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Research at the Tropical Field Station La Gamba in Costa Rica

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Front cover:
Euglossine bee *Euglossa tridentata* pollinating an orchid *Gongora sp.*
Photo: Thomas Eltz

There are many epiphytic plants in the Piedras Blancas National Park.
Photo: Peter Weish

Back cover:
The dining area of the station is situated in the middle of the garden.
Photo: Christian Hartmann

Students, volunteers and workers involved in the COBIGA project participate in an "action day" of reforestation.
Photo: Tropenstation La Gamba

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Inhalt – Content

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Preface

Over the past eight years the ZooBot (Austrian Zoological and Botanical Society) and the Association of the Tropical Station La Gamba have cooperated in organizing workshops on the research carried out at the rainforest field station of La Gamba in southern Costa Rica – the rainforest of the Austrians ("Regenwald der Österreicher"). This year we present a special issue of the ACTA dedicated to the broad range of science carried out in this area.

The field station, situated at the immediate fringe of a virgin lowland forest, the Piedras Blancas National Park, is ideally suited for studying pristine tropical rainforest ecosystems and their adjacent cultural landscapes. Starting in 1993, the station has developed from simple beginnings under the governance of the University of Vienna, and has increased its research capacities and laboratory facilities. In 2015, a small farmhouse called Finca Alexis was bought and can also be used as a base camp to do research at the transition zone between the lowland forests of the Golfo Dulce region and the mountain forests of the adjacent Fila Cal. The research facilities have attracted scientists from Austria and many international institutions to carry out field work in the La Gamba area, and also provide an excellent base from which to conduct student excursions and courses. The field station is well linked with the universities of Costa Rica as well as international research institutions on tropical ecology.

Besides providing immediate access to various tropical ecosystems, both terrestrial and aquatic, the station is ideally suited for studying applied aspects: a main focus is research related to reforestation programs of former agricultural areas. A further scope of the station is the function of migration corridors connecting isolated conservation areas in the region.

This volume demonstrates the high potential of the field station La Gamba for carrying out both basic and applied tropical research, and highlights the wide range of studies initiated during the last decade. They range from research on physiographic conditions, flora and fauna, the fascinating biotic interactions that are characteristic of tropical rainforest ecosystems, as well as applied aspects of reforestation programs and the function of migration corridors.

We hope that the publication is a stimulation for further utilization of the field Station La Gamba and for further research work on tropical biology and ecology.

Fritz Schiemer, Werner Huber, Anton Weissenhofer

Acta ZooBot Austria **156**, 2019, 3–11

The La Gamba Research Station in Costa Rica – History, Nature and Research

Werner HUBER & Anton WEISSENHOFER

Initiated by the Austrian nature conservation association "Rainforest of the Austrians", the tropical station was founded in 1993. It is now owned by the association "Förderverein des Tropenstation La Gamba", based at the University of Vienna. The station is located in the Pacific region of southern Costa Rica near the village of La Gamba and the Piedras Blancas National Park, one of the last remaining perhumid lowland rainforests on the Pacific coast of Central America. Due to its geographic, climatic and geological conditions, it is one of the most species-rich regions of Central America with about 2,700 plant, 370 bird, more than 90 reptile, 50 amphibian and 145 mammal species. The roots of Austrian scientific activity in this region reach back to an expedition of the Vienna Natural History Museum conducted in 1930. Since the establishment of the tropical station, research has been carried out on many tropical biology issues.

Huber W. & Weissenhofer A., 2019: Die Tropenstation La Gamba in Costa Rica – Geschichte, Natur und Forschung.
Ausgehend vom österreichischen Naturschutz-Verein "Regenwald der Österreicher" wurde die Tropenstation im Jahr 1993 gegründet und ist inzwischen im Besitz des „Fördervereins der Tropenstation La Gamba" mit Sitz an der Universität Wien. Die Station befindet sich im Süden Costa Ricas in der pazifischen Region in unmittelbarer Nähe der Ortschaft La Gamba und des Nationalparks Piedras Blancas, einem der letzten noch erhaltenen perhumiden Tieflandregenwälder an der Pazifikküste Mittelamerikas. Dieser gehört auf Grund seiner geographischen, klimatischen und erdgeschichtlichen Gegebenheiten mit etwa 2.700 Pflanzen-, 370 Vogel-, über 90 Reptilien- und 50 Amphibien- sowie 145 Säugetierarten zu den artenreichsten Regionen Mittelamerikas. Österreichische Wurzeln der wissenschaftlichen Betätigung in dieser Region reichen zurück bis zu einer Expedition des Naturkundlichen Museums/Wiens im Jahre 1930. Seit dem Bestehen der Tropenstation wird an vielen tropenbiologischen Fragestellungen gearbeitet.

Keywords: Field station, field course, rainforest, Neotropics.

Introduction

The La Gamba Research Station was founded in the year 1993. It is located near the small village of La Gamba and close to the Piedras Blancas National Park (Esquinas Rainforest). This national park exists thanks to the conservation efforts of the Austrian Michael SCHNITZLER, who collected financial donations in Austria and forwarded them to the Costa Rican government, which in turn purchased large tracts of forest from landowners. This resulted in the establishment of the Piedras Blancas National Park, with the section "Rainforest of the Austrians" and various private properties of the Tropical Research Station, which play a very important role in nature conservation and in the building of a biological corridor in the southern part of Costa Rica. The Piedras Blancas National Park harbors the last primary tropical lowland rainforest on the Pacific coast of Central America and boasts exorbitantly high biodiversity.

History

In 1993 the association "Rainforest of the Austrians" bought a small farmhouse (finca) for the first students (the authors of this article and Astrid Keber) who started their scientific activities in La Gamba. The farmhouse was the precursor of the Tropical Research Station La Gamba. Since 2002 the Austrian non-profit association "Förderverein der Tropenstation", closely linked to the University of Vienna, has taken over responsibility for the station. Together with the financial support of the association "Rainforest of the Austrians", the Austrian Ministry of Science and Research and the University of Vienna, the Tropical Research Station has been expanded and improved repeatedly during the last decade. It now comprises several buildings, including a laboratory, library and a botanical garden. It provides comfortable living and research facilities for about 40 visitors.

With support from the University of Vienna (specifically Prof. Anton Weber and Prof. Roland Albert) the scientific activities began to increase. Since those beginnings, many students and scientists have visited La Gamba on excursions or to work at the station (Albert 2013).

However, scientists have worked in the Golfo Dulce region for far longer than the first days of the station. In the 50s of the last century, the US-American botanist Paul H. Allen worked in the region and published the book "The rainforest of the Golfo Dulce" (Allen 1956). He was employed at the research department of the United Fruit Company.

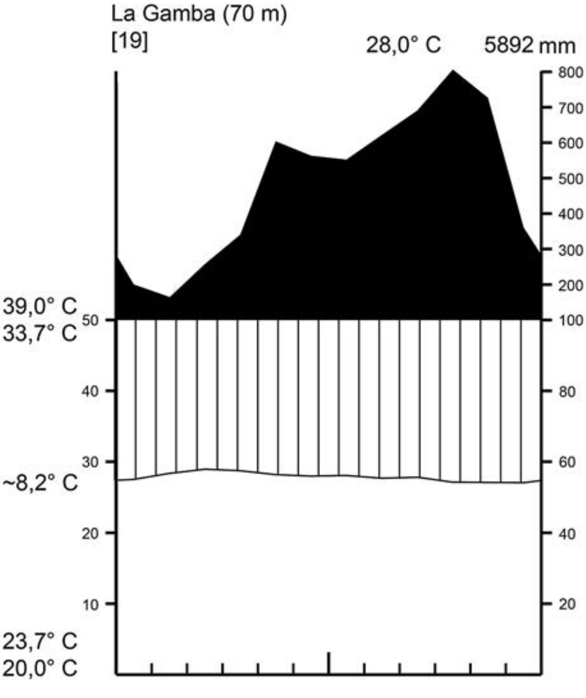
Even Austrians have worked in the region before. The roots of Austrian scientific work in Costa Rica date back to 1930, when an Austrian expedition led by the botanist Otto Porsch visited Costa Rica and the Golfo Dulce region.

In the 70s of the last century, scientific work increased and was mainly conducted by US scientists. Scientists were particularly attracted once the Corcovado National Park was founded in 1975. In 1989 the Instituto Nacional de Biodiversidad (INBio), the national institute for biodiversity and conservation in Costa Rica, was founded. A goal of the institute was to complete an inventory of the natural heritage of Costa Rica. For this purpose, INBio sent parataxonomists and field scientists to the region around the Golfo Dulce to gather more information on its diversity.

Location and nature

The Golfo Dulce region is located at the southern corner of Costa Rica's Pacific coast, close to the border to Panama, in an area comprising the Corcovado and Piedras Blancas National Parks (Esquinas forest). The region's forests are the only remaining moist and wet evergreen lowland forests on the Pacific slope of Central America. The elevation of the region ranges from sea level to a height of 745 m on the Cerro Rincón, Peninsula de Osa, and 579 m on the Cerro Nicuesa in the Esquinas Forest. Annual precipitation is about 6.000 mm, with a short dry season from December to March (Fig. 1).

The relief is strongly structured at the landscape level and contains many microhabitats and niches. The region is very diverse in soil types, and has high vegetation dynamics (i.e. many forest gaps). All these factors, together with the biogeographical position of



Quelle der Klimadaten: Tropenstation La Gamba, Diagramm erstellt von: Susanne Pamperl

Fig. 1: Climate diagram, tropical research station La Gamba. – Abb. 1: Klimadiagramm, Tropenstation La Gamba.

the Golfo Dulce region, have led to a highly species-rich forest, with about 2.700 species of higher plants and around 145 mammals, 50 amphibians, 90 reptiles, 370 birds and numerous insects (Weissenhofer et al. 2008). Much of the recently gathered biological data, together with older research, indicate that these lowland forests rank among the most highly diverse lowland rainforest ecosystems in Central America.

The station itself is located close to the primary tropical rainforests and in the vicinity of the village of La Gamba, with its mosaic of managed land, including secondary forests, reforestations, oil palm plantations, tree plantations, pastures, rice fields and home gardens. The field station therefore offers easy access to many types of natural and man-made ecosystems. Even marine ecosystems like mangroves, coastal forests and beaches are within reach. Thus, the station offers plenty of options to study different scientific issues in tropical environments.

Costa Rica’s geographical location on the connecting “bridge” between North and South America (Fig. 2) has led to remarkable biogeographical patterns, along with the fact that some parts of the country – like the region around the Golfo Dulce – formed a kind of refuge for tropical animals and plants during the last ice age. The region contains numerous range-restricted plants and animals (high endemism) of which many reach their northern limits in southern Costa Rica.

An extensive system of trails in the Piedras Blancas National Park (La Gamba) and on the properties of the station offers perfect insight into tropical rainforests, and the region is an excellent place for naturalists to learn about tropical nature.

