* 2010; Maunsell *et al.* 2015). Maunsell *et al.* (2015) found parasitoids of miners to be more generalists at lower elevations than in highland, where parasitoids were attacking only single host species. Hanson and Gauld (1995) proposed a theory that a higher proportion of tropical koinobiont ichneumonids are nocturnal at lower altitudes. It could relate to a lower temperature and easier searching activities during the night. Koinobionts are usually more host-specific, usually attacking external feeding hosts. With the changing altitude, temperature and feeding strategy of parasitoids we can expect species turnover, as was observed on parasitoids of geometrid caterpillars in Ecuador (Connahs *et al.* 2009). More braconids were observed at lower altitude while ichneumonids were more frequent at higher altitudes. If we take into account that ichneumonids-koinobionts have high host specificity and with increasing elevation, host specificity is increasing, it would be interesting to compare two different guilds of host – ectophagous caterpillars (e.g. Connahs *et al.* 2009) and endophagous larvae of mining insect (e.g. Maunsell *et al.* 2015).

1.6. Mortality of herbivore hosts

Herbivorous insects are attacked by a taxonomically and ecologically diverse collection of natural enemies including vertebrate predators, invertebrate predators, insect parasitoids, nematodes, fungi, protists, bacteria, and viruses (Hawkins *et al.* 1997). These organisms are representing “top-down” control agents (effect of natural enemies). For parasitoids, the host is a source of food supplies but also a source of living space. The parasitoids can thus be killed with the host by other predators or parasitoids on the same trophic level (intraguild predation; Van Veen *et al.* 2008). Other effects which affect hosts are “bottom-up” controls represented by host plants and their defensive strategies designed to avoid or reduce damage caused by herbivores (Hunter & Price 1992). The influence of plant factors is greatest in the early stages of larvae, but the older

stages are more affected by predators and parasitoids. Overall, parasitoids kill more herbivores in a temperate zone than do either predators or pathogens, however, in tropics predators and pathogens are possibly more important agents of mortality (Hawkins *et al.* 1997; Low *et al.* 2014).

“Bottom-up”

Host plants affect the rate of herbivory mainly through their defensive strategies (e.g. secondary metabolites, morphological adaptations or trichomes; Ehrlich & Raven 1964; Volf *et al.* 2015). As a response to these adaptations, we can find herbivores with different levels of host specialization (Redmond *et al.* 2019). Generalists respond to overall host plant chemistry and trichomes, whilst specialists respond to secondary metabolites and host plant phylogeny (Volf *et al.* 2015). Host plants and their secondary metabolites affect not only herbivores but also the parasitoid community through the trophic cascade. Herbivores can use secondary metabolites for their defense against parasitoids or predators (The NHH; Gauld *et al.* 1992)). For some groups of toxic caterpillars species, a trend of increasing diversity towards tropics was observed whereas their specialist parasitoids did not follow (Sime & Brower 1998). Another way, how host plants deal with their enemies is through releasing volatiles attracting predators and parasitoids to reduce herbivory (Dicke & van Loon 2000; Klemola *et al.* 2012).

“Top-down”

Arthropods, mainly parasitoids and ants, and birds represent the main source of mortality of herbivores (Lovei & Ferrante 2017). Avian predators are not attracted by volatiles directly, but they hunt caterpillars mostly visually (Lichter-Marck *et al.* 2015). Birds can play important role in intraguild predation because they can feed on herbivores attacked by other arthropods (Mooney 2007). In a tropical rainforest, Sam *et al.* (2015) tested the main sources of possible mortality on artificial caterpillars. They found that ants had a stronger influence at the lower elevation, however, in higher elevation birds were more important. Unfortunately, in their study they could not test an influence of parasitoids which play an important mortality factor, directly affecting host abundance and, indirectly, affect even host plants (Hawkins & Sheehan 1994; Stiling & Moon 2005). Libra *et al.* (2019; Chapter III.) tested how invertebrate predators and parasitoids affecting the predation rate of live caterpillars in tropical rainforest,

and they found that the predation rate of externally feeding caterpillars decreases with the elevation, however, did not find any difference in parasitism rate. This trend was also shown in a global study of Roslin *et al.* (2017) where they found a significant effect of invertebrate predators at lower elevation in tropics, and the importance of invertebrate predators decreased towards the poles. Mortality factors on external feeding hosts and herbivores in different habitats are not known and should be studied more deeply. A combination of traditional methods of observation, manipulative experiments and molecular methods would be very useful to describe interactions within food webs.

1.7. Classic taxonomy and molecular identification

The development of molecular methods, mainly DNA barcoding, allows the identification of species that are morphological uniform even to well-trained and skilled taxonomists (Hrcek & Godfray 2015; van Nouhuys 2016). A detailed recognition of species is necessary because it strongly affects how the whole food web structure and interactions within the web are perceived. Smith *et al.* (2006) conducted a study focused on host specificity of tachinid parasitoids which are widely presented as relative generalists in the species of hosts. DNA-barcoding has changed the original 16 morphospecies of apparently generalist tachinid fly parasitoids into a complex of at least 73 species. On the other hand, Condon *et al.* (2014) revealed a wider spectrum of hosts thanks to DNA barcoding. Parasitoid wasps hatched out of *Blepharoneura* fly hosts were referred to as highly host-specific.

However, a test of the presence of parasitoid tissue inside of host pupae revealed that the parasitoids are attacking multiple host species although they are not able to develop in most of them. The presence of foreign tissue in a sample tissue could be used to track trophic interactions in yet another way. Wirta *et al.* (2015) tested remaining tissue inside of parasitoid's guts with highly specific genetic markers which helped to identify the hosts of the parasitoids.

1.8. Aims and Scope of this thesis

Parasitoids shape communities of their herbivorous hosts and affect whole food web interactions (Memmott *et al.* 1993; Godfray & Hassell 1997). This dissertation aims to describe community patterns of parasitoids from tropical rainforest and help to understand how food web interactions of parasitoids and their hosts are structured in unexplored localities.

In **Chapter I.** we described parasitoid beta diversity of externally feeding caterpillars across the lowland tropical rainforest of Papua New Guinea. Because our data used in Chapter I. and II. comes from mass-rearing project, that allowed us to compare the dissimilarity in parasitoid diversity with the diversity of their hosts. In addition, we compared also tritrophic interactions and their dissimilarity with a distance. We also compared how less common plants and caterpillars affect the interactions within food web.

In **Chapter II.** we compared the diversity of parasitoid communities between low-elevation and high-elevation tropical rainforest of Papua New Guinea. The elevational comparison can indirectly substitute the latitudinal gradient with a decrease of temperatures from the equator to the poles. We found out that Ichneumonidae were more species-rich at high elevation in contrast to Braconidae and Tachinidae, which were more species-rich at low elevation. Further, we tested theories used for explaining the anomalous latitudinal gradient of Ichneumonidae, with higher diversity in a colder environment. Thanks to the mass rearing of caterpillars, we could compare also hyperparasitoids and their diversity between elevations.

Because one of the potential explanations of the difference in Ichneumonidae diversity is the higher predation rate of their hosts in warmer habitats, we conducted an experiment, where we exposed live caterpillars to invertebrate predators and parasitoids in different elevations (**Chapter III**). This was the first study where live externally feeding caterpillars were exposed to invertebrate predators and parasitoids in a tropical region.

The precise identification of all participants in food web interactions is crucial (Derocles *et al.* 2015). In **Chapter IV**, we described three new species of tachinid parasitoids that were reared from externally feeding caterpillars in Japan during mass rearing project (Volf *et al.* 2019). Except for the description of new species, we also provided new parasitoid host records and oviposition strategies for already known tachinid parasitoids. We also provided two identification keys for two genera with all currently described species.

