FACULTY OF SCIENCE
Charles University

TROPICAL INSECTS:

Diversity and interactions with other organisms

habilitation thesis

RNDr. Robert Tropek, PhD.

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This habilitation thesis is dedicated to Prof. Jan Žďárek

without whose popularising books I am not sure to become an insect ecologist.

"*With what pleasure had I looked upon every rare and curious insect I had added to my collection! How many times, when almost overcome by the ague, had I crawled into the forest and been rewarded by some unknown and beautiful species!*"

A.R. Wallace, 1853

"*To a rough approximation and setting aside vertebrate chauvinism, it can be said that essentially all organisms are insects*."

R.M. May, 1988

"*The only good bug is a dead bug!*"

An anonymous civilian in Starship Troopers by R.A. Heinlein, 1959

Acknowledgements

This habilitation thesis has resulted from my research activities since 2007 when I first joined a research project in Cameroon as a master student. I am grateful to all people who I have been collaborating during all the years of research on tropical insects, as well as on numerous ecological and entomological questions. Without them, this habilitation thesis would never have been written.

Namely, I am extremely grateful to Štěpán Janeček with whom I have spent many months (altogether even years) in the field in Cameroon and Czechia, firstly working within his research projects and later co-leading our shared projects. Thanks to him, I have discovered the amazing world of pollination which turned into my main research interest. I have also been working in tropical projects of Vojtěch Novotný, Petr Pyšek, and David Storch whose rich experience crucially helped to form a researcher in myself. Similarly, I am grateful to Szabolcs Sáfián and Torben Larsen who introduced me to the world of Afrotropical butterflies and moths which have become my passion and surely the favourite model groups for ecological studies. Big thanks are going to Vincent Maicher and Sylvain Delabye, my first doctoral students, who taught me at least as much as I hope to teach them. Unfortunately, there is not enough space to name all colleagues, students, and assistants but special thanks surely belong to Filip Tichánek, Vojtěch Kolář, Sailee Sakhalkar, Francis Luma, Yannick Klomberg, Jan Mertens, Eliška Chmelová, Mercy Murkwe, Kobe Ishmeal, Eric Fokam, Tomasz Pyrcz, Pavel Potocký, Ewelina and Karolina (in an alphabetic order!) Sroczki, Nestoral Fominka, Michael Bartoš, Jana Jersáková, Ondřej Sedláček, and many others. Guys, I am aware it has often not been easy with me but hopefully we have been unravelling interesting secrets of nature with enough fun and joy.

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V. Epilog

Overview of the habilitation thesis

This thesis collates key parts of my research focused on diversity and ecology of insects in tropical ecosystems. Although I find diversity of other parts of the world, especially Central Europe, highly attractive, the tropical (and especially Afrotropical) ecosystems became my main research interest (and a big personal joy), not only because most of the world terrestrial species lives and grows there. This is the main reason why my habilitation thesis deals solely with tropical insects, although a substantial part of my research activities has stayed outside the tropics.

The thesis is organised into three parts. The first part presents three selected papers focused on discovery and description of the understudied diversity of Afrotropical butterflies and moths. It starts with a simple taxonomic study describing two butterfly species from Mount Cameroon (**Paper 1**), followed by the first part of a few papers (two published, two under preparation) describing an unexpectedly high diversity of many-plumed moths (Lepidoptera: Alucitidae) in the newly discovered hotspot of the moth group diversity on Mount Cameroon (**Paper 2**). This part is framed by an example of using our abundant sampled material in the phylogenetic and phylogeographic study (**Paper 3**).

The second part focuses on drivers of uneven distribution of insect diversity in the tropics, together with the drivers responsible for the local assembly of insect communities. The core of this part is composed from a few studies on communities of butterflies and moths on Mount Cameroon (**Papers 4–6**), followed by a study from Afrotropical savannahs in southern Africa (**Paper 7**). Finally, I have decided to incorporate an outcome from the large collaborative project focused on comparison of arthropod diversity and interactions between tropical and temperate forests (**Paper 8**).

The last part focuses on interactions of insects with other organisms, specifically on plant-pollinator interactions. It combines detailed studies of selected plant species' pollination systems in Cameroonian montane ecosystems with community-wide studies focusing on the spatiotemporal changes in the interactions along the elevational gradient of Mounts Cameroon. The first two studies focused on pollination systems of three plant species (**Papers 9 and 10**), they are supplemented by the two following reports on the competitive interactions among pollinators in the same communities (**Papers 11 and 12**). **Paper 13** analysed spatiotemporal importance of floral traits in shaping tropical plant-pollinator interactions and confronted it with the pollination syndrome hypothesis. In the last two studies, we described elevational changes in the pollinator communities of a butterfly-pollinated plant species (**Paper 14**) and elevational and seasonal patterns in the role of butterflies and hawkmoths in pollination networks on Mount Cameroon (**Paper 15**).