The field Station La Gamba

The station is located close to the Pan-American Highway and near the small cities of Golfito and Rio Claro. The station itself is close to the village of La Gamba and provides comfortable accommodation and research facilities for about 40 visitors at a time. A small botanical garden, a laboratory and library are also part of the installation (Fig. 3). The sta-

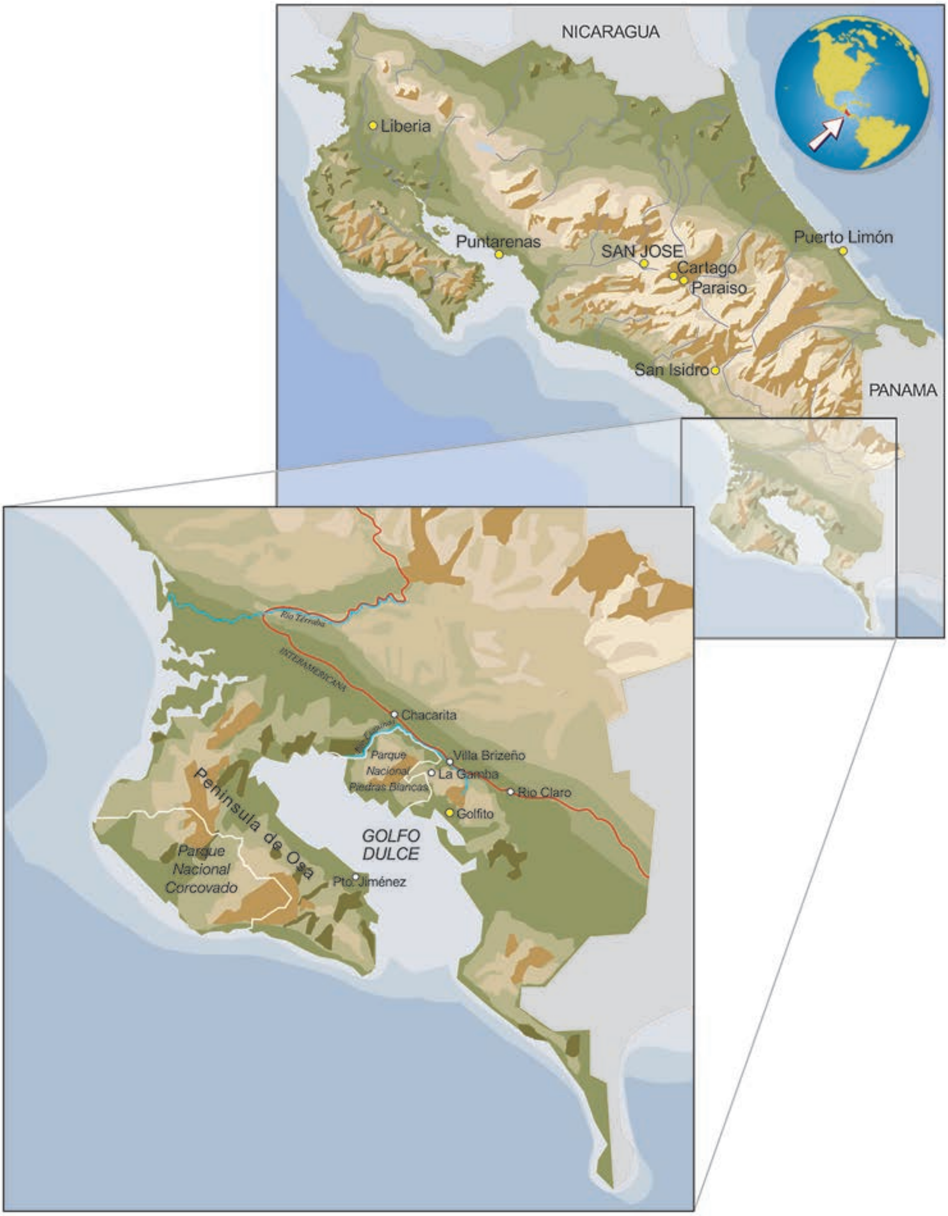


Fig. 2: Geographical location of Costa Rica and the Golfo Dulce region with the Corcovado National park (Parque Nacional Corcovado) and Piedras Blancas National Park (Parque Nacional Piedras Blancas). Preparation: Bettina Berger-Zimmermann. – Abb. 2: Geografische Lage Costa Ricas und des Golfo Dulce, mit dem Corcovado Nationalpark (Parque Nacional Corcovado) und dem Piedras Blancas Nationalpark (Parque Nacional Piedras Blancas). Gestaltung: Bettina Berger-Zimmermann.

Fig. 3: Aerial photo of the station and its surroundings. – Abb. 3: Luftbild der Station und ihrer Umgebung.

tion owns about 300 hectares of pasture, reforestations, and primary and secondary forests in various stages in the vicinity of La Gamba and in the region.

Thanks to its location close to various tropical habitats and to its infrastructure, the station has gained international attention as a research institution and educational centre focusing on scientific exploration and conservation of Neotropical rainforests (Wanek et al. 2017). At about 300 m a.s.l. lies the rustic farmhouse Finca Alexis, an outpost of the station that provides space for about six visitors in two rustic rooms. The Finca Alexis is located close to a lower mountain rainforest about one hour by car from La Gamba.

Overview of scientific work at the Station

Since 1993 numerous scientific publications have resulted from research performed at the Tropical Research Station La Gamba, including around 170 doctoral and diploma theses. Studies of the flowering plants of the region resulted in the book "An Introductory Field Guide to the Flowering Plants of the Golfo Dulce Rain Forests" (Weber et al. 2001), as did further scientific work in the book "Natural and Cultural History of the Golfo Dulce Region, Costa Rica" (Weissenhofer et al. 2008). Catalogues about groups of animals, plants, the history of the station, the biological corridor, the stream ecosystems and about the life of the people in La Gamba have been published. Various exhibitions on the nature of the Golfo Dulce region and on the scientific work carried out at the field station have been presented in Austria, and one even in La Gamba itself. The scientific work initially focused on the flora and vegetation of the Esquinas forest but has now broadened to include a wide range of other topics, such as animal-plant interactions, herpetology, ornithology, entomology, limnology, plant ecophysiology, biogeochemistry, geography and sociology. Around 100 field courses and excursions have been conducted, enabling students and scientists from universities all over the world to visit and study the Piedras Blancas National Park (Fig. 4). Some of the most exceptional scientific work will be described in more detail in this book and mentioned below. All publications resulting from work done at the field station are cited in the "Wissenschaftlicher Bericht" at www.lagamba.at.

Fig. 4: The Piedras Blancas National Park. – Abb. 4: Der Piedras Blancas Nationalpark.

Vegetation and Botany: Many publications were completed on the plant diversity and vegetation of the region – mainly by the authors of this article. More work on different groups of plants like Rubiaceae (Berger et al. 2015, Berger et al. 2017), Orchids (Hetherington-Rauth & Ramirez 2016, Rakosy et al. 2013), Lichens (Baloch & Grube 2009, Breuss & Neuwirth 2007, Grube et al. 2004, Lücking et al. 2013) and many more has been carried out.

Ecophysiology: Under the guidance of Wolfang Wanek, research into primary production and nutrient cycling has led to various publications (Hofhansl et al. 2015). Wolfgang Wanek (Wanek et al. 2002) and Wolfram Weckwerth (Wang et al. 2016) are interested in the metabolism of the genus *Clusia*.

Plant-Animal Interactions: Research into pollination in plants is a research topic of Florian Etl, Anton Weber and Jürg Schönenberger (Etl et al. 2016). For almost 20 years Veronika Mayer (Mayer et al. 2018) has focused on the relationship of ants, plants and fungi.

Limnology: Students of Fritz Schiemer (Schiemer et al. 2010) and Leopold Füreder (Füreder et al. 2014) have worked on ecology and diversity in freshwaters of the region.

Animal Biology: Many scientists are focusing on bees, including Thomas Eltz, Santiago Ramirez and Tamara Pokorny (Pokorny et al. 2017), Stefan Jarau (Flaig et al. 2016), and Johannes Spaethe (Spaethe et al. 2014). Research on other invertebrate groups (dragonflies, butterflies) has been conducted by Christian Schulze and students (Schulze 2008) and Harald Krenn and Martin Wiemers (butterflies) (Krenn et al. 2010). Meanwhile, Gerlinde Höbel (Höbel 2000), Michael Franzen (Franzen & Kollarits 2018), Walter Hödl (Hödl 1996, Mangold et al. 2015) and Dennis Kollarits (Kollarits et al. 2017) have been working on amphibians and reptiles, and Christian Schulze (Seaman & Schulze 2010) and Gerhard Aubrecht (Aubrecht et al. 2013) have studied the region's birdlife. An inventory of the mammals was completed by Armin Landmann (Landmann et al. 2008).

Conservation Biology: Christian Schulze (Freudmann et al. 2015, Gallmetzer & Schulze 2015) has worked on various animal groups and their abundance and diversity in different anthropogenic habitats and reforestations. Peter Hietz (Hietz & Kleinschmidt 2018) and Wolfgang Wanek (Wania et al. 2002) have worked on natural succession, soils and CO2 sequestration in reforested habitats within the Biological Corridor COBIGA, mainly on Finca Amable.

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Contributions to the climatology around La Gamba, Costa Rica

Birgit Eibl & Reinhold Steinacker

When observing weather phenomena in the greater area of the tropical research station in La Gamba, Costa Rica, high rain rates and precipitation sums are to be expected. This is due to the fact that the described area is influenced by the south-westerly trade winds, forcing warm moist air from the Pacific to rise at the leeward sides of 3 orographic elevations on its way east. Deep convective cloud development is highly variable in the predominantly complex terrain and is reinforced when the inner-tropical convergence zone passes the area from the south in June. To back up the frequently used description "heavy precipitation" with numbers, several measuring campaigns were carried out. An evaluation of the droplet spectrum of various precipitation events revealed that a difference between stratiform and convective precipitation can be observed in the distrometer measurements. Radiation investigation of light availability on vertically and horizontally oriented surfaces in vegetation of different densities provides an indication of the quantitative influence of various cloud appearances on the light intensity reaching the ground. To determine the significance of these findings, and whether they constitute the norm or more of an exception, long-term observations would be necessary.

Eibl B. & Steinacker R., 2019: Beitrag zur Klimatologie um La Gamba, Costa Rica.