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CHAPTER I.

Beta diversity in a tropical rainforest is higher for parasitoids than their caterpillar hosts

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Abstract

The patterns of multitrophic interactions are key to understanding mechanisms of biodiversity origin and maintenance. Here we analyze beta diversity of all trophic levels in the parasitoid-herbivore-plant interactions across 500 km of continuous lowland rainforest in Papua New Guinea. The beta diversity was higher for parasitoids than their caterpillar hosts. Both beta diversity values increased with distance and were driven by species turnover rather than nestedness. The dissimilarity of interactions between parasitoids and caterpillars was significantly increased with increasing distance, and it was caused by species turnover. Moreover, the dissimilarity of interactions between parasitoids and caterpillars was higher than for caterpillars and their host plants. That indicates the tri-trophic network of plants, caterpillars and their parasitoids can generate highly heterogeneous environment for the upper trophic level despite a largely homogenous environment of lowland tropical forest.

Keywords: parasitoid, beta diversity, food web interactions, species turnover

CHAPTER II.

Elevational contrast in parasitoid community structure in tropical rainforest: Ichneumonidae are more diverse in highlands.

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Abstract

Ichneumonidae parasitoids represent a rare example of an anomalous latitudinal gradient of diversity, decreasing from temperate to tropical regions. Here we used quantitative rearing data on caterpillar-parasitoid communities from Papua New Guinea rainforests to show that Ichneumonidae, but not other parasitoids, exhibit a similarly unusual gradient of decreasing diversity from low to high elevations. The latitudinal trends have been explained by several theories based on host ecology, but not tested for the lack of data. Our analysis of elevation trends showed that caterpillar diversity and feeding mode, plant diversity, and parasitoid host specificity did not have a significant effect on Ichneumonidae diversity so that the gradient is not due to host-related factors. Instead, an increase in body size with elevation in the parasitoid community is consistent with Bergmann's rule and thermal ecology could explain anomalous diversity gradients in Ichneumonidae.

Keywords: parasitoid, diversity, Ichneumonidae, altitude, latitude

CHAPTER III.



Elevational contrast in predation and parasitism risk to caterpillars in a tropical rainforest

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Key words: mortality factor, parasitoid, sentinel prey, intraguild predation, Lepidoptera, Crambidae, *Talanga excelsalis moresbyensis*, Moraceae, Papua New Guinea, *Ficus copiosa*

Abstract

Invertebrate predators and parasitoids are among the most important natural enemies of insect herbivores. Yet, the strength of natural enemy pressure along an altitudinal gradient and interactions between the groups of natural enemies (such as predation on parasitized prey) are not well known. Various methods are used to reveal the mortality factors of herbivores. Predation pressure is usually assessed through exposure of artificial prey. However, this method cannot provide information about the attacks of parasitoids, or their eventual interactions with predators. Furthermore, artificial or dead prey might not attract predators because they do not show expected host behavior, and this method mostly cannot distinguish between predation and scavenging. For the first time in a tropical rainforest, we quantified elevational contrast in mortality factors using exposure of live caterpillars. We exposed a total of 800 live caterpillars of *Talanga excelsalis moresbyensis* Strand (Lepidoptera: Crambidae) on saplings of *Ficus copiosa* Steud. (Moraceae) at two elevations in primary tropical rain forest in Papua New Guinea (200 and 1 200 m a.s.l.). We exposed the caterpillars in two treatments: exposed to and protected from invertebrate predators and parasitoids. Disappearance of caterpillars was significantly higher in the exposed treatment. Furthermore, caterpillar disappearance was significantly higher in lowlands than in highlands (43 vs. 12%). We consider the vast majority of the disappearance to be due to predation, as migration of the caterpillars from the focal trees was not observed (except one caterpillar). This estimate of invertebrate predation rate corresponds with studies which used artificial caterpillar models. No significant difference in parasitism rate between the two elevations was observed (12 vs. 13%). The combination of the disappearance and parasitism rate patterns means that larval parasitoids face stronger pressure from invertebrate predators through higher predation of their hosts in the lowlands than in the highlands.

Introduction

Predators and parasitoids are the major sources of insect herbivore mortality and influence the structure and stability of food web interactions (Ehrlich & Raven, 1964; Cornell & Hawkins, 1995). It is therefore essential to understand the effect of various natural enemies on herbivore communities, not only in agricultural habitats, where natural enemies are used for biological control of insect pests, but also in natural forest habitats (Jervis, 2005; Howe

et al., 2015; Morris et al., 2015; Buczkowski, 2017). Furthermore, the groups of natural enemies can interact, and we therefore need to quantify their interactions as well to understand their natural combined effect on the herbivore community (Polis & Holt, 1992; Stireman et al., 2017). Most parasitoids of herbivores attack the herbivore larvae, and predation of the herbivore larva is therefore fatal for the parasitoid as well (Hawkins, 1994). However, predation and parasitism rates are rarely quantified together.

Multiple approaches can be used for assessing predator–prey interactions (direct observation, examination of prey remnants in gut contents or feces, exclusion of predators, molecular gut content analysis), but exposure of baits is the most commonly used method (reviewed by Birkhofer et al., 2017; Lovei & Ferrante, 2017). The baits can be either

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artificial (dummy) or live (sentinel) prey (Birkhofer et al., 2017). Artificial prey is typically made of plasticine or dough (Low et al., 2014). The imprints of mandibles or beaks in soft material allows to identify the attacker and provide the estimates of attack rates on generalized models of prey. Artificial prey lacks the natural attributes and behavior of the live prey (e.g., surface chemicals, defensive movement, vibrations, frass). These attributes are important for prey recognition by predators and parasitoids, therefore data obtained by artificial prey exposure might be biased (Sunderland, 1988; Vet & Dicke, 1992; Agelopoulos & Keller, 1994; Mäntylä et al., 2004; McCormick et al., 2012). In some cases, when dead insects or tuna baits are used to mimic artificial prey, the smell can influence the attraction of predators and scavengers (Novotny et al., 1999). Moreover, methods based on artificial prey are not suitable for the estimation of parasitism rate, and parasitism rate is therefore rarely estimated together with predation rate.

Artificial prey simulating herbivorous insects concern in vast majority imitation of lepidopteran larvae, because the larval stage is most commonly attacked by predators (Howe et al., 2009; Lovei & Ferrante, 2017). Easy manipulation and standardization of artificial caterpillars allows the method to be used across large areas and long gradients (Sam et al., 2015b; Seifert et al., 2016). Roslin et al. (2017) tested global predation trends on artificial caterpillars, and found a strong dominance of invertebrate predators, mainly in the lowlands of tropical forests. In temperate or tropical montane regions, the dominant predators of artificial caterpillars were birds or mammals (e.g., Drozdova et al., 2013; Sam et al., 2015a; Ferrante et al., 2018).

The exposure of live prey attracts not only polyphagous but also more host-specific predators or parasitoids, and thus provides a more accurate estimation of predation and parasitism rates than the artificial type of prey (Strand & Obrycki, 1996; Hrcek et al., 2013). On the other hand, this method has also several problems, such as a lack of direct evidence of the cause of death, prey mobility, or requirement for mass rearing of the prey (Kneib & Scheele, 2000; Molleman et al., 2016). For evidence of parasitism of the exposed host, the host must be kept until the adult stage, dissected for the presence of parasitoid larvae, or screened by molecular detection methods (Franck et al., 2017; Šigut et al., 2017).