I find uncomfortable to summarise the work of many people in a single thesis, especially considering the used language. Therefore, the text of this overview is mostly written in plural to express the gratitude to all my collaborators, except the parts where my personal opinions are expressed (although all my opinions have surely come from numerous discussions with my co-authors and collaborators).

Tremendous diversity of (Afro)tropical insects

Insects surely belong among the most speciose and abundant organisms in the terrestrial ecosystems. As recently reviewed by Nigel Stork (2018), over a million insect species has been described so far. However, the estimates of insect diversity have recently stabilised between some 6 and 8 million species (Stork 2018), an overwhelming majority of this tremendous diversity occurring in the tropics (Novotny & Miller 2014; Stork 2018). Nevertheless, application of DNA methods in taxonomy have revealed an unexpected cryptic diversity in some insect groups, especially dipterans and parasitic wasps, and have re-opened the debates if the Erwin's original estimates of 30 million insect species (Erwin 1982) could not have been realistic (Stork 2018; García-Robledo et al. 2020). Despite these doubts on the total number of the insect species on Earth, it is clear that most of the diversity of insects remains undiscovered and undescribed, especially in tropical ecosystems. Unfortunately, the Afrotropical ecosystems remain strongly understudied (as obvious from an example of distribution records of butterflies and moths in the database of the Lepidopterists' Society of Africa, Fig. 1a; LeSoc 2022), despite the centuries of extensive entomological research. During our research projects, we have been doing our best to contribute a bit to the knowledge of the rich regional communities of especially butterflies and moths.

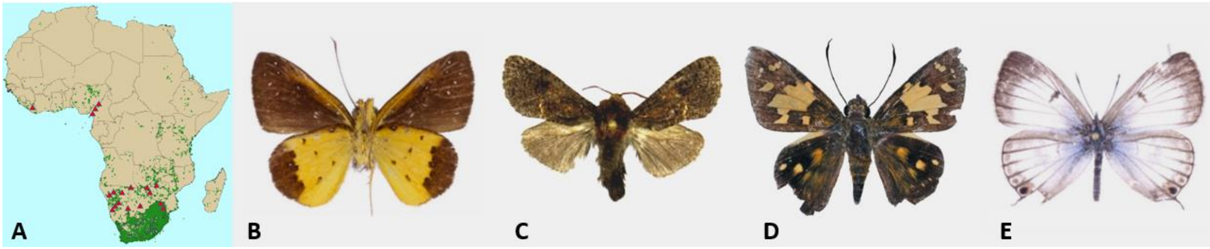

Figure 1. (A) The overview of the distributional data of butterflies and moths in the Sub-Saharan Africa (green dots) as available in the LepiMAP project (LepSoc 2022), with areas from which I co-authored taxonomic or faunistic studies (red triangles). (B–E) Examples of lepidopteran taxa discovered and described in the material collected during our projects in the Afrotropics: (B) Ceratrichia fako *Sáfián & Tropek, 2016, Mount Cameroon, Cameroon; (C)* Geraldocossus durrelli *Yakovlev & Sáfián, 2016, Mount Cameroon, Cameroon; (D)* Scopulifera sagamase tropeki *Libert, 2014, Mount Swa, Liberia; (E) Lepidochrysops liberti Sáfián & Tropek, 2016, Mount Cameroon, Cameroon.*

The poor knowledge of Afrotropical moths can be documented by our recent discovery of the diversity hotspot for many-plumed moths (Alucitidae) on Mount Cameroon. During our extensive sampling on the southwestern slope of the mountain (e.g. Maicher et al. 2020a), we have sampled several dozens of many-plumed moths resulting in a truly unexpected number of species. Prior our sampling, 58 *Alucita* species had been known from the Afrotropics (**Paper 2**: Ustjuzhanin et al. 2018). So far, we have described 16 new species from Mount Cameroon (Ustjuzhanin et al. 2018, 2020a). Moreover, we are preparing description of additional nine new species (Ustjuzhanin et al., in prep.), whilst we have decided to postpone description of other five taxa because we have not yet sampled enough specimens for the responsible taxonomic decision. Together with a new species described from our material without our co-authorship (Kovtunovich & Ustjuzhanin 2016) and six previously described species we recorded in our samples, Mount Cameroon is known to host over 35 species of many-plumed moths. Although Mount Cameroon is known to harbour high diversity in many groups, including plants (Cable & Cheek 1998), birds (Hořák et al. 2019), and even butterflies and moths (e.g., Larsen 2005; Ballesteros-Mejia et al. 2013; Przybyłowicz et al. 2019a), such local diversity of the Alucitidae family (22 of 72 described Afrotropical species, De Prins & De Prins 2022; plus numerous not formally described species; Ustjuzhanin et al. 2020a) is unprecedented on the African continent, despite the intensive sampling of these moths in numerous localities in the past decades (Ustjuzhanin et al. 2018). Additionally, we have drawn attention to another critically endangered but overlooked ecosystem in the area, the virtually vanished littoral forests (Ferenc et al. 2018). We suppose that numerous similarly important local diversity hotspots for various insect groups are still waiting for their discovery in the Afrotropics.