Werden Wettererscheinungen im Großraum um die Tropenstation in La Gamba, Costa Rica untersucht, so erwartet man in jedem Fall hohe Niederschlagsmengen und Regenraten. Das liegt vor allem daran, dass das Untersuchungsgebiet direkt vom Südwestpassat beeinflusst wird. Dieser transportiert feuchtwarme Luftmassen vom Pazifik an die Leeseiten verschieden hoher topografischer Erhebungen. Damit werden räumlich höchst variable, hochreichende konvektive Systeme ausgelöst, die teilweise von der innertropischen Konvergenzzone verstärkt werden können. Um die Variabilität und Intensität der Niederschläge zu messen wurden verschiedene Messkampagnen durchgeführt. Die Auswertung des Niederschlagstropfenspektrums unterschiedlicher Niederschlagsereignisse ergab, dass eine Unterscheidung zwischen stratiformem und konvektivem Niederschlag auch durch Distrometermessungen möglich ist. Strahlungsuntersuchungen über die Lichtverfügbarkeit an vertikal und horizontal orientierten Oberflächen in unterschiedlich dichter Vegetation quantifizieren den Einfluss des Bedeckungsgrads und -charakters auf die Lichtbedingungen am Boden. Eine signifikante Aussage darüber, ob die Ergebnisse der vorliegenden Untersuchungen die Norm oder eher eine Ausnahme darstellen, kann nur nach langzeitlichen Beobachtungen getroffen werden.

Keywords: Heavy precipitation, light intensity, convection, droplet spectrum, radiation.

Introduction

Equatorial fully humid climate (Kottek et al. 2006), as it is observed in the South of Costa Rica, is characterized by higher diurnal than annual temperature changes and a total precipitation amount of up to 6000 mm per year. To understand the following investigations and results, a brief description of the surrounding environment is provided. The orography in the South of Costa Rica can be described as complex terrain with three elevated terrains oriented southeast – northwest like the Pacific coastline. The outer border is marked by the Osa Peninsula, with a mountain ridge of 600 m in height. Between the peninsula and the mainland lies the Golfo Dulce, a shallow warm water bay with temperatures of around 30° C throughout the year.

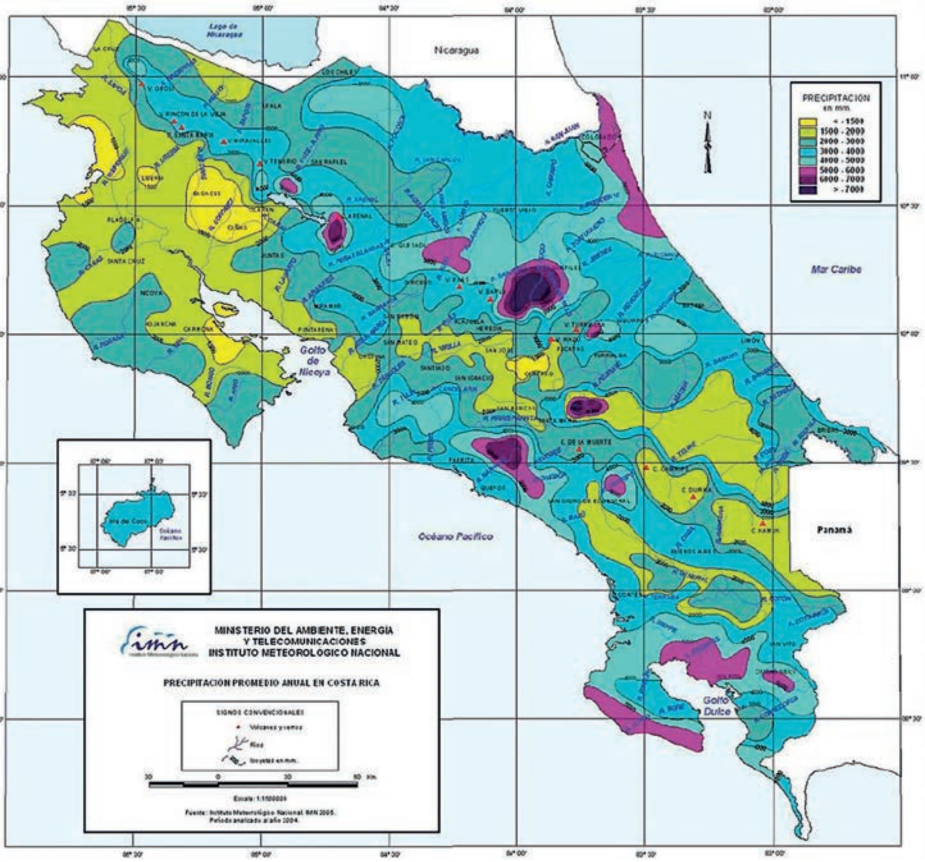

Fig. 1: Map of the annual precipitation sum in Costa Rica. In the South, the purple precipitation field at the leeward side of the mountain ridge on the Osa Peninsula and at the shore of the mainland of the Golfo Dulce (including the area of the "Regenwald der Österreicher") indicates up to 6000 mm of precipitation per year (Instituto Meteorológico Nacional, 2014). – Abb. 1: Karte der jährlichen Niederschlagssummen in Costa Rica. Die violetten Felder im Süden Costa Ricas an der an der Leeseite der Halbinsel Osa und an der Leeseite des Küstengebirges auf der Festlandseite des Golfo Dulce (inklusive des Gebiets des „Regenwald der Österreicher") markieren eine jährliche Niederschlagssumme von bis zu 6000 l m^{-2} (Instituto Meteorológico Nacional, 2014).

Continuing towards the Caribbean side, the next elevation relevant to the investigations is the Fila Cal, a mountain ridge of 800 m, followed by the Talamanca mountains (Fig. 1). The main mesoscale dynamics influencing the daily weather conditions in the Piedras Blancas Region are the south-westerly winds, bringing warm, moist air from the Pacific first to the Osa Peninsula, where air masses are lifted as they reach the mountainous terrain. The consequences are convection and heavy rain showers, nearly every day. If the wind from the southwest is very weak or the weather conditions are very calm, convective clouds form on top of the mountain ridge due to heating and evaporation from the rainforests covering the Osa Peninsula. Short, heavy showers and downbursts can be seen from the Golfo Dulce, as is shown in Figure 2.

Fig. 2: Downburst over the Osa Peninsula, seen from Golfo Dulce (Photo: B. EIBL). – Abb. 2: Fallwindböe aus einer Schauerwolke über der Halbinsel Osa, vom Golfo Dulce aus gesehen (Foto: B. EIBL).

As air moves forward towards the mainland of Costa Rica, moisture from the Golfo Dulce is soaked up by the downflow of air overflowing the Osa mountain ridge. This now very moist and warm air flow is forced to an uplift again at the coastal mountain ridge, producing another area with high precipitation amounts throughout the year. Since the mountain ridge is only 400 to 600 m high, a significant amount of the moist air continues further inland until it reaches the next mountain ridges, triggering more convective clouds which each lead to heavy precipitation in turn. Additionally, mesoscale convective systems arising in the Pacific Ocean around Panama's North are often advected through the south-westerly winds to the Pacific coast of Costa Rica (DURAN-QESADA et al. 2010). Depending on the wind speed and intensity of the spatially widespread cloud formations, the systems penetrate inland more or less intensely. Observations in June and July 2008 showed that, not rarely, only stratiform rain out of the edge zones of these convective systems reaches parts of the Piedras Blancas regions. It is interesting to note that, in contrast to the Pacific Northwest of Costa Rica and the central part of Panama, The Golfo Dulce region receives considerable precipitation amounts even during the northern hemisphere winter. The reason for this is that the high Central Cordilleras of Costa Rica block the north-easterly trade winds and both deflected branches in the west and east of the High Cordilleras lead to a south-westerly counterflow towards the Pacific southeast coast of Costa Rica.

With this in mind, an investigation of convective clouds and the resulting precipitation in the surroundings of La Gamba was conducted. The first aim was to find objective evidence for subjectively experienced differences in precipitation amounts within the region. The second aim was to find differences in the precipitation events, whether they are of a stratiform or convective character, by examining the cloud droplet spectra.

The droplet size distribution is different and exclusive for every precipitation event, since the cloud regions from where precipitation drops originate are never the same. A precipitation drop spectrum can therefore be seen as the fingerprint of a precipitating cloud. Since clouds are basically categorized in cumulus-like and stratus-like types, precipitation is distinguished as being either convective or stratiform. When it comes to deep convection, high convective clouds and extended warm convective clouds, cloud droplet distribution, and in consequence the precipitation drop distribution, are not so easy to distinguish. Droplet development in warm convective clouds is expected to lead to an according precipitation droplet spectrum, just as stratiform precipitation is mostly expected to be observed originating from stratus clouds. Mesoscale convective systems and large deep convection clouds themselves have parts with so-called stratiform precipitation. During the observational period, a distrometer was used to determine the precipitation fingerprint of clouds passing the tropical station in La Gamba. The overall subjective impression in a tropical wet forest – that every precipitation event is of a heavy character with large drops and of short to extended duration, depending on rainy/dry seasonality, could not be verified in this observational period.

The precipitative temperature cooling during precipitation events and heavy rainfalls is also shortly discussed. One heavy downburst event was documented directly at the tropical station in La Gamba, with an associated temperature drop of 5 degrees within half an hour. The precipitative cooling is caused by melting and evaporation of hydrometeors on the way downwards. Due to the high melting level of precipitation (above 4000 m msl) only longer-lasting intense precipitation events may cause a significant cooling at the earth's surface, whereas evaporative cooling needs a certain layer in the lower troposphere, where a significant spread (difference between air temperature and dew point temperature) occurs. The generally high relative humidity in the tropical climate makes significant evaporative cooling less frequent than in drier climates.

Aside from precipitation climatology, solar radiation conditions have also received considerable interest in tropical rainforests, because the high degree of cloudiness and the dense canopy of vegetation leads to an extreme reduction of radiation, which can prohibit the germination and growth of plants. There are not many investigations on how different types of vegetation with different densities affect solar irradiance. Reforestation attempts may profit greatly from better knowledge about changing radiation conditions in the course of plant growth. Different height growth of different plant species may have a selective effect.

The basic principles of global solar radiation on the earth are shortly outlined in the following (see e.g. Landolt-Börnstein 2005): the extraterrestrial solar radiation-flux-density (solar irradiance) shows a distinct gradient on our planet. Due to the angle of inclination of the earth's axis, the average annual solar irradiance decreases from nearly 540 Wm^{-2} in the tropics to roughly 210 Wm^{-2} in the polar regions. Annual solar energy supply is often indicated instead of average irradiation: extraterrestrial 4700 $kWhm^{-2}a^{-1}$ in the tropics versus 1800 $kWhm^{-2}a^{-1}$ in the polar regions. The factor to obtain the annual solar energy supply ($kWhm^{-2}a^{-1}$) from the average solar irradiance (Wm^{-2}) is 8,766. For solar energy considerations as well as biologically relevant processes (e.g. photosynthetically active radiation), the extraterrestrial solar irradiation is only of limited theoretical value. The spectral solar irradiance is strongly affected by absorption (by atmospheric gas components, aerosol), as well as reflection and scattering processes (clouds, hydrometeors, aerosol) within the atmosphere. Due to the high degree of cloud cover in the tropics, the extraterrestrial solar irradiance is reduced by roughly 60 % to roughly 210 Wm^{-2}, i.e. the same value as the extraterrestrial irradiation in the polar regions! Only in the subtropical dry regions with low average cloudiness is the solar irradiation at the earth's surface higher, up to a global maximum of roughly 300 Wm^{-2} in the high desert regions of the Andes (Altiplano) or on the Tibetan plateau. If we compare typical average solar irradiance values for Austria (approximately between 120 Wm^{-2} and 160 Wm^{-2}) to tropical values, we still see a considerably higher value in the tropics, despite the large reduction against the extraterrestrial value due to tropical cloud cover. This means that, for solar energy production as well as for photosynthesis, there is still plenty of solar radiation available in the tropics.