Live (sentinel) insects, mostly represented by lepidopteran eggs or larvae, have been used mainly in temperate and subtropical regions, primarily for studies of interactions between pests and their enemies in agricultural habitats (Lemessa et al., 2015; Macfadyen et al., 2015; Begg et al., 2017; Lovei & Ferrante, 2017). To our

knowledge, the only previous study conducted in a tropical rainforest exposed caterpillar of an endemic crambid at three different islands of the Hawaiian archipelago, and found a 14% per day predation rate and a 5% parasitism rate (Kaufman & Wright, 2009). There were also four studies which used egg exposure in tropical agricultural habitats (Lovei & Ferrante, 2017).

Using live prey in natural settings allows quantification of intraguild predation, at least in relative terms. Predation of parasitized hosts (called intraguild predation) is expected to be common (Polis & Holt, 1992), but quantitative data are rare. In essence, intraguild predation is a combination of competitive and trophic interaction (Polis et al., 1989). The predators can sometimes discriminate between parasitized and unparasitized hosts and prefer one of the two types of prey (López, 1982; Harvey & Eubanks, 2005).

In this study, we aimed to investigate how factors contributing to mortality of caterpillars vary with altitude in primary tropical rain forest and what the interaction is between predation and parasitism. We also aimed to evaluate whether the disappearance rate recorded using exposed live caterpillars is comparable with the predation rate measured using artificial caterpillars at the same altitudinal gradient (Sam et al., 2015a). We predicted that exposed live caterpillars face stronger predation pressure by invertebrates in lower than in higher elevations, as previously reported using artificial caterpillars (Sam et al., 2015a). Furthermore, we expected a higher parasitism rate of caterpillars at higher elevations because of lower expected predation rate on the hosts and thus greater chance for parasitoids to avoid predation while inside the host.

Material and methods

Study sites

The experiment was conducted along a rainforest altitudinal gradient of Mt Wilhelm (4 509 m above sea level) in the Central Range of Papua New Guinea. The experimental exposure of caterpillars in primary rainforest was conducted at two low-elevation sites near Kausi village (ca. 200 m a.s.l.; site I: -5.738 S, 145.332 E; site II: -5.738 S, 145.334 E); and two high-elevation sites near Memeku village (ca. 1 200 m a.s.l.; site III: -5.723 S, 145.270 E; site IV: -5.722 S, 145.266 E; Figure 1A). The two elevations were ca. 7.5 km apart. We used another site at mid-elevation (ca. 700 m a.s.l.; site V: -5.737 S, 145.270 E; Figure 1A) in between the two experimental areas, near Numba village, as a source of eggs and caterpillars used in the experiments. The experiment was performed during the dry season, from May to July 2016 with average

temperatures of 26.8 °C at 200 m a.s.l., 24.7 °C at 700 m a.s.l., and 19.7 °C at 1 200 m a.s.l.

Experimental caterpillars

We used externally feeding caterpillars of *Talanga excelsalis moresbyensis* Strand (Lepidoptera: Crambidae) as live sentinel prey (Figure 1D,E). Caterpillars used for the experiment were reared from eggs collected by hand from host trees (*Ficus copiosa* Steud., Moraceae) at ca. 700 m a.s.l. A part of the caterpillars used for the experiment (13%) was reared from eggs obtained from wild-caught adults. Younger instars (\leq third) of this species are gregarious, whereas older caterpillars are solitary. Caterpillars are sessile: they usually make thin silk webs on the underside of leaves (Figure 1F). The threads of silk are used by adults to lay eggs on (M Libra, field observation; Figure 1E). The caterpillars only feed on trees of the genus *Ficus* but are not specific to particular species of this genus (Miller et al., 2007; V Novotny, unpubl.). In addition to eggs, we also collected caterpillars of different instars for rearing parasitoids to provide additional information on the natural parasitism rate, and to obtain adult moths. The sampling of eggs and caterpillars was performed in May and June 2016. All leaves for caterpillar feeding were obtained from a single tree to standardize food source. Caterpillars were

fed every 2nd day with fresh leaves. The containers were cleaned every day with paper tissue to reduce condensation and prevent mold growth. Groups of caterpillars were split into smaller groups as they were growing, keeping a maximum four caterpillars per rearing container once they reached the third and fourth instars. The species identification was confirmed upon reaching the third instar, when the caterpillars have eight well-visible dots on the thorax, which distinguishes them from locally occurring related species, that is, *Talanga sexpunctalis* Moore, feeding on the same host plant. The identification was re-confirmed on reared adults at the end of the experiment. The size of live caterpillars used in the experiment was 1.6 ± 0.4 cm (fourth and fifth instars) and caterpillars were randomly mixed before being assigned to experimental trees.

Experimental host trees

We used saplings of *F. copiosa* as experimental trees. *Ficus copiosa* grows in secondary and primary forest, where it requires high light environments, typically in naturally open areas such as around streams or in tree gaps, and at disturbed areas with secondary vegetation in abandoned food gardens or at roadsides. *Ficus copiosa* occurs from sea level to 1 800 m a.s.l. Local density (number of stems ha^{-1}) and basal area ($\text{m}^2 \text{ha}^{-1}$) of *F. copiosa* are 78 and



Figure 1 (A) Map of the localities (sites I and II at 200 m a.s.l., III and IV at 1 200 m a.s.l., and V at 700 m a.s.l.). Experimental treatments: (B) exposed and (C) protected. (D) Caterpillar of *Talanga excelsalis moresbyensis* with parasitoid larvae hatching from it. (E) Egg on silk and first-instar caterpillar. (F) Caterpillars with silk made during acclimatization phase

0.01 at 200 m, 306 and 0.061 at 700 m, and 34 and 0.038 at 1 200 m, respectively. The *Ficus* genera community along the Mount Wilhelm elevational gradient (ca. 75 species) represents almost half of all occurring *Ficus* species in Papua New Guinea (Berg et al., 2005). Accessible saplings from primary forest were selected as experimental trees [height = 3.4 ± 1.03 m (mean \pm SD), range: 1.2–6.1 m]. The selected saplings had at least two branches with a comparable number of leaves of a similar size (the average leaf area on one tree inside each treatment was $3\ 131$ cm²). For all experimental trees, we measured total leaf area from at least 10 randomly selected leaves from experimental branches (mean \pm SD = 19.45 ± 7.23 leaves).

Experimental design

The experiment manipulated access by vertebrate and invertebrate predators to experimental caterpillars. Ten experimental trees, at least 10 m apart, were selected at each site (i.e., 20 trees per elevation, 40 trees in total). Two similar branches were selected on each tree, and randomly assigned to ‘exposed’ or ‘protected’ treatment (Figure 1B, 1). In the ‘exposed’ treatment, caterpillars were protected only from vertebrate predators, but they were accessible to invertebrate predators and parasitoids. This was achieved by enclosing caterpillars in ‘bird’ nylon netting sleeves with 2.5×2.5 cm mesh size. The ‘protected’ treatment had additional protection represented by an additional layer of ‘insect’ nylon netting with ca. 250 μ m mesh size and the branches were protected in addition from access by crawling invertebrate predators with a 7-cm strip of Tanglefoot glue. The ‘protected’ treatment was intended as complete protection against all natural enemies. However, although it did protect against predation, it did not protect against parasitoids (see Results). This was unexpected but allowed us to separate the effect of predation (allowed in the ‘exposed’ treatment, but not in ‘protected’ treatment) from the effect of parasitism (allowed in both treatments). The design thus also allowed two separate estimates of the parasitism rate. At the beginning of the experiment, before the exposure of caterpillars, all potential invertebrate predators were collected by hand from focal branches and stored in ethanol. This allowed us to eliminate predation during the acclimatization period, and, together with analogous collection after the exposure, to obtain the information about predator communities (only ants and spiders were found).