Although I am mainly interested in the insect life history and ecology, I am convinced that material sampled during ecological projects should be used also for improving the knowledge of biodiversity, especially in the understudied tropical areas. Therefore, our group has always been collaborating with various specialists for particular insect groups and has shared our abundant material (just lepidopterans sampled during our African projects exceeded 100,000 specimens already some time ago) with them for collaborative studies, or for other scientific usage. As a results, already 20 lepidopteran species was described in publications I co-authored (**Paper 1:** Sáfián & Tropek 2016; Ustjuzhanin et al. 2018, 2020a; Fric et al. 2019; Sáfián et al. 2019; Fig. 1). Moreover, I am aware about several other species described from our material without my co-authorship (e.g. Libert 2014; Yakovlev & Sáfián 2016; Kovtunovich & Ustjuzhanin 2016; Przybylowicz et al. 2019b; Ustjuzhanin et al. 2020b; Fig. 1), whilst most of our material has been stored in public collections of several museums and other institutions and various taxonomists have been working with it without my knowledge. Last but not least, with our colleagues we described newly previously unknown sexes of four nymphalid butterfly species (Sáfián et al. 2019) and of an arctiin moth species *Amerila femina* (Przybylowicz et al. 2019a).

According to my opinion, the good knowledge of local biodiversity is crucial for setting priorities in the nature conservation, as described above on the example on many-plumed moths on Mount Cameroon. Especially on Mount Cameroon, we have always tried to educate the local communities about the tremendous biological value of the pristine forest ecosystems in the area. Therefore, we dedicated names of some described lepidopteran species to the local places, communities, and persons: *Ceratrichia fako* (named after Fako, the local name for Mount Cameroon), *Alucita bakingili* and *A. bokwango* (named after the villages on Mount Cameroon), *A. bakweri* (named after the local tribe), and *A. fokami, A. escobari* and *A. besongi* (named after our local collaborators). All these species have been publicly "donated" to the local communities who have obviously appreciated the gesture. I am highly convinced that creating of such a "formal bound" of the local communities to the local biodiversity can contribute to the protection of the local ecosystems.

To improve the systematic knowledge of (Afrotropical) butterflies and moths, we have participated in phylogenetic and phylogeographic studies. In the study led by Zdeněk Fric (**Paper 3**, Fric et al. 2019), we studied the phylogeny and phylogeography of the Leptotini tribe of lycaenid butterflies, including the detailed study of *Leptotes pirithous*, one of the most widespread butterfly species. We have hypothesised this species originated in Madagascar from where in colonised most of the African continent and Europe (Fric et al. 2019), unlike most of the other studied butterfly lineages which are known to radiate in Madagascar after its colonisation from the continental Africa (Kodandaramaiah et al. 2010; Aduse-Poku et al. 2015), or even using Madagascar as a stepping-stone when spreading to

India from Africa (Condamine et al. 2013). In the detailed phylogenetic and phylogeographic study of *Lepidochrysops* butterflies led by Marianne Espeland (Espeland et al., under review; preprint available as Espeland et al. 2019), we have revealed the phyto-predation of the genus to be the key innovation for the diversification of the genus during the historical aridification of the African ecosystems. Additionally, our material was used in a few studies without my co-authorship: Oskar Brattström added our specimens of satyrin butterflies into analyses of the *Bicyclus* genus phylogeny (Brattström et al. 2015), biogeography (Aduse-Poku et al. 2022), and 'evo-devo' (Brattström et al. 2020); Lukasz Przybyłowicz used our specimens of arctiin moths as the key part of his analysis of the *Amerila* genus phylogeny (Przybyłowicz et al. 2019b).

Besides the taxonomic work, our sampling also improved the knowledge of the already described, but often poorly known, species' distribution and/or life history. Because most of our research has been performed in the highly undersampled areas of the Afrotropics, it is not surprising we made numerous interesting faunistic findings, including extensions of species' distributions into new countries, sometimes even extensions of the known ranges for thousands of kilometres and/or into the previously unknown biogeographic regions (e.g. Delabye et al. 2020a,b, 2022; Tropek et al. 2013b, 2015; Maicher et al. 2016; Przybylowicz et al. 2019a). Through our collaborators, these data are always entered into the existing distributional databases, such as Afromoths (De Prins & De Prins 2022), or African Butterfly Database (ABDB; Sáfián & Siklosi 2022) to make such records publicly available. We have also described external and internal morphology (e.g. Przybylowicz et al. 2019a), elevational ranges (e.g. Maicher et al. 2016, 2020a; Delabye et al. 2020a), phenology (Maicher et al. 2018), habitat preferences (Tropek & Konvička 2010; Maicher et al. 2020b), endosymbionts (Duplouy et al. 2020), or adult food plants (Mertens et al. 2020, 2021) of numerous lepidopteran species in our community-wide studies, mostly mentioned in the following two chapters.