The availability of low-cost, robust and easy to handle radiation sensors has opened the way for detailed investigations of differences in irradiance in different parts of a rainforest with high temporal and spatial resolution. As solar radiation is not isotropic (radiation is rather concentrated around a specific space angle), not only the radiation flux to a horizontal plane but also to vertical planes can and should be observed. This leads to a more realistic estimate of the absorptive potential of differently inclined surfaces (e.g. leaves).

The experiment carried out in the immediate area of the biological Station in La Gamba may be seen as a pilot study for more specific investigations on the radiation climate of a tropical rainforest.

One basic problem has to be pointed out here: whereas usually in climatology the "global radiation" (the complete direct solar plus diffuse sky irradiance in the solar spectrum with wavelengths between 0,3 and 3 µm) hitting a horizontal surface is measured with pyranometers, the illuminance (the part of the solar irradiance in the wavelengths perceptible for the human eye, e.g. 0,4 to 0,7 µm) is measured by LUX-meters. Some radiation instruments restrict the spectrum to the photosynthetically active radiation (PAR-meters). Not only are the physical units of the three different radiation sensors different (Wm^{-2}, LUX, µmol of photons $m^{-2}s^{-1}$), but direct conversion of the units is also not possible due to the variable spectral irradiance of the solar radiation.

Methods and Data

Precipitation

Data for precipitation evaluation was available from different measuring campaigns carried out by staff of the Meteorology department of the University of Vienna.

For the precipitation studies, ONSET (HOBO) tipping bucket devices were set up at the tropical station in La Gamba (Fig. 3), at the Finca Modelo near the tropical station at the same altitude, and at the Finca Alexis. The latter is located 16 km further east on a mountain ridge, surrounded by tropical forest at an altitude of 400 m (Fig. 4). The measurement principle of the tipping bucket is that precipitation droplets are collected in a tilt tray with a volume equivalent of 0.2 mm of precipitation water. Every tilting and the exact time of the tilting moment are recorded. In a post processing step, the rain rate and precipitation

Fig. 3: Rain gauges at the Tropical Station La Gamba (Photo: B. Eibl). – Abb. 3: Regenmesser auf der Tropenstation La Gamba (Foto: B. Eibl).

Fig. 4: Rain gauges at Finca Alexis (Photo: A. Weissenhofer). – Abb. 4: Regenmesser auf der Finca Alexis (Foto: A. Weissenhofer).

sums in different time resolutions can be calculated. Additionally, and for validation purposes, daily precipitation amounts from totalizator measurements at the tropical station, recorded by the staff of the tropical station are available. The tipping bucket measurements include a temperature sensor which is not used for proper temperature declarations but can be used for quantitative precipitation cooling effects. Temperature measurements are available for La Gamba only.

For the investigation of the droplet spectrum of different precipitation events, a THIES distrometer was installed 1 m above the laboratory's roof in the tropical station in La Gamba. When droplets fall through a laser beam emitted and reflected horizontally by the distrometer, the disturbances of the beam are measured. Since disturbances are different for every droplet, an algorithm, containing the droplet calculation of Marshall & Palmer (1948) directly calculates the droplet size and fall velocity of every droplet. Hence the droplet spectrum is directly calculated by the device, as is the precipitation amount and precipitation rate. Due to power loss during heavy precipitation events, droplet spectrum data was not available for some events with high precipitation amounts and high rain rates. Data for the precipitation droplet distribution is available during recorded events in minutely detailed resolution.

Fig. 5: Setup of the radiation sensors, one positioned horizontally (top) and four vertically in the main directions (Photo: R. Steinacker). – Abb. 5: Aufstellung der Strahlungssensoren, einen horizontal (oben) und vier vertikal, die in die Haupthimmelsrichtungen zeigen (Foto: R. Steinacker).

Radiation

There exist only a few high-quality long-term radiation measurement sites in the tropical belt of our planet. The reason for this lies in the difficulty to keep radiation instruments running under continuously moist and rainy conditions. Dew or rain droplets and dirt or insects on the protection cover may produce biased values and require more or less permanent maintenance and frequent re-calibration. Moreover, standard radiation measurements according to WMO standards (WMO 1986) should be carried out at locations with no or minimal horizontal obstruction (mostly on towers of observatories). These values cannot be directly transferred to a vegetated tropical site. Therefore, several publications deal with the possibility of estimating radiation conditions from satellite observations, or to model the radiation field by geometrical assumptions, see e.g. Etuk

& Akpabio 2002, Maracchi & Miglietta 1988, McCaskill 1990, Samani et al. 2007 or Turton 1991.

Aside from the climatological distribution of the global radiation according to WMO standards, a biological application should also consider the variability of the radiation within a vegetated zone, because plant growth usually starts on the ground below the canopy. To receive comparative values for solar radiation in different parts of the canopy, instruments were set up in the area of the biological station in La Gamba in the framework of a field study of the Institute of Meteorology and Geophysics of the University of Vienna.

The radiation sensors were mounted on a pole, roughly 1 m above the surface, positioned horizontally as well as vertically in the 4 main directions N, E, S and W (see Fig. 5). The instruments were low cost Lux-meters (ONSET Hobo Pendant Temperature/Light 8K Data Logger). They were chosen because they are suited for wet, even immersed, conditions, which is an important requirement for long-term functioning under a tropical wet climate. Four such setups with five sensors each were deployed a) in a clearing in the garden of the tropical station, b) under low vegetation (bushes), c) at the edge of dense tropical rainforest, and d) inside dense tropical rainforest.

Before the initial setup at the different locations, a calibration period of the instruments was carried out, during which all setups were positioned close together in the clearing of the garden. The relative difference of the sensors was mostly within ± 10 % of the average value of each direction and was used to correct the values of the single sensors during the whole experiment. Instead of evaluating the recorded LUX-values, all values were transferred to dimension-less relative values (in %) according to the maximum observed values (= 100 %).

Whereas the time interval of measurements was set to 1 min during the three-day calibration period, the interval during the six-month observing period was set to 10 min, so as not to exceed the capacity of the data loggers.

Results

Spatial precipitation distribution and variability

Precipitation measurements were taken at Finca Werner Klar (N8°46’50,6” W83°10’1,6”, 568 m msl), Finca Alexis (N8°45’53,0” W83°9’49,8”, 400 m msl) and at the tropical station and Finca Modelo (N8°42’1,8” W83°11’37,4”, 80 m msl) in La Gamba (N8°42’2,3”, W83°12’8,2”, 80 m msl) for different, short time periods. Due to the high variability of precipitation and the short time series, no climatological statements as to significant differences in precipitation amount between the aforementioned locations can be made. The physically explainable altitude-dependency explains some of the higher precipitation amount at Finca Alexis and Finca Werner Klar.

Figure 6 shows an evaluation of different monthly precipitation sums of the devices located at La Gamba (tropical station and Finca Modelo) and at Finca Werner Klar. Data for Finca Modelo was not available for the time span marked by the red box because the device was blocked by plant material and sheltered by a nearby tree. The hand sample was performed by staff of the tropical station. Since tipping bucket devices tend to underes-

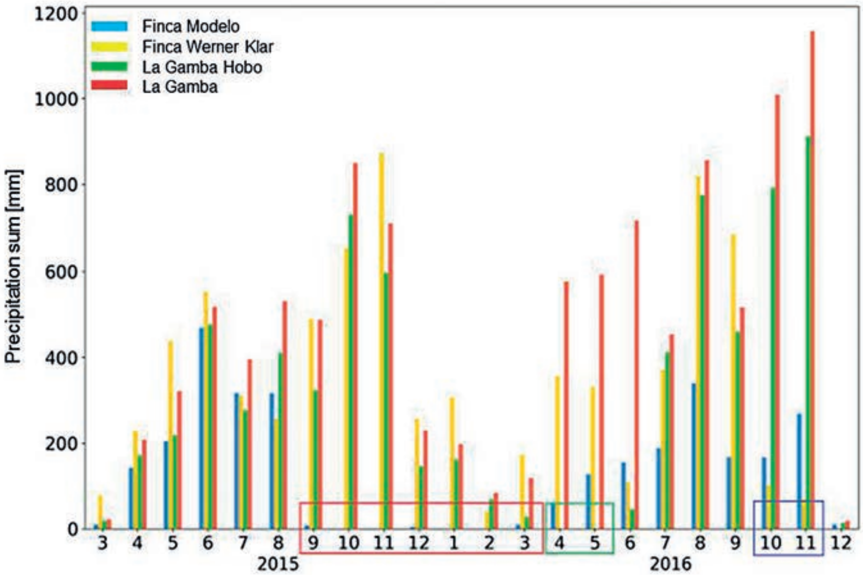

Fig. 6: Monthly precipitation sum for Finca Modelo, Finca Werner Klar and tropical station La Gamba (Bachelor's thesis, Th. Aistleitner, 2018). – Abb. 6: Monatliche Niederschlagssummen gemessen an der Finca Modelo, an der Finca Werner Klar und an der Tropenstation La Gamba. (Bachelorarbeit, Th. Aistleitner, 2018).