Ten randomly chosen caterpillars were placed on each selected branch on each experimental tree, thus using 400 caterpillars per elevation and 800 caterpillars in total. The caterpillars were kept on experimental branches for 4 days (96 h). The first 48 h were allocated for an acclimation

period, during which caterpillars were kept on branches under ‘insect’ netting, whereas the second 48-h period was used to measure disappearance and attack rate under the two treatments. Netting was used during acclimation period to encourage caterpillars to settle on a new host tree. After the acclimation period, the ‘insect’ nylon sleeve was removed from the ‘exposed’ treatment, whereas the ‘bird’ netting remained in place, restricting vertebrate predator access. At this point, the caterpillars were observed to be settled on the leaves within the silk webs they spin. At the end of the exposure period, all remaining caterpillars were hand collected, placed in plastic containers, and reared to adults or parasitoids. The entire experimental trees were also checked for possibly escaped caterpillars (only a single escaped caterpillar was found, and this was at 1 200 m). The retrieved caterpillars were fed with leaves from the same tree on which they were placed during the experiment. For logistical reasons affecting where the rearing facility could be placed, due to landowner restrictions, experimental caterpillars from 1 200 m a.s.l. were moved back to 700 m a.s.l. (a 0.5-h walk) for rearing, whereas caterpillars exposed at 200 m a.s.l. stayed at the same elevation to reduce potential mortality due to difficult transport up to 700 m a.s.l. (a 5-h walk). Rearing parasitoids were identified and morphotyped by ML.

Disappearance vs. predation rate

In the results, we conservatively report the disappearance rate rather than the predation rate, because observing predation directly in an experiment with realistic caterpillar densities is currently technically impossible. However, we interpret disappearance to be mostly due to invertebrate predation, because (1) during our preliminary work and rearing of the caterpillars for the experiment we have observed the caterpillars to be sessile within their silk webs (Figure 1F), (2) during the experiment we have recorded only a single escaped caterpillar, and (3) we have added an acclimation period as an extra precaution. If any caterpillar would have left the host tree, the chances that it would be able to reach another host tree without being predated are very small [the mean (\pm SD) distance between *Ficus* host trees within each site at 200 m a.s.l., site I: 49.5 ± 5.19 m, site II: 443.7 ± 45.36 m; at 1 200 m a.s.l., site III: 376.1 ± 37.16 m, site IV: 103.0 ± 12.61 m—and this is when measured in a straight line, whereas a real minimal distance the caterpillar would have to cover due to forest complexity would be several times longer].

Data analysis

Five categorical outcomes were used for the analysis: reared to adult, dead caterpillars (died during the experiment for unknown reason, during rearing or as pupa),

missing caterpillars after exposure ('disappearance'), reared to parasitoid (parasitoid adults or pupae), and unavailable samples (lost or damaged caterpillars after the experiment—there were only eight such cases).

Disappearance rate was quantified as the percentage of missing caterpillars from all caterpillars exposed in the given treatment. Unavailable samples were included in the total. Parasitism rate was measured as the percentage of parasitoids reared from caterpillars collected alive following the exposure (caterpillars which entered the rearing). Unavailable samples were excluded from the analysis because of the missing information about the outcome.

We used binomial generalized linear mixed effect models (GLMM) for testing the effect of elevation and treatment on caterpillar mortality factors. Disappearance rate and parasitism rate were included as binomial dependent variables. Elevation, treatment, and site within elevation were modeled as fixed categorical predictors and tree identity was included as random factor (Dependent variable \sim Altitude * Treatment + Site + (1|Tree identity)).

Furthermore, the effect of invertebrate assemblages of predators (pooled from pre- and post-experimental search) on invertebrate predation rate was tested using GLMM as above, with ant and spider presence/absence added as explanatory factor. The effect of altitude on the presence of ants and spiders was tested using binomial GLMM with altitude and site fixed categorical predictors and tree identity was included as random factor. Two of the 40 experimental trees were excluded from the analyses because they were damaged by a falling branch during the experiment. All analyses were conducted in R software v.3.3.1 (R Core Team, 2017) and lme4 package (Bates et al., 2015).

Results

Mortality factors of caterpillars

We exposed 800 conspecific caterpillars to invertebrate predators and parasitoids on 40 trees for 48 h at two elevations of primary rainforest in Papua New Guinea. We found that the disappearance (measured as the percentage of missing caterpillars) of caterpillars exposed to invertebrate, but not vertebrate, predators was higher at 200 than at 1 200 m a.s.l. (43 vs. 12%; logit coefficient 1.220 ± 0.610 ; $\chi^2 = 15.07$, d.f. = 1, $P < 0.001$; Figure 2). The percentage of missing caterpillars was higher in the 'exposed' treatment than in the 'protected' treatment (logit coefficient 2.037 ± 0.230 ; $\chi^2 = 56.32$, d.f. = 1, $P < 0.001$; Figure 2). However, we found also some missing caterpillars in the 'protected' treatment (200 m a.s.l.: 9.5%; 1 200 m a.s.l.: 2.6%). The interaction between

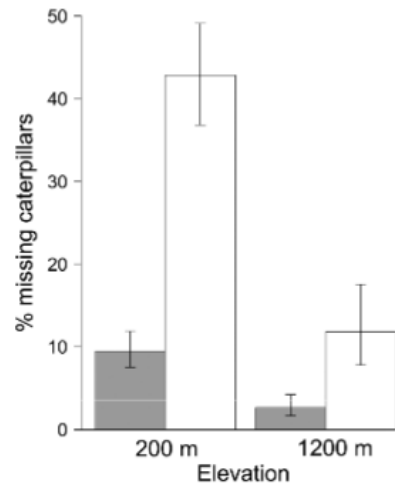


Figure 2 Mean (\pm SE; $n = 19$) percentage of missing *Talanga excelsalis moresbyensis* caterpillars from all caterpillars in protected (gray bars) and exposed (white bars) treatments at 200 and 1 200 m a.s.l.

treatment and elevation was not significant (logit coefficient 0.442 ± 0.588 ; $\chi^2 = 0.57$, d.f. = 1, $P = 0.45$).

The parasitism rate (percentage of reared parasitoids from retrieved live caterpillars) was not different between 200 and 1 200 m a.s.l. (14 vs. 13%; logit coefficient 0.156 ± 0.723 ; $\chi^2 = 0.26$, d.f. = 1, $P = 0.61$; Figure 3). We found no difference in parasitism rate between 'exposed' and 'protected' treatment (logit coefficient 0.004 ± 0.409 ; $\chi^2 = 0.84$, d.f. = 1, $P = 0.36$; Figure 3). Interaction between treatment and elevation was not significant (logit coefficient 0.441 ± 0.543 ; $\chi^2 = 0.66$, d.f. = 1, $P = 0.43$). Rearing success was different between elevations: 56% of caterpillars were successfully reared from 200 m a.s.l. compared to 39% from 1 200 m a.s.l. ($\chi^2 = 10.95$, d.f. = 1, $P = 0.001$). The rearing success and the mortality may have been affected by moving the caterpillars from high elevation to the campsite at 700 m a.s.l., which we had to do for logistical reasons.