I am aware our sampling is insufficient to efficiently discover and describe even the diversity of the single insect order in the few areas studied by us. Nevertheless, I hope it helps at least a bit to its better knowledge, necessary for its efficient protection.

Complicated patterns of insect diversity and its drivers

Ever since the foundation of biogeography by A. von Humboldt, the understanding to uneven distribution of diversity on Earth and its drivers have become the central question in ecology (MacArthur 1972; Pontarp et al. 2019). Although insect species dominate diversity of most terrestrial ecosystems, the current (macro)ecological research is strongly biased towards vertebrates and plants (Hortal et al. 2015; Beck & McCain 2020). Although for vertebrates (especially birds and mammals; e.g. Jetz et al. 2012; Jenkins et al. 2013) and plants (especially trees; Liang et al. 2022), strong analyses of their global diversity patterns based on the very detailed datasets have been already published, we are still lacking any high-quality data even for some smaller arthropod taxa (e.g. Beck & MacCain 2020; Fig. 2). The few existing analyses of the global (or at least intercontinental) analyses of the insect diversity patterns are based on approximated distributional data gained by the species distribution modelling (e.g. for Sphingids, Ballesteros-Mejia et al. 2017), or strongly biased by the uneven sampling in particular areas, especially in the Old World tropics (e.g. for geometrid moths, Beck et al. 2017; or for bees, Orr et al. 2021; Fig. 2). In both these cases, we cannot be sure about the power such analyses to explain the general diversity patterns of insects. Nevertheless, without reliable data and analyses of the most speciose terrestrial animal group, we can hardly understand the general patterns of the diversity distribution and its drivers. This can be represented by the example that most of the known aberrant (inverse or other) latitudinal diversity patterns were described for insects (e.g. aphids, Dixon et al. 1987; freshwater insects, Vinson & Hawkins 2003; galling insects, Price et al. 1998; grasshoppers, Davidowitz & Rosenzweig 1998; braconid and ichneumonid wasps, Santos & Quicke 2011; bees, Ollerton et al. 2006; sawflies, Kouki et al. 1994).

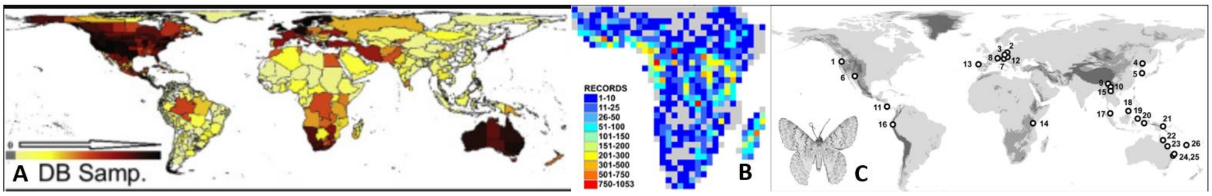

Figure 2. Examples of geographic bias in global and other large-scale analyses of insect diversity patterns towards the (Afro)tropical ecosystems: (A) sampling effort of bee diversity in individual world countries (Orr et al. 2021); (B) available records of Afrotropical sphingid moths (Ballesteros-Mejia et al. 2013); (C) available datasets on elevational diversity patterns of geometrid moths (Beck et al. 2017).

Because we are still far from having strong data on the global distribution of insect diversity, even research of its regional or local patterns and their drivers is a challenge because of the high demands on sampling of the high-quality data on all insect diversity (Beck & MacCain 2020). Therefore, most of the studies focused on various insect groups hoping they are the suitable indicators for insect general diversity. As such a model we mostly selected butterflies and moths, the hyperdiverse group of insect herbivores (Stork 2018). In the studies included in this thesis, we focused on lepidopteran communities along an elevational gradient of Mount Cameroon, the only complete gradient of primary tropical forests ranging from lowlands to the natural timberline in the African continent (Maicher et al. 2020a; Fig. 3A), and on moth communities along a cross-continental gradient of environmental productivity in south African savannahs, unique by its independency on environmental temperature (Delabye et al. 2022; Fig. 3C). The last study in this part compares abundances of several groups of arthropods in tropical and temperate forests (Mottl et al. 2021; Fig. 3B).