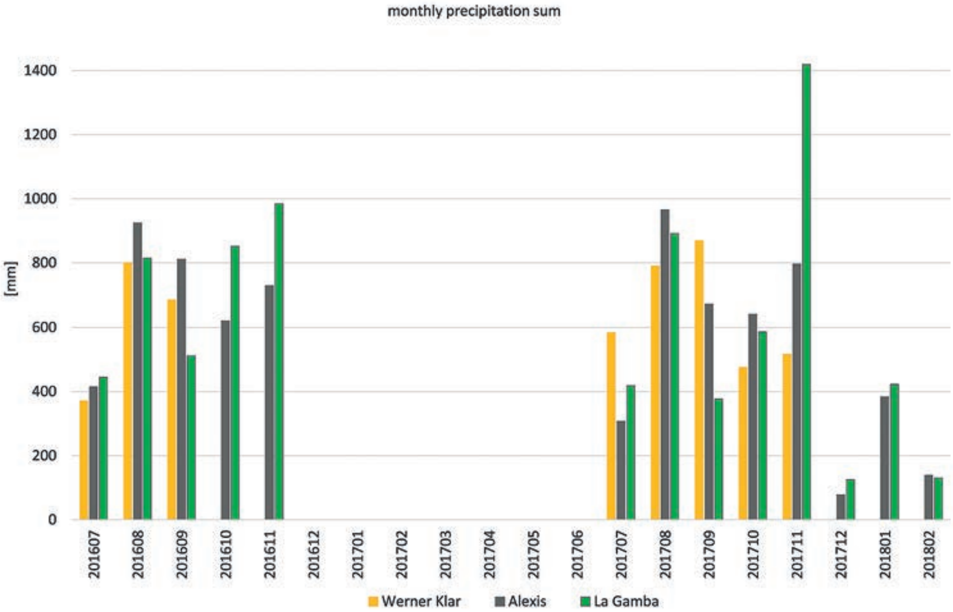

Fig. 7: Monthly precipitation sum from tipping bucket measurements at Finca Werner Klar, Finca Alexis and the tropical station La Gamba. No measurements were available from December 2016 until June 2017. – Abb. 7: Monatliche Niederschlagssummen, mit Niederschlagswippen an der Finca Werner Klar, an der Finca Alexis und an der Tropenstation La Gamba gemessen. Von Dezember 2016 bis Juni 2017 sind keine Messdaten verfügbar.

timate high precipitation rates, the monthly sums measured by the tipping bucket at La Gamba station (green) are lower than the hand samples (red). The high spatial variability of the precipitation events and the short observation time do not allow for a significant conclusion as to whether the precipitation amount was higher at Finca Werner Klar at 600 m height or La Gamba at 80 m. There are months where the sum is higher at the elevated Finca (yellow), for example in May, June and December 2015, and vice-versa in April and May of 2016.

In Figure 7, monthly precipitation sums for Finca Werner Klar, Finca Alexis and the tropical station for July until November 2016 and from July 2017 until February 2018 are shown. No significantly higher precipitation amount for a single location can be ascertained. For some months, precipitation sums are highest at Finca Werner Klar (July, September 2017), while in August 2017 and 2018 precipitation sums are highest at Finca Alexis. In October and November 2017, as well as in November 2018, the highest precipitation amount was observed at the tropical station. For a climatological conclusion as to systematically higher precipitation sums in any one location, longer time series would have to be collected.

Precipitation cooling effect

During heavy rain showers, a significant temperature drop due to evaporative and melting energy loss of the surrounding air can be observed.

As shown in Figure 8, within a precipitation event that did not last longer than 10 minutes, a temperature drop of nearly three degrees was observed. Two sensors, one within a tip-

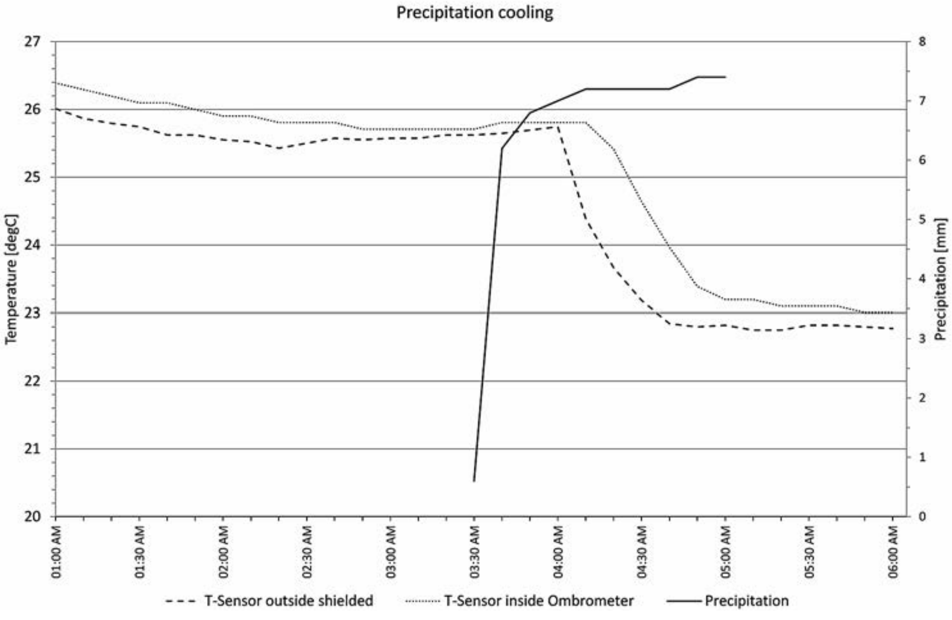

Fig. 8: Precipitation cooling during a short but heavy precipitation event at the tropical station in La Gamba. - Abb. 8: Niederschlagsabkühlung während eines kurzen aber heftigen Regenschauers an der Tropenstation La Gamba.

ping bucket and the other one in a radiation shield outside the bucket, were compared. The temperature was nearly equal before the precipitation event. Shortly after the beginning of the precipitation event, the temperature drop began. With decrease of the precipitation rate, the temperature stops declining. This phenomenon is not restricted to the tropical zone and can be observed at higher latitudes as well, in cases of heavy precipitation. But the high recurrence in the tropics brings an additional cooling effect to the nightly radiation cooling on a regular basis.

Droplet size distribution differences

Analysis of droplet distribution from different precipitation events revealed that, for heavy deep convective precipitation, droplet distribution was broader and droplet density per minute was much higher than the distribution from precipitation originating from stratiform parts of convective clouds. At several precipitation events in July 2018, distrometer measurements were performed and satellite images (IR and VIS) were simultaneously studied to ascertain whether deep or low convection was prevalent.

Examples of stratiform precipitation droplet distribution are shown in Figures 9–11. It can be seen that droplet number per minute can vary, but the variation is slight. The raindrops nearly have the same diameter with a maximum at 0.125 mm in Figures 10 and 11. This is due to the lower collision rate between the droplets during precipitation drop development

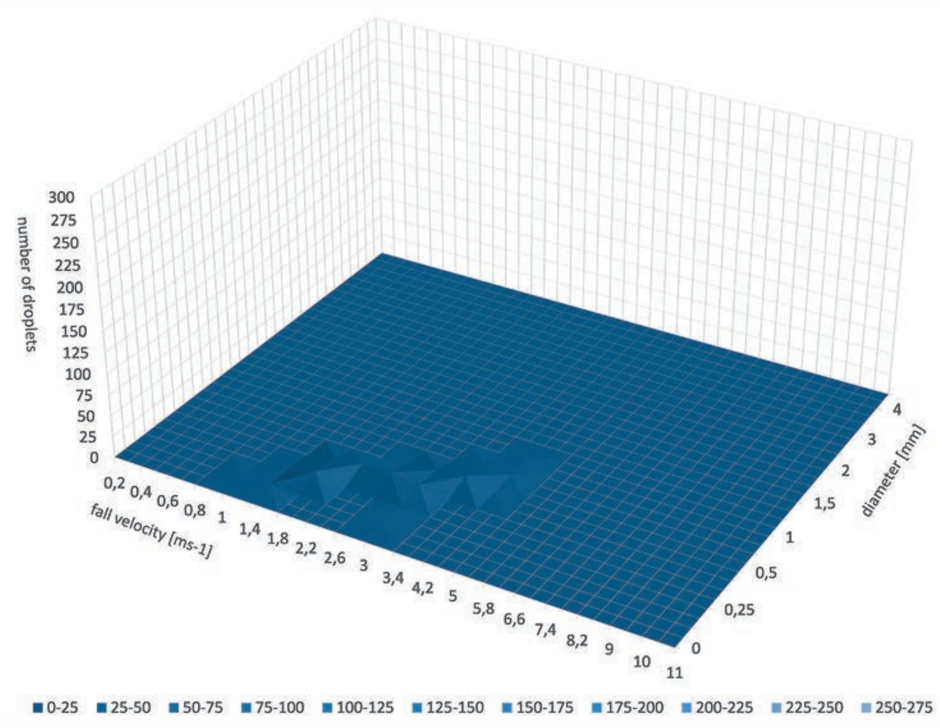

Fig. 9: Precipitation droplet distribution for 1 minute of precipitation within a stratiform precipitation event on 10 July 2018. – Abb. 9: Niederschlagströpfchenverteilung der einminütigen Niederschlagssumme während eines stratiformen Niederschlagsereignisses am 10. Juli 2018.

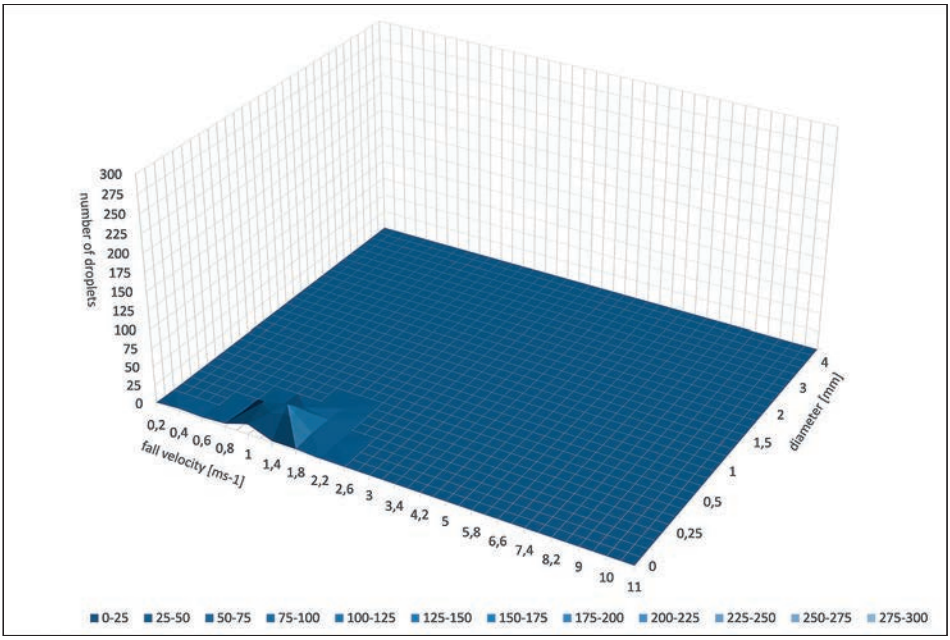

Fig. 10: Precipitation droplet distribution with an amount of 111 droplets per minute during a stratiform precipitation event. – Abb. 10: Niederschlagströpfchenverteilung von 111 Regentropfen, die innerhalb 1 Minute während eines stratiformen Niederschlagsereignisses gefallen sind.