Predators

We found no effect of presence of either ants or spiders on the focal trees before and after the experiment on disappearance of caterpillars (ants: logit coefficient 0.478 ± 0.560 ; $\chi^2 = 1.27$, d.f. = 1, $P = 0.26$; spiders: logit coefficient 0.203 ± 0.571 ; $\chi^2 = 0.139$, d.f. = 1, $P = 0.71$). There was no difference in the presence of ants and spiders between altitudes (ants: logit coefficient 0.272 ± 0.827 ; $\chi^2 = 0.108$, d.f. = 1, $P = 0.74$; spiders: logit coefficient 0.028 ± 0.571 ; $\chi^2 = 0.01$, d.f. = 1, $P = 0.97$; Figure 4).

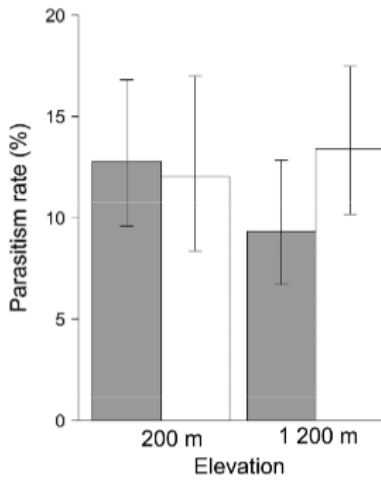


Figure 3 Mean (\pm SE; $n = 19$) percentage of reared *Talanga excelsalis moresbyensis* caterpillars retrieved at the end of the experiment that were parasitized in protected (gray bars) and exposed (white bars) treatments at 200 and 1200 m a.s.l.

Reared parasitoids

Six morphospecies of parasitoids were reared from exposed caterpillars collected at 200 and 1200 m a.s.l. (Table 1), including five morphotypes of larval parasitoids (Braconidae, Microgastrinae) and one probable hyperparasitoid species (Ichneumonidae: Mesochorinae, based on the known natural history of this subfamily). Two morphotypes were unique for the higher elevation, the remaining four were obtained from both elevations.

To acquire general knowledge about parasitoids of this host species, additional parasitoids were reared from 639 caterpillars collected at 700 m a.s.l., initially collected to obtain adults and eggs; 7% of those were attacked by parasitoids and 5.6% were infected by entomopathogenic helminths (Nematoda) which kill the host caterpillar. The parasitoids were represented by six morphospecies, including two dipteran (Tachinidae) and four

Table 1 Abundance of parasitoids reared from *Talanga excelsalis moresbyensis* from three elevations: 200, 700, and 1200 m above sea level. Parasitoids from 200 and 1200 m a.s.l. were reared from exposed caterpillars. Parasitoids from 700 m a.s.l. were reared from free-living caterpillars and eggs collected for the experiment, and provide additional information on natural parasitism rate

| Parasitoid morphotype | Elevation (m a.s.l.) | | |
|-----------------------------------|----------------------|-----|------|
| | 200 | 700 | 1200 |
| Macrocentrinae sp. 1 | 0 | 1 | 0 |
| Macrocentrinae sp. 2 | 0 | 1 | 0 |
| Mesochorinae sp. 1 | 0 | 0 | 1 |
| Microgastrinae sp. 1 | 0 | 0 | 2 |
| Microgastrinae sp. 2 | 4 | 0 | 7 |
| Microgastrinae sp. 3 | 10 | 18 | 9 |
| Microgastrinae sp. 4 | 13 | 11 | 8 |
| Microgastrinae sp. 5 | 1 | 0 | 1 |
| Tachinidae sp. 1 | 0 | 1 | 0 |
| Tachinidae sp. 2 | 0 | 1 | 0 |
| Mymaridae sp. 1 (egg parasitoid) | 0 | 1 | 0 |
| Encyrtidae sp. 1 (egg parasitoid) | 0 | 1 | 0 |
| Total | 28 | 71 | 28 |

hymenopteran morphospecies (Braconidae: Macrocentrinae and Microgastrinae); in addition, two egg parasitoids were reared from eggs collected at 700 m a.s.l. (Encyrtidae and Mymaridae). The two most common Microgastrinae morphospecies were also retrieved from larvae exposed at 200 and 1200 m elevation.

Discussion

In this study, we set out to record how different mortality factors of exposed live caterpillars change with altitude in a tropical rainforest. We show that caterpillars in the 'exposed' treatment disappeared more often at the lower altitude (43% at 200 m a.s.l.) than at the higher altitude (12% at 1200 m a.s.l.). The disappearance of caterpillars was most probably caused by invertebrate predators, which corresponds with the trend of decreasing predation by invertebrate predators with increasing elevation

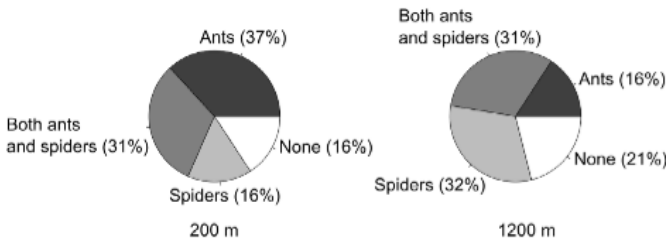


Figure 4 Colonization of experimental trees by invertebrate predators at 200 and 1200 m a.s.l. The presence of spiders and ants was recorded on all 19 experimental trees per elevation. Data are combined from pre- and post-experimental search

observed on artificial caterpillars by Sam et al. (2015a) along the same elevation gradient (Mt. Wilhelm, Papua New Guinea). Roslin et al. (2017) also showed that the predation of artificial caterpillars by invertebrates was highest in lowlands of tropical forests. Our observed disappearance rate (average of 21.5% per day) was higher than 12% per day observed by Sam et al. (2015b) on artificial caterpillars in the same lowland locality, which might suggest that live caterpillars are more attractive to predators. The drawback of using live caterpillars is that it is difficult to prove predation because video recording is not currently feasible at this scale (Kaufman & Wright, 2009; Lovei & Ferrante, 2017), but it is similarly difficult to prove that marks on an artificial caterpillar were really made by the presumed predator and, even if they were, that the attack would have been successful. Live caterpillars can theoretically leave the host plant, but as we describe in the methods section, our study species is sessile and even if a caterpillar left, the chances that it will reach another host plant without being predated are extremely low. We thus consider most of the recorded disappearance to be due to predation. However, some caterpillars also disappeared from the 'protected' treatment. We expect that they might have died from some disease and it might therefore be more accurate to estimate predation rate as disappearance rate in the 'exposed' treatment minus disappearance rate in the 'protected' treatment.

The main advantage of using live caterpillars in comparison to artificial prey, is the possibility to obtain information about additional mortality factors (parasitoids or parasites). Despite our original intention, parasitoids were able to attack caterpillars in the 'protected' treatment to the degree that the parasitism rate did not differ between the two treatments. That a similar estimate of parasitism rate was recorded with two quite different treatments suggests that the estimate is relatively accurate. Parasitism rate was similar in exposed treatments between the two studied elevations (12 vs. 13%). This is in contrast with the previously reported general decrease of parasitism of externally feeding caterpillars with increasing elevation of ca. 0.44%/100 m, with a relative loss of ca. 15%/100 m (related to a median parasitism rate of 3.7%; Péré et al., 2013). A decrease of parasitism rate with increasing elevation was also observed in systems of cavity nest bees (Morris et al., 2015) or leaf-mining weevils (Maunsell et al., 2015). Hrcek et al. (2013) reports an average 12% parasitism rate of multiple species of semi-concealed caterpillars from lowland rainforest of Papua New Guinea, a category which includes leaf-tying caterpillars. The exact species studied here (*T. excelsalis moresbyensis*) had a parasitism rate of 9% in that study. Kaufman & Wright (2009) obtained a ca.