In the first included project, we primarily focused on the elevational patterns of lepidopteran diversity. Similar to latitudinal gradients, various environmental factors and biological interactions changes spatially, but elevation offers steeper gradients in the restricted spatial scales (McCain & Grytnes 2010). On Mount Cameron, we have described a mid-elevation diversity peak for all studied groups of butterflies and moths (**Paper 4**, Maicher et al. 2020a). Nevertheless, we have revaled the seasonal shifts of these diversity peaks along elevation, caused presumably by interseasonal shifts of elevational ranges of individual species, with a minor contribution of the interseasonal community assembly turnover among elevations (Maicher et al. 2020a). We are not sure if these shifts are caused by the elevational migrations of individual lepidopteran species, or by phenological shifts at individual elevations. Our recent unpublished results from metabarcoding of pollen loads from a papilionid butterfly *Papilio zoroastres* detected pollen of numerous lowland plant species on the butterfly specimens collected close to the timberline, which would support the first hypothesis (Tropek et al., unpublished data). Nevertheless, the strong influence of phenology on composition of lepidopteran species was already described from many lowland tropical forests (e.g. Aduse-Poku et al., 2012; Grøtan et al., 2014), including lowlands of Mount Cameroon (Maicher et al. 2018). Additionally, we used the data sampled along the elevational gradient also for the analysis of habitat requirements of the fruit-feeding lepidopteran communities, showing that butterfly communities are sensitive mostly to the forest structure (mainly amount of sunlight in the habitats), whilst moth communities are more strongly driven by the composition of plant communities (**Paper 5**, Delabye et al. 2021). Such results support the need to sample as diverse communities of insects as possible to allow generalisation of the given results. Our ongoing and future research is focused on disclosing of the described elevational shifts (Maicher et al. 2020a). Besides the mentioned metabarcoding of pollen carried by butterflies, we are also planning stable isotope analyses of selected species to reveal in which elevation they developed. Using the already collected material, we also already measured and analysed intra-specific elevational and seasonal differences in the body size revealing that species developed in colder environment (higher elevations, or colder season) are larger and follow Bergmann's cline (Papandreou et al., under review). Additionally, we have started to gather all available datasets of COI sequences of geometrid moths from elevational gradients across the world to perform a meta-analysis of phylogenetic diversity patterns along elevation.

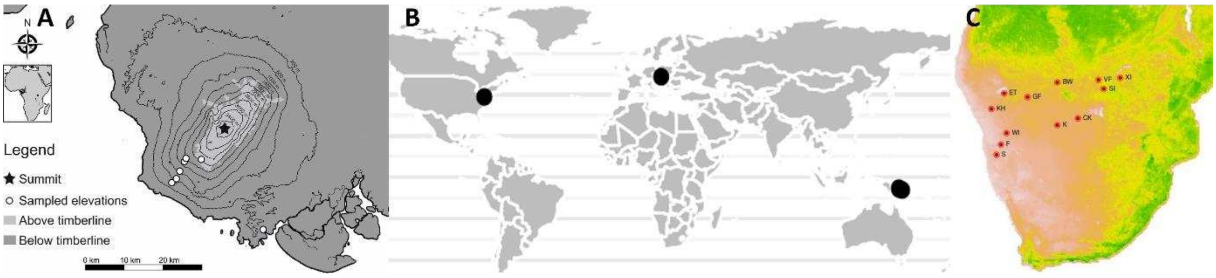

*Figure 3. Overview of sampling sites used for the large-scale studies in this thesis. (A) Sampling sites along the elevational gradient on Mount Cameroon used for **Papers 1, 2, 4, 6, 7, 10, 14, and 15**. (B) Sampling sites for sampling of arthropod abundances used for **Paper 8**. (C) Sampling sites along the cross-continental gradient of environmental productivity used for **Paper 5**.*

The unique situation on Mount Cameroon allowed also to study the impacts of natural disturbances by forest elephants on diversity of insects (again butterflies and moths) and trees (**Paper 6**, Maicher et al. 2020b). Mount Cameroon hosts one of the last persisting populations of forest elephants in West/Central Africa (MINFOF 2014). Nevertheless, the forests on the southern slopes of the mountains were split by a lava flow which is not crossed by elephants in 1980s' and 1990s', offering a unique large-scale 'natural enclosure experiment' with elephants present in forests west of the lava flow and missing in forests east of the lava flow (Maicher et al. 2020b). Our sampling revealed the changes in the forest structure and species composition under those two forest regimes, followed by the changes of lepidopteran communities. We found higher diversity of butterflies in the disturbed forests, corroborating our previous findings of more butterfly species preferring forests with open structure (Delabye et al. 2021). Moreover, the elephant-less forests hosted butterflies with wider distribution, whilst the endemic and distribution-restricted butterfly species preferred the disturbed forests (Maicher et al. 2022). Such findings did not surprise me because they confirmed results of a smaller study done during my MSc. studies in Bamenda Highlands in the same montane range where I found that all three studied endemic species of the Gulf of Guinea Highlands prefer open habitats and forests with open structure which I interpreted as they involved in naturally disturbed montane landscapes (Tropek & Konvička 2010). Altogether, our results confirmed the key role of African forest elephants for maintaining of the diversity of Afrotropical rainforests.