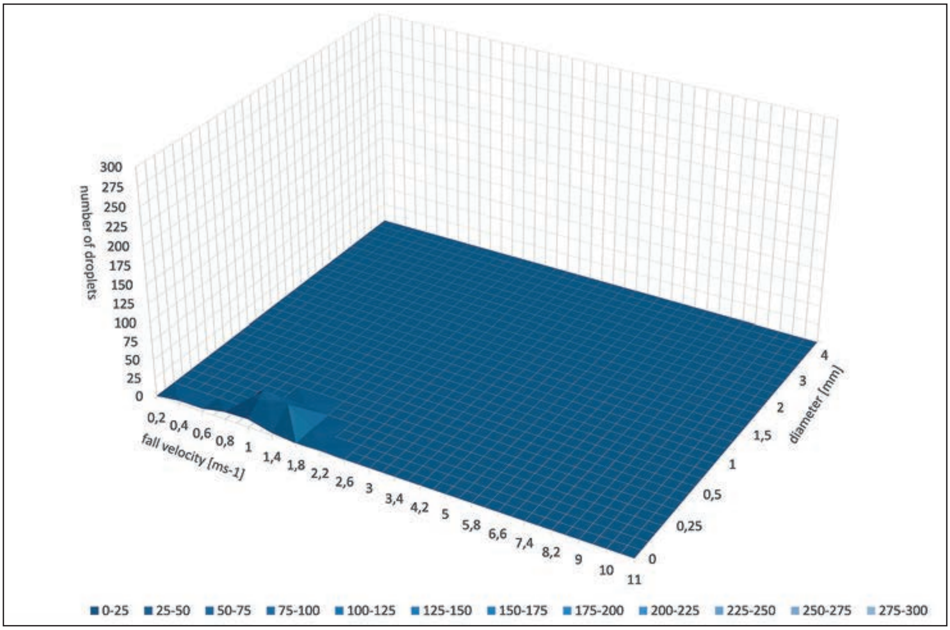

Fig. 11: Precipitation droplet distribution with an amount of 92 droplets per minute during a stratiform precipitation event. – Abb. 11: Niederschlagströpfchenverteilung von 92 Regentropfen, die innerhalb 1 Minute während eines stratiformen Niederschlagsereignisses gefallen sind.

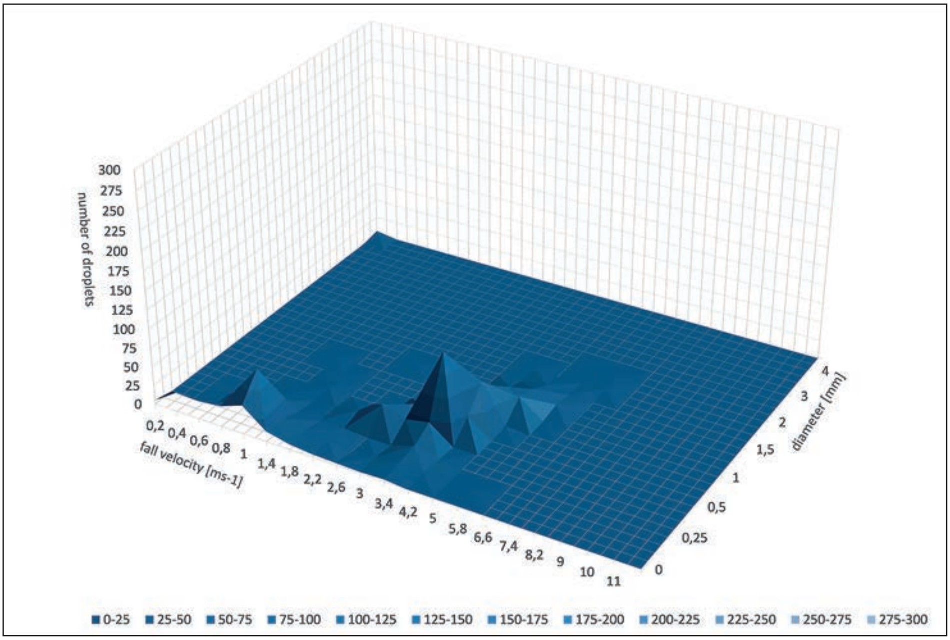

Fig. 12: Precipitation droplet spectrum with an amount of 721 droplets per minute during a convective precipitation event. – Abb. 12: Niederschlagströpfchenverteilung von 721 Regentropfen, die innerhalb 1 Minute während eines konvektiven Niederschlagsereignisses gefallen sind.

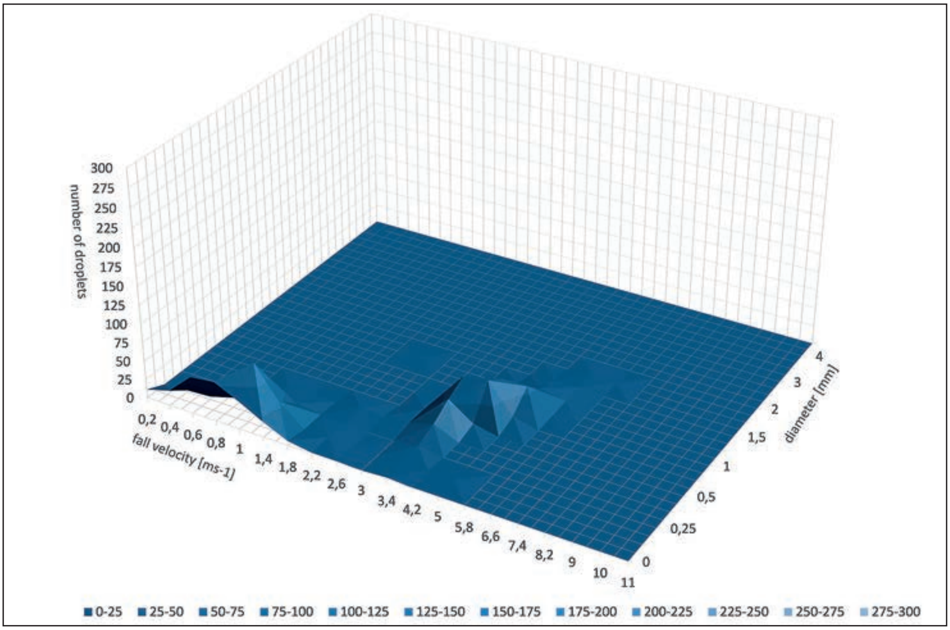

Fig. 13: Precipitation droplet spectrum with an amount of 630 droplets per minute during a convective precipitation event. – Abb. 13: Niederschlagströpfchenverteilung von 630 Regentropfen, die innerhalb 1 Minute während eines konvektiven Niederschlagsereignisses gefallen sind.

in stratiform clouds. The updrafts are not as high as in deep convective clouds, where it is more likely for droplets to collide with each other when higher up- and downdrafts are present within the cloud.

For precipitation originating in deep convective clouds, the droplet distribution was rather broad with two maxima, as is shown in Figures 12 and 13. The droplet distribution in Figure 12 with a total of 721 droplets per minute shows a maximum of faster rain drops of 1 mm diameter. The total number of droplets is the result of summing up all droplets at every fall velocity and diameter. In Figure 13, the maximum with smaller droplet diameters was at 0.25 mm. A second maximum with much faster drops was at 1 mm diameter. Both figures show that the rain was heavier and the rain rate higher, as more droplets per minute came down in comparison to the example of stratiform precipitation (Fig. 10). Droplets were only a little larger than the stratiform droplets, but were falling faster, accelerated by the downdraft of a deep convective cloud. For heavy tropical rain events, droplets in this example were rather small. Due to power loss during heavy rainfalls with large raindrops, it was not possible to capture a droplet distribution with droplet diameters larger than 3.5 mm.

Figure 14 shows the distribution of 1726 droplets per minute, most of which were under 1 mm in diameter and had a small fall velocity. Faster and slightly larger droplets fell at the same time, as can be seen in a smaller and broader maximum of the distribution in Figure 14.

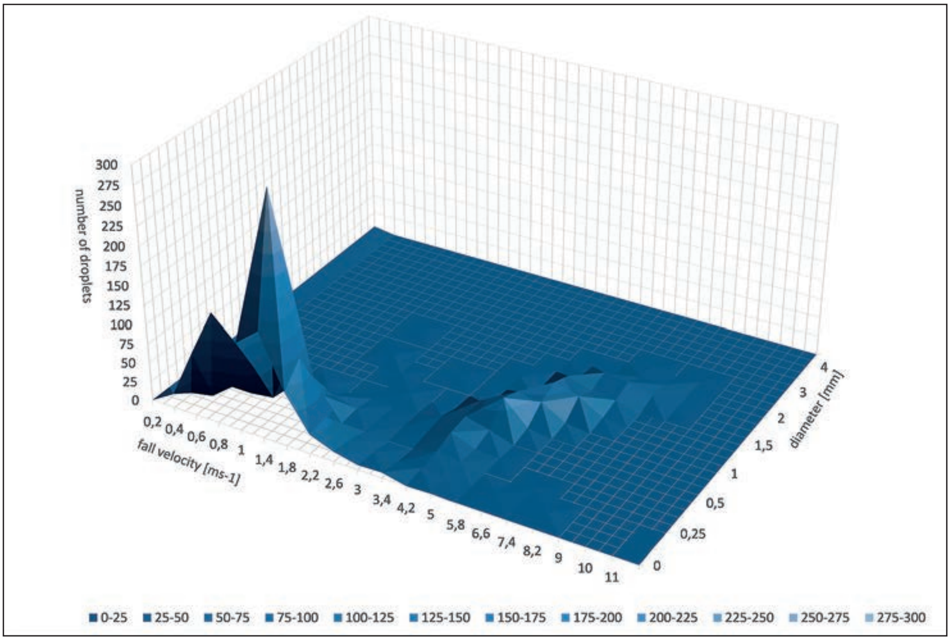

Fig. 14: Precipitation droplet spectrum with an amount of 1726 droplets per minute during a convective precipitation event. – Abb. 14: Niederschlagströpfchenverteilung von 1726 Regentropfen, die innerhalb 1 Minute während eines konvektiven Niederschlagsereignisses gefallen sind.

Radiation conditions

The radiation conditions were analyzed with regard to orientation.

The plot for the horizontal plane is shown in Figure 15. It is interesting to note that, despite the location of La Gamba being close to the equator, a definite annual cycle is visible. Around 1 September the sun is at its zenith at noon and around 21 December the sun is only at an elevation of 68 degrees at noon. Hence the average daily global radiation in the clearing on a horizontal plane is significantly lower (by roughly a factor of three!) in December as compared to September. One has to consider, however, that the variation is not only explained geometrically but also that the average cloudiness differs from month to month. The variation between the maximum and minimum daily value in the whole period is roughly a factor of 10! The values for the edge of the rainforest and the bushes are quite similar, however the annual cycle seems to be different due to different shading. As could be expected, the lowest average values were observed in the rainforest location. There, the average global radiation was roughly 10 % of the clearing site. The ratio varies considerably, which is an indication that even on the ground beneath a rainforest canopy there are some angles where more (direct solar) radiation can penetrate. An upward-facing fish-eye photograph could easily prove this hypothesis (Turton 1991). The day with the absolute minimum in the rainforest was only 1 % of the maximum value in the clearing. The day to day variability was also considerable. On 19 November, a distinct minimum was recorded on a day with continuous rain and deep cloud layers. The global radiation was only about 10 % of the day with the maximum global radiation on a sunny day at the end of September. It is interesting to note that the ratio of roughly 10 between the maximum and minimum daily global radiation is rather constant for all locations and all orientations. This means that the ratio must be the same for the direct as well as the diffuse part of the global radiation.