9% parasitism rate (3%/day) during the exposition of single host species across altitudes, which was still lower than our result (6%/day).

As parasitism rate is similar between elevations, but predation rate is much higher in the lowlands, being a larval parasitoid is a much riskier life-history strategy in the lowlands. Assuming random predation of hosts in respect to parasitism (as the lack of difference in parasitism between our treatments would suggest), we can quantify the difference for the 2-day period for which we exposed the hosts: in the lowlands, a female wasp must parasitize 3.6× more hosts to achieve the same fitness as in the highlands. The decrease in predation rate with increasing elevation was not compensated for by increased parasitism. Our data indicate that predation either generally decreases at higher elevations, or invertebrate predators are replaced by vertebrate predators (e.g., birds, not studied here) at higher elevations. Sam et al. (2015a) found ants to become relatively less important predators than birds at 1 700 than at 1 200 m a.s.l., but observed a general decrease of predation with increasing elevation, just a larger decrease in the case of ants. Based on their results, compensation from birds at higher elevations is therefore not likely.

A limitation of this study is that it uses only two sites per elevation, relatively close to each other. We were able to use only a limited number of sites mainly because of a limited knowledge about the distribution of the study species. The major strength in using live caterpillars lies in allowing interactions with a natural set of predator and parasitoid species and we only had data on occurrence of *T. excelsalis moresbyensis* in this area. However, we believe that the major strength outweighs this limitation, and that further studies will extend the results to more altitudinal gradients and host species.

This study measured disappearance and parasitism rate by introducing a defined amount of resource into the studied ecosystems. Such studies often use artificial resources, such as tuna baits to measure ant predation, or artificial prey, such as plasticine caterpillars (e.g., Ferrante et al., 2014). Although the artificial baits are useful to judge relative trends in predation, they are not suitable to measure absolute predation or parasitism rate as such estimates can be heavily influenced by the type of artificial baits (Sam et al., 2015b). The present study uses reared conspecific caterpillars of a native species instead, aspiring thus for direct measurement of disappearance and parasitism rates. It is, to our knowledge, the first such study in which a live local prey species was used at multiple elevations of primary tropical rainforest to measure predation and parasitism rates. Surprisingly, we found that caterpillars experienced parasitoid attack in protected treatments. The

direct contact of some branches and protecting nets might have allowed the parasitoids to attack the caterpillars through the net, which indeed has been observed at one occasion. Therefore, some caterpillars may have been exposed to parasitoids for four, rather than only 2 days.

We did not find any statistically significant effect of collected invertebrate predators (ants and spiders) on missing caterpillars in our experiment. This might be due to different food preferences of the predators collected. Not all ant species feed on live caterpillars, and sometimes they can even co-occur on the same leaf with caterpillars (M. Libra, field observation). Hence the exact species identity and diet should ideally be considered in further studies (Orivel et al., 2018). Another reason why the presence of predators was not significant could be the lack of other foraging invertebrate predators such as wasps (Fáveri et al., 2008; Molleman et al., 2016). Wasps (e.g., Polistinae, Eumeninae) use caterpillars as prey to feed their larvae (Goulet & Huber, 1993). Tvardikova & Novotny (2012) observed the predation attacks of artificial caterpillars by wasps in Papua New-Guinean rainforest, which are not possible to detect without the usage of artificial preys or direct field observation. During the experiment, we did not observe any other predators attacking the caterpillars; however, we observed predation of the experimental caterpillar species by a mantis during the egg-collection.

Another mortality factor affecting caterpillar communities are entomopathogenic helminths (Nematoda). These nematodes have a similar life history to insect parasitoids and can kill the host. We did not rear any nematodes from the exposed caterpillars; however, 5.6% of caterpillars collected at 700 m a.s.l. were infected by nematodes.

The impact of predators and parasitoids in habitats as complex as primary tropical forests could be examined in further studies by a variety of experiments, including enclosure of ants, spiders, day- and night-foraging vertebrates (birds and lizards vs. bats and other mammals), and using a wider range of sentinel larval instars and herbivore species. Our study suggests that intraguild predation is strong in low-elevation rainforest. This should be investigated further, and such investigations should also extend to agricultural habitats in the tropics, where biological control can be considered.

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CHAPTER IV.

Tachinid flies (Diptera, Tachinidae) reared from lepidopteran larvae living in the canopy of temperate deciduous forest of Hokkaido, Japan, with descriptions of three new species

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Abstract

Dipteran parasitoids are important natural enemies of herbivorous insect, however, very little is known about their diversity and their life history. In this study, we described tachinid parasitoids reared from caterpillars sampled from tree canopies (0.2 ha plot) of a deciduous forest at Hokkaido, Japan (Tomakomai Experimental Forest). From 16 405 sampled caterpillars, we reared 77 tachinid parasitoids to belong to seven species. Three of the species were newly described (*Blepharomyia brevicornis* sp. nov., *Cyzenis equifacialis* sp. nov., *Cyzenis tetrasetosa* sp. nov.). For both, the new and already described species the host records have been added. For reared *Eulasiona zimini* Mesnil the female was described for the first time. The parasitism rate and natural history of these tachinids were briefly discussed. We also noted that the genus *Eulasiona* (Townsend) was moved to the subfamily Tachininae from the current taxonomic position in Dexiinae subfamily.

Keywords: Tachinidae, diversity, parasitoid, Diptera, Lepidoptera

Introduction

SUMMARY

The aim of this dissertation was to describe the community structure and diversity trends of parasitoids in the species-rich tropical rainforest of Papua New Guinea. In addition to, the potential predation risk to caterpillars and indirectly also affecting parasitoids was also documented. Finally, new species of parasitoids were described, as the whole thesis is based on thorough species identification.

Chapter I. describes the beta diversity of parasitoids in the continuous lowland rainforest of Papua New Guinea reared from more than 27 000 externally feeding caterpillars, their hosts. In addition to it shows how interactions between parasitoids and their hosts change across the forest. Parasitoids had lower site occupancy than caterpillars and their metapopulation dynamics was, therefore, faster than that of their caterpillar hosts. Parasitoid beta diversity was higher than the hosts', and the dissimilarity of parasitoid communities increased with the distance much steeper than was recorded for caterpillars. It seems that the tri-trophic network of plants, caterpillars and their parasitoids can generate a highly heterogeneous environment for the upper trophic levels despite a largely homogeneous environment of lowland tropical forest. Moreover, the changes in the dissimilarity of interactions were caused mainly by species turnover, which indicates that parasitoids are quite stable with their host preferences and they do not "rewire" their interactions with other hosts.

Chapter II. shows how the diversity of parasitoid communities changing between low and high elevational sites within the tropical rainforest. The parasitism rate did not differ between low and high elevations (10.2 % vs 13.4 %). The diversity of Ichneumonidae family was reversed in comparison to two other important parasitoid families (Braconidae (Hymenoptera) and Tachinidae (Diptera)) and showed higher diversity in high than low elevation. Because with increasing elevation the temperature decrease, we tested the potential explanation of this anomalous trend used previously to explain the latitudinal anomalous diversity trend of Ichneumonidae. It seems that their reverse altitudinal diversity trend is not explained by host-related factors, where we found no effect of caterpillar diversity and their feeding mode, or plant diversity. Instead of this, we found that the body-size of the parasitoid community was bigger in a colder

environment thus an increase in body size with elevation in the parasitoid community is consistent with Bergmann's rule and thermal ecology could explain anomalous diversity gradients in Ichneumonidae.