Paper 7 (Delabye et al. 2022) represent the recent shift of our research interest into the tropical savannahs in southern Africa. In this study, we focused on the effect of one of the key drivers of diversity patterns, environmental productivity (Storch 2012; Bohdalková et al. 2021; Hejda et al. 2022). For this purpose, we extensively sampled moth communities along a cross-continental gradient of environmental productivity, unique by its perpendicularity to latitude and independency to environmental temperature (Delabye et al. 2022). We revealed a linear relationship of all studied moth groups to environmental productivity, confirming its key role for the insect diversity patterns. To follow this study, we are participating in the large-scale multitaxon study led by Prof. Petr Pyšek in savannahs of the Kruger National Park, where we are trying to disclose the effects of environmental variables, disturbances of large herbivores, seasonal availability of water, and inter-guild interactions on diversity of plants, several insect groups, birds, large mammals, and bats.

The last study in this part (**Paper 8**, Mottl et al. 2021) represents my collaboration in large projects led by Prof. Vojtěch Novotný which focus on comparison of tropical and temperate forest communities (I have also participated in the sampling protocols for spiders in the plot-based approach, Volf et al. 2019). Mottl et al. (2021) was the first study comparing abundances of several trophic guilds (leaf-chewing and leaf-mining herbivores, together with ant and spider predators) of arthropods across biomes, using the well-standardised sampling protocols in forests of three continents. Unlike the top-down regulation hypothesis (Walker & Jones 2001; Floren et al. 2002), we did not find any negative correlations between the predatory and herbivory guilds. Moreover, the strength of the correlations did not depend on environmental temperature, according to the niche breadth-latitude hypothesis predicting more intensive interspecific interactions towards lower latitudes (MacArthur 1972). Therefore, we interpreted the results as the support of the bottom-up limitation when insect abundances depend rather on availability and quality of resources, and on abiotic variables (Walker & Jones 2001; Welti et al. 2020). Once all material from the eight forests in the tropical and temperate ecosystems will by sampled, we will continue in analyses of species richness and interspecific interactions.

Insects in interactions with other organisms

Besides number of species, biodiversity also comprises interspecific interactions, including those among plants and animal visitors of their flowers (e.g. Ollerton 2017; Andresen et al. 2018). Especially knowledge on these interdependences on the community level is crucial for understanding past coevolution processes and recent ecosystem functioning, as well as for predictions related to local and global environmental changes. Paradoxically, the key role of interspecific interactions in the most fundamental biodiversity patterns, such as latitudinal patterns of species richness, was discussed already by some classical ecologists, including A.R. Wallace and R.H. MacArthur. Nevertheless, despite their importance, the underlying mechanisms responsible for the large-scale geographical patterns in biotic specialization remain poorly understood (Moles & Ollerton 2016; Ollerton 2017).

The specialisation-generalisation continuum (hereinafter simplified to 'specialisation' as a value characterising species position along this ecological gradient), one of the key properties of these biotic interactions, plays a central role in species coexistence and both plants and pollinators diversification (Van der Niet & Johnson 2012, Andresen et al. 2018). After all, already since Darwin the diversity of flowers has been attributed to selection by specialised pollinators. Pollinators commonly act as selective agents on floral traits and therefore the evolutionary development of specialised plant-pollinator interactions is commonly hypothesised as one of the key drivers of the historical radiation of angiosperms (e.g. Van der Niet & Johnson 2012, Kay & Sargent 2009). As recently reviewed by Armbruster & Muchhala (2009), plant specialization in pollination interactions promotes initial reproductive isolation and reinforcement of reproductive isolation upon secondary contact, as well as reduces the extinction rate by promoting tighter species packing. Simultaneously specialisation of a plant pollination system increases competition for pollinators and niche partitioning among related species in communities, and thus again support diversification (Armbruster & Muchcala 2009). Nevertheless, we are often missing even the basic information on pollination systems of individual plant species in the tropical ecosystems.