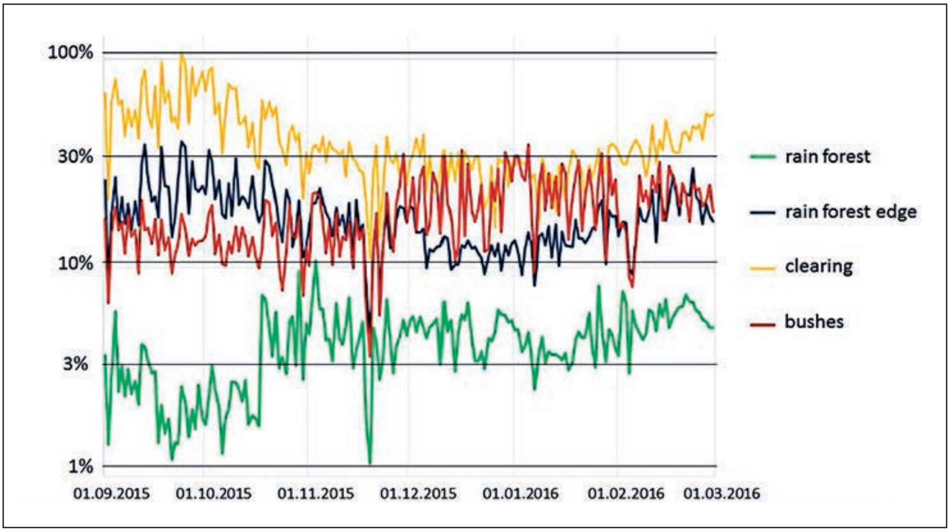

Fig. 15: Daily average global radiation at a horizontal surface in relative units on a logarithmic scale. – Abb. 15: Tägliche mittlere Globalstrahlung an einer horizontalen Fläche in relativen Einheiten, auf einer logarithmischen Skala.

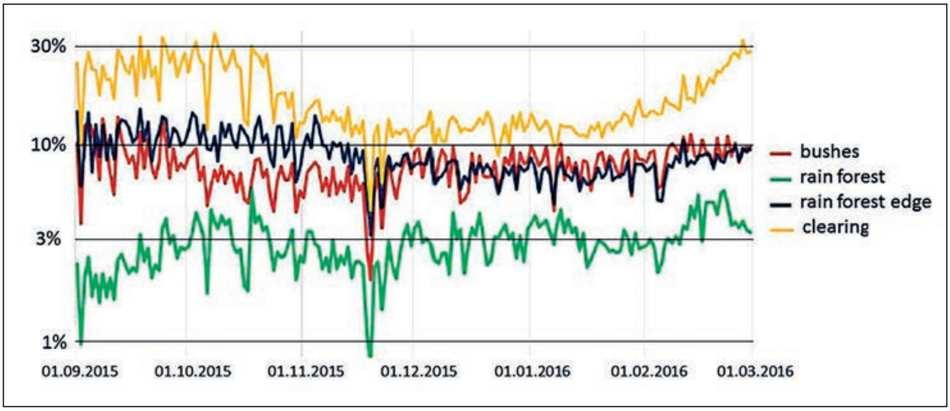

Fig. 16: Daily average global radiation at a vertical surface, facing East, in relative units on a logarithmic scale. – Abb. 16: Tägliche mittlere Globalstrahlung an einer vertikalen Fläche, nach Ost ausgerichtet, in relativen Einheiten, auf einer logarithmischen Skala.

If we compare the average global radiation on a vertical plane, facing East (Fig. 16), in the clearing a reduction by roughly a factor of 3 is apparent. The East-facing plane in the rainforest, however, shows much less reduction, which means that the tiny spots where the direct radiation comes through the canopy are not only found around the zenith, but also at lower angles. Hence, under the canopy, the orientation becomes less important than in a clearing. The results for the other directions of vertical planes (not shown), confirm the findings of Figure 16. The South-facing surface shows a somewhat different behavior concerning the annual cycle. In this case, in winter, when the solar elevation angle is lowest, no minimum like with all other orientations was observed, because the South-facing plane receives more direct solar radiation at this time.

Discussion

For long-term climate assertions, at least 30 years of observational data are required. When it comes to the tropics, especially, simply setting up high-quality devices for data collection is by far not enough. Since only long-distance measurements without regular maintenance of the instrumentation were manageable from our side, a proper timeline of meteorological parameters is pending. The higher the variability of a weather phenomenon, the longer the timeline of observation has to be to reach a significant conclusion. This is even more important if different sites are compared and a systematic difference is to be deduced. The long-term precipitation measurement provided by the tropical station is the most robust available for the moment.

Measuring extreme weather phenomena is always challenging. Instrumentation is generally built for the most likely range it will be operating in. What we learned from this investigation was that heavy precipitation rates made the distrometer automatically record those events as failures. The reason is that the device is not able to dissolve rainfall if more than one droplet falls through the laser beam at the exact same time, which is the case when it comes to very heavy rainfall.

To reach a more complete picture of the precipitation droplet distributions, an additional observation period with a distrometer should definitely be conducted in October or November in this same region, when heavy convective precipitation events are more frequent and huge amounts of rain are measured. But even then the bottle up effects on the different mountain ranges would lead to the presumption that several spectra would be of stratiform character.

One interesting experiment could focus on the change of radiation conditions during a reforestation period, while another could investigate the impact of topography, slope inclination, orientation, altitude, etc. on the specific radiation conditions.

Concerning solar radiation, the field study pointed towards the importance of taking into account the spatial variability of global radiation due to the vegetation canopy. As the vegetation is not a constant, the investigation of the feedback mechanism between the radiation conditions and the vegetation dynamics would be an interesting field of research. Long term investigations are quite difficult to perform under humid tropical conditions. Therefore, a different approach would appear promising. Instead of placing several instruments in different vegetation zones, one could profit from mobile equipment making radiation measurements along fixed transects in the rainforest. Especially the profiling of an area of reforestation could give interesting insights into the vegetation dynamics. Measurements along such transects could be carried out in certain intervals or on a selection of days (sunny, rainy, etc.) during the annual cycle extended over years without too much personal and technical effort. It is clear, that we can still learn a lot more about the relation and feedback mechanism between radiation and vegetation growth in the tropics.

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Diversity and composition of tropical forest plant communities in the Golfo Dulce region

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The Golfo Dulce region located in southwestern Costa Rica represents one of the remnant global biodiversity hotspots containing the largest Pacific lowland tropical forest in Central America. Recent biogeographical analyses revealed a close relation of taxonomic tree species composition to northern South America. However, the underlying factors leading to the extraordinary taxonomic richness of the region have so far remained elusive. In this study, we analyzed the composition of tropical forest plant communities (trees, lianas and palms with a diameter at breast height ≥10 cm) occurring in one-hectare permanent forest inventory plots. To that end, we established 20 forest sites across the Golfo Dulce region, i.e. 5 geographic locations each comprising 4 forest habitat types in ridge, slope and ravine positions, as well as in secondary forest stands. Our study highlights the oligarchic dominance of tropical forest plant communities in the Golfo Dulce region, and further suggests that plant species composition is associated with topography, disturbance history and edaphic properties. The finding that oligarchic and rare species are affected by environmental filtering has important implications for assessment and modeling of important ecosystem functions, such as carbon storage and productivity, as well as nutrient cycling and trophic interactions and thus could potentially improve biodiversity conservation and management strategies.

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Die Golfo Dulce-Region im Südwesten von Costa Rica ist einer der letzten globalen Biodiversitäts-Hotspots mit dem größten verbliebenen tropischen Wald im pazifischen Tiefland in Mittelamerika. Jüngste biogeografische Analysen der taxonomischen Baumartenzusammensetzung zeigten eine enge Beziehung zu den Pflanzengesellschaften Südamerikas. Die zugrundeliegenden Faktoren, die zu dem außerordentlichen taxonomischen Reichtum der Region führten, sind jedoch bislang schwer fassbar. In dieser Studie haben wir die Zusammensetzung der tropischen Vegetation (Bäume, Lianen und Palmen mit einem Durchmesser in Brusthöhe ≥ 10 cm) auf 20 Inventarflächen von jeweils 1ha Größe (5 geografische Standorte mit jeweils 4 Habitatstypen in Kamm-, Hang- und Schlucht- Position, sowie einem Sekundärwald) analysiert. Unsere Studie beschreibt die Dominanz häufiger tropischer Pflanzenarten und legt nahe, dass die Zusammensetzung der Vegetation mit der Topographie, der Störungsgeschichte und den edaphischen Eigenschaften zusammenhängt. Die Feststellung, dass die Zusammensetzung von sowohl häufig vorkommenden als auch eher seltenen, Großteils endemischen Arten von denselben Umweltfaktoren beeinflusst wird, birgt interessante Erkenntnisse über wichtige Ökosystemfunktionen wie die Kohlenstoffspeicherung und die Produktivität des Ökosystems und könnte somit helfen Strategien zur Erhaltung und Bewirtschaftung der Artenvielfalt in tropischen Ökosystemen zu entwickeln.

Keywords: Biodiversity, biogeography, Costa Rica, Golfo Dulce, tropical plant communities.

Introduction

Tropical forest ecosystems typically persist on strongly weathered nutrient-poor soils but comprise high species richness and store large amounts of carbon. Hence, tropical forest ecosystems provide crucial ecosystem functions, such as carbon sequestration from the atmosphere via plant carbon metabolism (Dusenge et al. 2018). However, despite their global importance in mitigating global warming, tropical ecosystems are still increasingly exploited by human activities, such as deforestation and logging, to support the increasing demands of global populations (Barlow et al. 2018). It is therefore of the utmost importance to understand how these important ecosystems function in order to derive and implement management plans that succeed in maintaining crucial ecosystem processes under projected future scenarios, while concomitantly providing ecosystem functions, such as continuous extraction of food and timber under realistic socioeconomic pathways.