Chapter III. demonstrates the contrast in the predation pressure to parasitoid's hosts – externally feeding caterpillars. For the first time in a tropical rainforest, with exposure of live caterpillars, the contrast in mortality factors was quantified. A total of 800 live caterpillars of *Talanga excelsalis moresbyensis* Strand (Lepidoptera: Crambidae) was exposed on saplings of *Ficus copiosa* Steud. (Moraceae) at two elevations in a primary tropical rainforest in Papua New Guinea (200 and 1 200 m a.s.l.). The disappearance of caterpillars exposed at low elevation was higher (43 %) than those exposed at high elevation (12 %). The disappearance was considered mainly because of the predation by invertebrate predators because the potential migration of caterpillars was not recorded. The estimated predation rate was corresponding with studies using artificial caterpillar models (Roslin *et al.* 2017). In terms of parasitism rate, there was no difference between elevations (13 vs 12 %). The combination of the disappearance and parasitism rate indicates that caterpillar hosts suffer a much higher mortality risk at a low elevation of tropical rainforest than those caterpillars living in higher elevations.

Chapter IV. is based on the taxonomy and description of unknown species of dipteran parasitoids (Tachinidae) from Japan. General knowledge of trophic interactions is rarely known, especially from species-rich areas. The data used in Chapters I., II. & IV. comes from massive rearing projects, due to which the interactions between parasitoids, caterpillars and host plants are documented. In the last chapter, seven dipteran parasitoid species were obtained from caterpillars sampled at 0.2ha of species-rich temperate forest in Japan. Three species were newly described, four of them were already known. For all reared species, their host species and ovipositing strategies were documented. For *Cyzenis* and *Blepharomyia* genera, the identification key was provided, and for *Eulasiona zimini* (Mesnil, 1963) missing description of female was finally provided. The last chapter represents the necessity of proper description of all participants in food web interactions for more accurate results and better interpretation of observed results.

Discussion and future directions

In the past, studies focused on food web interactions were very often limited to only several species, however, thanks to large rearing projects, we can extend the knowledge up to the large scale and get deeper into mechanisms affecting each trophic level in broad context. Our results showed how parasitoid communities can be dissimilar even within the homogenous rainforest, and how drastically the parasitoid diversity can differ between two elevational sites (even on the family level). Hence, drawing conclusions based only on local small datasets might be misleading. This can be reflected even on larger scales, as it might be in the case of the anomalous diversity gradient, where higher diversity of Ichneumonidae at high latitude was proposed from a small dataset (Owen & Owen 1974), without sufficient explored tropical mountain habitats (Santos & Quicke 2011). Then, for revealing the latitudinal trend of Ichneumonidae, multiple site comparison along latitudinal gradient will be needed, also including sites from high elevations from tropics. Our studies are focused on parasitoids of externally feeding caterpillars, and it allow us to test possible explanations of diversity trends. However, similar large rearing projects including multiple sites located in high elevations will probably not happen in near future (unfortunately). Possible substitution might be in global diversity comparison programs such e.g., LIFEPLAN project led by Tomas Roslin and his colleagues or The Global Malaise Trap Program (GMP) led by Paul Hebert and his team. They use standardized methodology (Malaise traps) across whole world, and the obtained data will be analyzed via modern molecular techniques (GMP: DNA barcoding; LIFEPLAN: metabarcoding). Important part of such comparison is the long-term sampling, because of the seasonality of some taxa (Shapiro & Pickering 2000; Sääksjärvi *et al.* 2004). That standardized methodology allows to make a proper comparison (Volf *et al.* 2019), where diversity of particular parasitoid taxa can be compared on global scale.

Rearing projects on such scale as the team of V. Novotný conducted, are very important, despite they are extremely time-consuming (...and resource demanding). However, without such observations, we cannot properly describe even basic ecological questions, such as whether the beta

diversity of parasitoids and their hosts differ. This thesis is based on such data, where thorough species concept was used for all types of analyses. As far as possible, we combined morphological identification with molecular techniques for accurate species determination. The precise identification of species is important for interpreting interactions within food webs (Smith *et al.* 2007). With current advanced molecular techniques (e.g. DNA metabarcoding of light trap material; Zenker *et al.* 2020) there are possibilities to obtain information on local diversity, however, the natural history of the species is mostly unknown, and the chance of getting better insight into different theories explaining differences in diversity is low. Additionally, advanced statistical techniques allow us to perform metacommunity analyses which could explain observed patterns of species distribution, their abundance and eventually the interactions of species within food webs (Start & Gilbert 2016)

As was already mentioned, studying of trophic interactions on large scale is difficult. Another possibility of revealing the mechanics affecting the interactions is to switch from large-scale surveys to experiments, which allow to test more focused questions tasked for particular system or phylogenetically related organisms (Segar *et al.* 2020; Jeffs *et al.* 2021). Our experiment shows how important higher trophic levels, as predators and parasitoids, are in affecting the community of herbivorous insect. It shoes that the mortality agents in combination with defense mechanisms of plants are also an important part of the mosaic explaining the higher diversity of tropical insect in comparison to those occupying temperate regions.

The experimental approach might help us to reveal mechanisms affecting the structure of food web interactions, whereas e.g. we can test and predict how the global warming and, therefore, increase of temperature affect the interactions (Maunsell *et al.* 2015; Thierry *et al.* 2021). Furthermore, such manipulative experiments in combination with knowledge of life history of parasitoids (e.g., host specificity; Yousuf *et al.* 2021)) can provide valuable information for potential biological control of pests and invasive species and their impact to native communities (Henneman & Memmott 2001; Meineke *et al.* 2014).

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- Yousuf, F., Follett, P., Conrad, ·, Gillett, C., Honsberger, ·, Chamorro, L., *et al.* (2021). Limited host range in the idiobiont parasitoid *Phymasticus coffea*, a prospective biological control agent of the coffee pest *Hypothenemus hampei* in Hawaii. *J. Pest Sci.*
- Zenker, M.M., Specht, A. & Fonseca, V.G. (2020). Assessing insect biodiversity with automatic light traps in Brazil: Pearls and pitfalls of metabarcoding samples in preservative ethanol. *Ecol. Evol.*, 10, 2352–2366.

APPENDIX

Martin Libra

Date of Birth: 8.1.1990

EDUCATION

- 09.2015 – present **Ph.D. study**
Department of Zoology, Faculty of Sciences,
University of South Bohemia
Community structure of caterpillar parasitoids from the tropical rainforest.
Supervisor: prof. RNDr. Vojtěch Novotný, CSc,
Supervisor - specialist: M.Sc. Jan Hrček Ph.D.
- 09.2013 – 05.2015 **M.Sc. of Biology**
Department of Zoology, Faculty of Sciences,
University of South Bohemia
Diversity of gall-inducing arthropods in two different host communities in temperate forests.
M.Sc. Thesis, in Czech
Supervisor: M.Sc. Martin Volf, Ph.D.
- 09.2009 – 02.2013 **B.Sc. of Biology**
Department of Zoology, Faculty of Sciences,
University of South Bohemia
Intraguild predation of predators on caterpillar parasitoids. Bc. Thesis, in Czech
Supervisor: M.Sc. Jan Hrček, Ph.D.