The first two studies in this part of the thesis represent detailed research of pollination systems of three plant species in the Bamenda Highlands, NW Cameroon: a phenotypically specialised *Hypoestes aristata* (Acanthaceae) with mellitophilous flowers (**Paper 9**, Padyšáková et al. 2013) and two phenotypically generalised *Hypericum* species (Hypericaceae; **Paper 10**, Bartoš et al. 2015). Both studies combine identification and quantification of flower visitors, observation of their behaviour with the specific focus on their contacts with the plants' reproductive organs (stigmas and anthers), and exclusion experiments (Fig. 4C) followed by counting of the produced seed sets to reveal the real contribution of individual visiting species to the plant reproduction success (although the experiments with both *Hypericum* species were unsuccessful because of the high infestation of their fruits by herbivorous insects). Although numerous insect and sunbird species visited the studied plants, we revealed a surprisingly high level of specialisation of all studied pollination systems. *Hypericum aristata* was visited only by three bee species (*Xylocopa lugubris, X. caffra, Apis mellifera*) and a sunbird species (*Cinnnyris reichenowi*) in high enough abundances to be considered as efficient pollinators (Padyšáková et al. 2013). Nevertheless, the experiments revealed that whilst both carpenter bee species were efficient pollinators of the plant, the sunbird had no effect on its pollination, and the honeybee's visits even decreased the produced seed set. Interestingly, the sunbird's effect on the plant was later revealed as negative because we have observed and described that it has defended the plant's nectar against the pollinating carpenter bees (**Paper 11**, Tropek et al. 2013). Surprisingly, despite the species-rich communities of insects visiting flowers of the two studied *Hypericum* species, we also revealed the high specialisation of their pollination systems (Bartoš et al. 2015). Only a few medium-sized bee species (mainly *A. mellifera* and *Meliplebeia ogouensis*) were observed to be able efficiently pollinating the nectar producing *H. roeparianum*, whilst the nectarless flowers of *H. revolutum* can be efficiently pollinated only by a single carpenter bee species (*Xylocopa caffra*) during common collecting of its pollen (Bartoš et al. 2015). We described a similar partitioning of floral resources in the Bamenda Highlands by two eusocial bees, although appeared to vary interseasonally (**Paper 12**, Tropek et al. 2018).

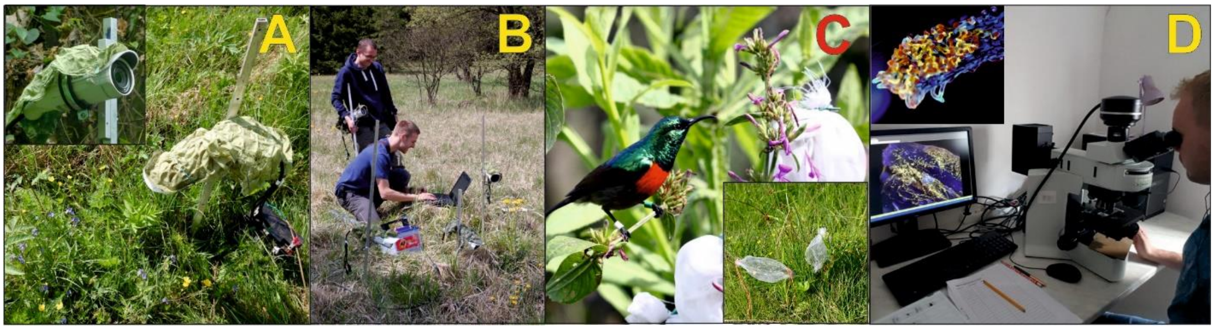

Figure 4. Selected methods used in our pollination research: *(A) Video-recording of flower visitors. (B) Setting of video cameras in the field. (C) Experimental excluding of pollinators. (D) Counting of germinating pollen tubes using fluorescence microscopy.*

Considering phenotypical specialisation of flowers, the concept of pollination syndromes has become an important part of the modern pollination biology. The pollination syndrome represents a set of convergent floral traits (such as shape, colour, odour, or production, composition and display of floral rewards) evolved as adaptations to a particular pollinator group (Faegri & van der Pijl 1979; Willmer 2011). In the past two decades, this hypothesis has been under an intensive discussion with studies approving or disproving its validity (e.g. Ollerton et al. 2009; Rosas-Guerrero et al. 2014; Dellinger 2020). Our case studies of individual pollination systems repeatedly confirmed our predictions of the main pollinators based on the pollination syndrome hypothesis (e.g. Janeček et al. 2011, 2021; Bartoš et al. 2012, 2015; Padyšáková et al. 2013; Mertens et al. 2020, 2021; Chmel et al. 2021). To challenge the hypothesis on the broader scale, we analysed the role of numerous floral traits of 117 plant species in shaping of plant-pollinator interactions (**Paper 13**, Klomberg et al. 2022). We revealed various flaws in the rigid definition of the pollination syndromes: some floral traits were more important than others for individual pollinator groups, and their importance also differed among the studied elevations and seasons. Based on such spatiotemporal and pollinator-specific differences, we have therefore suggested to not refuse the pollination syndrome concept, but to step back from its rigid definition and rather improve our understanding to the role of floral traits in plant-pollinator interactions at the community level under different environmental conditions (Klomberg et al. 2022). In another study of communities on Mount Cameroon, we revealed spatiotemporal distribution of floral traits to be driving elevational and seasonal changes in nectar robbing and thieving behaviour (the study is under review, its preprint can be found as Sakhalkar et al. 2022).