Currently involved in following projects

Explaining latitudinal trends in global biodiversity: why are there more species of insect herbivores in tropical than temperate forests? (V. Novotný – ERC)

Why is the world green: testing top-down control of plant-herbivore food webs by experiments with birds, bats, and ants (K. Sam – ERC)

Global monitoring of Hymenoptera (M. L. Buffington, Washington, USA)

PUBLICATIONS

Libra M., Tulai S., Novotný V., Hrček J. (2019) Elevational contrast in predation and parasitism risk to caterpillars in a tropical rainforest. *Entomologia Experimentalis et Applicata* 167: 922-931. DOI: 10.1111/eea.12851

Mottl O., Fibich P., Klimeš P., Volf M., Tropek R., Anderson-Teixeira K., Auga J., Blair T., Butterill P. T., Carscallen G., Gonzalez-Akre E., Goodman A., Kaman O., Lamarre G., **Libra M.**, Losada M.E., Manumbor M., Miller S., Molem K., Nichols G., Plowman N., Redmond C. M., Seifert C. L., Vrána J., Weiblen G., Novotný V. (2020) Spatial covariance of herbivorous and predatory guilds of forest canopy arthropods along a latitudinal gradient. *Ecology Letters* 23: 1499–1510. DOI: 10.1111/ele.13579

Seifert C. L., Volf M., Ré Jorge L., Abe T., Carscallen G., Drozd P., Kumar R., Lamarre G., **Libra M.**, Losada M.E., Miller S., Murakami M., Nichols G., Pyszko P., Šigut M., Wagner D.L., Novotný V. (2020) Plant phylogeny drivers arboreal caterpillar assemblages across the Holarctic. *Ecology and Evolution* 10: 14137-14151. DOI: 10.1002/ece3.7005

Volf M., Klimeš P., Lamarre G., Redmond C. M., Seifert C. L., Abe T., Auga J., Anderson-Teixeira K., Basset Y. F., Beckett S., Butterill P. T., Drozd P., Gonzalez-Akre E., Kaman O., Kamata N., Laird-Hopkins B., **Libra M.**, Manumbor M., Miller S., Molem K., Mottl O., Murakami M., Nakaji T., Plowman N., Pyszko P., Šigut M., Šipoš J., Tropek R., Weiblen G., Novotný V. (2019) Quantitative assessment of plant-arthropod interactions in forest canopies: A plot-based approach. *PLOS One* 14: e0222119. DOI: 10.1371/journal.pone.0222119

Szefer P., Carmona C., Chmel K., Konečná M., **Libra M.**, Molem K., Novotný V., Segar S. T., Švambergová E., Topliceanu T., Lepš J. (2017) Determinants of litter decomposition rates in a tropical forest: functional traits, phylogeny and ecological succession. *Oikos* 126: 1101-1111. DOI: 10.1111/oik.03670

Volf M., Pyszko P., Abe T., **Libra M.**, Kotásková N., Šigut M., Kumar R., Kaman O., Butterill P. T., Šipoš J., Abe H., Fukushima H., Drozd P., Kamata N., Murakami M., Novotný V. (2017) Phylogenetic composition of host plant communities drives plant-herbivore food web structure. *Journal of Animal Ecology* 86: 556-565. DOI: 10.1111/1365-2656.12646

PROFESSIONAL APPOINTMENTS

- 2010 – PRESENT **INSTITUTE OF ENTOMOLOGY,
BIOLOGICAL CENTRE AS CR (ENTU CAS)
Technical assistant, part job**
Sorting of parasitoid samples from PNG – J. Hrček
Sorting and determination of ants from PNG – P. Klimeš
Identification of bark beetles from PNG – K. Zimová
04. 2018 – 05. 2019 **RESEARCH STAY – TOEF, JAPAN
leading of the research team
Research stay at Tomakomai Experimental
Forest, Hokkaido, Japan**
Cooperation with University of Chiba
ENTU CAS + Faculty of Science
(Part of ERC project of K. Sam)
10. 2018 – 12. 2018 **RESEARCH STAY – EUCFACE, SYDNEY,
AUS
Research stay at EucFACE, Hawkesbury
Institute for the Environment, West. Sydney
University**
Cooperation with Western Sydney University
ENTU CAS + Faculty of Science
(Part of ERC project of K. Sam)

07. 2018 – 07. 2018 **RESEARCH STAY – TESRI, TAIWAN**
Research stay at TESRI, Jiji, Taiwan
 Research stay + Thy HYMcourse workshop
 Supervised by Prof. D. Quicke, Chulalongkorn
 University, Bangkok
05. 2018 – 05. 2018 **RESEARCH STAY – TOEF, JAPAN**
**Research stay at Tomakomai Experimental
 Forest, Hokkaido, Japan**
 Cooperation with The University of Tokyo & Chiba
 ENTU CAS and Faculty of Science
 (Part of ERC project of K. Sam)
- 03.2016 – 08.2016 **RESEARCH STAY – BINATANG RESEARCH
 CENTER, PAPUA NEW GUINEA**
**Research stay - *Survival of caterpillars in different
 altitudes*, published as **Libra et al. (2019)** Elevational
 contrast in predation and parasitism risk to caterpillars
 in a tropical rainforest. EEA**
05. 2015 – 07. 2015 **RESEARCH STAY – TOEF, JAPAN**
 05. 2014 – 08. 2014 **Research stay at Tomakomai Experimental
 Forest, Hokkaido, Japan**
 Cooperation with The University of Tokyo & Chiba
 ENTU CAS and Faculty of Science, USB (Part of ERC
 project, V. Novotný)

SCIENTIFIC CONFERENCES, TALKS AND WORKSHOPS

- 08.2020 **ESA Annual Meeting conference, Salt Lake City, USA - talk**
Elevational contrast in parasitoid communities in tropical rainforest
- 12.2019 **BES conference, Belfast, UK - talk**
Diversity of parasitoid communities along an altitudinal gradient in tropical rainforest
- 05.2019 **Chiba University + Kyoto University, Japan – talk**
Diversity of parasitoid communities along an altitudinal gradient in tropical rainforest
- 07.2018 **The HYMcourse, TESRI, Jiji, Taiwan**
Workshop focused on identification, diversity, and the life history of Hymenoptera.
Under the supervision of Prof. D. Quicke.
- 12.2017 **BES conference, Ghent, Belgium**
Predation and parasitism risk to caterpillars along an altitudinal gradient in tropical rainforest
poster + The Lightning talk (**1st place**).
- 11.2016 **Kyoto University, Japan – talk (2x)**
Insect sampling methods used for global comparison of diversity, and Diversity of gall-inducing arthropods in temperate forests.
- 02.2016 **Zoological Days – conference talk, C. Budejovice, CZE – talk**
Diversity of gall-inducing arthropods in temperate forest.

ADDITIONAL INFORMATION

- Language English – fluent (TOEFL exam – 2015)
French – passive
Czech – native
PNG Pidgin – passive
- IT Skills Common computer programs (MS Word, Excel etc.)
Statistical programs (R; CANOCO, Statistic, EstimateS)
Sound and graphic editors (Audacity, Adobe Photoshop)
- Statistics ANOVA, GLM, LME, diversity comparison, data visualization, food-web analysis, phylogenetic comparative methods
- Other skills Communication, Leading of team, Teamwork, Initiative, Problem solving, Flexibility, Driving license (B)
- Reviewing Reviews of manuscripts for Arthropod-Plant Interactions and Current Opinion in Insect Science
- Other activities Science outreach presentations about my fieldwork in Papua New Guinea and Japan (intended for kindergartens, schools, home for the elderly, public lectures). Science outreach within CAS Biology Centre for kindergartens and primary schools

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