Besides the already discussed effects of environmental variables on the uneven diversity distribution on Earth, biotic interactions are often proposed among the most important drivers of LDG (Brown 2014; Pontarp et al. 2019). The originally proposed latitude-niche breadth hypothesis (MacArthur 1972) predicted decreasing niche breadth (i.e. higher specialisation) with increasing species richness towards the tropics. In the stable and productive tropical ecosystems, the importance of biotic interactions increases over abiotic stress, which triggers diversity-generating evolutionary processes (Brown 2014; Granot & Belmaker 2019; see my overview of the potential mechanisms responsible for the positive feedback processed between high species richness and high specialisation in Fig. 5a). Although this hypothesis has never been sufficiently confirmed by the data (e.g. Moles & Ollerton 2016), it has been modified also into the altitude-niche breadth hypothesis predicting lower specialisation of interspecific interactions at higher elevations (Rasmann et al. 2014). Nevertheless, almost any data on the plant-pollinator interactions in communities along elevational gradients are missing from the tropical ecosystems.

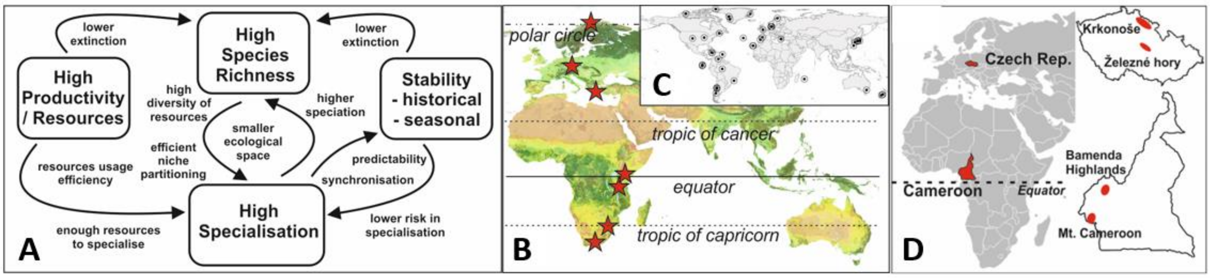

Figure 5. *(A) Schematic relationships between high species richness and high specialisation of individual species. (B) Study sites of our ongoing research along the latitudinal gradient. (C) An example of the geographic bias of a synthetic analysis by Trøjelsgaard & Olesen (2013). (D) Study sites of our ongoing research comparing plant-pollinator interactions in tropical and temperate montane ecosystems.*

To address the altitude-niche breadth hypothesis, we studied a very intensive dataset of plant-pollinator interactions from four elevations on Mount Cameroon. During the optimisation of methodology necessary for such intensive studies in tropical forests (Fig. 4), we studied elevational changes in pollination system of psychophilous *Scadoxus cinnabarinus* (Amaryllidaceae; **Paper 14**, Mertens et al. 2020), including analyses of germinating pollen grains after individual pollinators' visits (Fig. 4). Although the most common and most efficient pollinators of the plant were a few species of butterflies, we have revealed elevational changes in the pollination communities with proportionally more non-pollinators thieving rewards from the flowers at both lower and upper elevational limit of the plant (Mertens et al. 2020). Another case study performed during the methodology optimisation focused on pollinators of *Uvariopsis dioica* (Annonacea; Mertens et al 2018), although we failed to find enough plant specimens for the elevational changes. Nevertheless, we proposed orthopterans and cockroaches as the most probable pollinators of the studied plant based on the gathered data (Mertens et al. 2018).

Although the huge data from the mentioned community study of the elevational patterns in plant-pollinator interactions specialisation (>28,000 recorded hours of flowers of 217 plant species, resulting in >46,000 interactions with >500 morphospecies of insect and vertebrate visitors; Tropek et al., in prep.) are still under process, we performed a detailed analysis of flower visits by butterflies and hawkmoths (**Paper 15**, Mertens et al. 2021). We found both groups to be relatively rare flower visitors, however overwhelming majority of their visits included contact with the plants' reproduction organs, and they appeared to be potential key pollinators of some studied plant species. Specialisation of pollination interactions showed no apparent elevational or seasonal patterns, potentially because of the already high specialisation of all studied networks (Mertens et al. 2021). These specialised interactions probably resulted from the high trait matching between butterflies and the visited flowers, confirmed by the significant relationship between proboscis and floral tube lengths. Similarly, individual lepidopteran families showed different preferences to the floral traits indicating their high specialisation for floral rewards (Mertens et al. 2021).

Because the topic of the spatiotemporal changes of specialisation in plant-pollinator interactions appeared very interesting for me, most of my current and future research is going to aim this question. At this moment, we are finalising sampling of the plant-pollinator interactions in the Afromontane grasslands above the timberline on Mount Cameroon. We have added experimental studies of intraspecific elevational patterns in self-compatibility and pollen limitation in several selected Afromontane species along this gradient. Simultaneously, we are sampling the standardised data in Cameroonian and Czech montane grasslands to analyse potential differences related to latitude (Fig. 5). Last but not least, we have succeeded to start a large intercontinental project focused on the latitudinal patterns in plant-pollinator interactions (Fig. 5). I hope that these and other projects of our research group will contribute to the knowledge of tropical (and other) insects and their role in the global ecosystems.

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