Tropical tree communities are characterized by high species richness, often surpassing 100 species per hectare (Valencia et al. 1999). What drives this exuberant diversity has been a matter of scientific debate, but in general the number of co-occurring species depends on the number of available niches determined by local environmental factors, such as climate, parent material, topography and soil type (Jucker et al. 2018). A structurally diverse environment was found to increase the available niche space and thereby enable a high diversity of co-occurring biota (Figueiredo et al. 2018). As a result, distinct floristic communities with distinct functional properties may emerge on different parent material and soil types (Prada & Stevenson 2016). Both species diversity and taxonomic composition of tropical plant communities might therefore differ in association with geological substrate and topoedaphic factors by affecting the availability of resources, such as light, water and nutrients along environmental gradients.

Topography strongly influences local-scale variability in soil chemistry, hydrology and microclimate (Werner & Homeier 2015, Jucker et al. 2018) thus being one of the main factors determining species-habitat associations in tropical forests (Zuleta et al. 2018). Due to subtle differences in elevation controlling soil chemistry and hydrology, such environmental filtering was found to profoundly influence forest-level patterns of community assembly by affecting diversity and composition of tropical tree communities (Jucker et al. 2018). Furthermore, more diverse communities should have the potential to exploit available resources more efficiently due to niche complementarity and positive species interactions (Tilman 1999). Therefore, it has been proposed that biodiversity positively affects ecosystem functioning in hyperdiverse tropical forests (Poorter et al. 2015). However, because a high proportion of biodiversity in tropical regions stems from closely related species, species diversity is not proportional to functional diversity (Safi et al. 2011), but higher levels of functional diversity imply larger numbers of functional types and therefore a greater breadth of functional response-spectra to climatic signals. Hence, it might not necessarily be species diversity of a location but rather species composition in a given environment, and particularly the functional composition of a given community, that will determine ecosystem functioning under future scenarios of projected environmental changes.

In this case study we examined tropical tree communities growing on nutrient-poor soils in a topographically heterogeneous environment located in southwestern Costa Rica. The study region is located in the largest remaining tropical rainforest in the Pacific lowlands of Central America (Gilbert et al. 2016), and constitutes one of the remnant global biodiversity hotspots. The region therefore is especially suited for studying biodiversity and species composition of tropical plant communities. We compiled a unique dataset comprising 10,007 individuals and 485 species recorded in twenty one-hectare permanent inventory plots established across five sites (differing in climate, parent material and soil type), each one comprising four forest habitat types (differing in topographic position and disturbance regime). Based on this set-up we investigated the following hypotheses: (1) Plant species richness varies between sites, along spatial gradients in climate, soil parent material, landform and soil types; (2) plant species composition varies between forest habitat types, in association with local topography and disturbance regime and thus; (3) the abundance of hyperdominant plant species varies in relation to habitat preference among co-occurring plant species distributed across the Golfo Dulce region.

Material and Methods

The study was conducted in tropical lowland forests located between 50 and 450 m a.s.l. in the Área de Conservación Osa (ACOSA) at the Pacific slope of southwestern Costa Rica (08.6°N, 83.2°W). The region is considered a "hot spot" of biodiversity with 700 tree species among 2,369 species of ferns, fern allies and flowering plants recorded in total (Quesada et al. 1997). The terrain is characterized by parent material originating from the Cretaceous, Tertiary and Quaternary (i.e. basalt, alluvium and sediment) and is divided into six different landforms (i.e. denudational, volcanic, alluvial, structural, littoral, tectonic) and four soil orders, i.e. Entisols, Inceptisols, Mollisols and Ultisols (Lobo 2016). The dominating, highly weathered, strongly acidic Ultisols on ridges and upper slopes are replaced by younger, moderately weathered Inceptisols in ravines and lower slopes and little-developed Mollisols in fluvial deposits (Lobo 2016). Daily climatologic data for temperature and precipitation (starting in 1997) are available from La Gamba field station; https://www.lagamba.at/en/tropical-field-station/scientific-data-of-the-golfo-dulce-region/. Mean annual precipitation for the period 1998–2017 accounted for 5892 mm with no month receiving less than 180 mm on average. The rainy season lasts from April to December, the driest months are January to March. Mean annual temperature for the period 1998–2017 was 28.0° C and ranged between 23.7° C and 33.7° C.

Study plot locations were selected based on a stratified sampling design (Clark & Clark 2000), i.e. the set of ecosystem types to be studied was pre-selected upon a thorough evaluation of the relative spatial contribution of different ecosystem types in the ACOSA region. Dominant regional ecosystem types are broad-leaved evergreen well-drained lowland forests (38.3 %), dense tropical evergreen well-drained lowland (woody) herbland dominated by graminoids (pastures) (22.0 %) and dense tropical broad-leaved evergreen well-drained lowland shrubland with early to late successional re-growth (9.7 %). The old-growth lowland forests stock on narrow ridges (3.2 %), steep slopes (94.0 %) and valleys (2.8 %) passed by streams and small rivers (Weissenhofer et al. 2008). To account for the spatial variability of climate, parent material and soil types, permanent forest inventory plots were installed in replicates across five sites, i.e. La Gamba (LG), Riyito (RY), Agua Buena (AB), Rancho Quemado (RQ), and Piro (PR), each comprising four plots: three plots of old-growth forest in habitat types differing in topographic position, i.e. forest stands in hilltop (ridge), intermediate (slope) and valley bottom (ravine) positions and one plot in secondary re-growth forest stands. Plots were of 1-ha in size and subdivided into subplots of 10 × 10 m following the standards of Alder & Synnott (1992). Plot shape was adapted to the physiography of the terrain, ranging from regular (100 × 100 m) to irregular shapes, especially in the case of ravine forest plots where subplots were situated along the small streams and adjacent terraces. For further information and an interactive map showing respective locations of forest plots including numbers of surveyed tree individuals and identified taxonomic species please visit http://www.univie.ac.at/bdef/.

Plant species were identified in the field (Eduardo Chacon) and, where direct ID in the field was not possible, samples were collected for taxonomic identification and deposited in the herbarium of the Biology School of the University of Costa Rica (USJ, acronym). The number of species analyzed in this study was 485 species of trees, palms and lianas from a total of 11,786 individuals with a diameter at breast height ≥ 10 cm, of which only 11,514 represented live trees, 272 dead trees, and around 86 % were identified to species level (96 % at genus level), thus representing 10,007 individuals analyzed subsequently. Plot survey and data collection involved all live stems from individuals with a diameter above breast height larger than 10 cm (DBH ≥10 cm at 1.3 m), thus representing 485 species, 280 genera and 77 families of trees, palms and lianas recorded in the 2012–2015 census interval. To compare the diversity of species between locality and forest habitat types we used rarefaction analysis of Hill numbers based on the abundance of species (Hill 1973). We estimated the diversity of Hill numbers of order 0, 1 and 2, which represent the richness (number of species), the effective number of rare species and the effective number of abundant species (Hsieh et al. 2016), respectively. For each forest site we estimated the

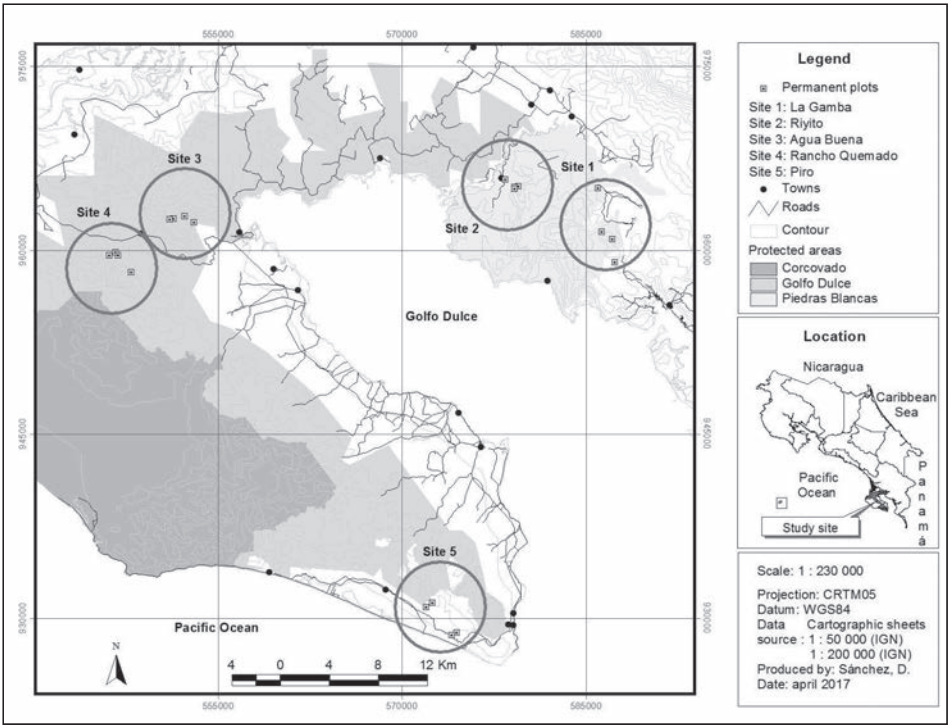

Fig. 1: Map of the study region showing the distribution of permanent plots surveyed per geographic location i.e. Site 1: La Gamba; Site 2: Riyito; Site 3: Agua Buena; Site 4: Rancho Quemado; Site 5: Piro, situated across the Golfo Dulce region in southwestern Costa Rica. – Abb. 1: Karte des Untersuchungsgebiets in der Golfo Dulce Region im Südwesten von Costa Rica inkl. Verteilung der geografischen Standorte, d. H. Standort 1: La Gamba; Standort 2: Riyito; Standort 3: Agua Buena; Standort 4: Rancho Quemado; Standort 5: Piro.

diversity by extrapolation using rarefaction analysis to 3,500 individuals per site and for forest habitat type we estimated the richness by extrapolation using rarefaction analysis to 1,000 individuals for each forest habitat type in each site. Variation in species composition among forest plots was assessed using hierarchical cluster analysis and non-metric multidimensional scaling (NMDS) based on a double-standardized, square root-transformed species abundance Bray–Curtis dissimilarity matrix. To assess the relationships between tropical forest plant community composition, environmental variables and geographic distance we correlated the respective matrices of species composition, environmental variation and a matrix of three-dimensional Euclidean geographic distances calculated from GPS coordinates. Based on these parameters we performed variation partitioning among factors controlling variation of plant community composition (i.e. beta diversity) among twenty 1-ha permanent inventory plots established in the Golfo Dulce region, Costa Rica (Fig. 1).

Results and Discussion

Species richness (alpha diversity) of rare and abundant tropical plants

Tropical forests are characterized by extraordinary species richness, however, less is known about variation in species richness between rare and abundant species. We found that alpha diversity (species richness) varied throughout the study region (Fig. 1). However, while some regions, such as Rancho Quemado and Agua Buena, had high species richness with over 300 estimated species, other regions, such as Piro, hardly reached 200 estimated species based on extrapolation using rarefaction analysis (Fig. 2). Since all sites showed similar diversity of abundant species, the variation in alpha diversity was mainly due to differences in the number of rare species (Fig. 2). Within each region, plots in

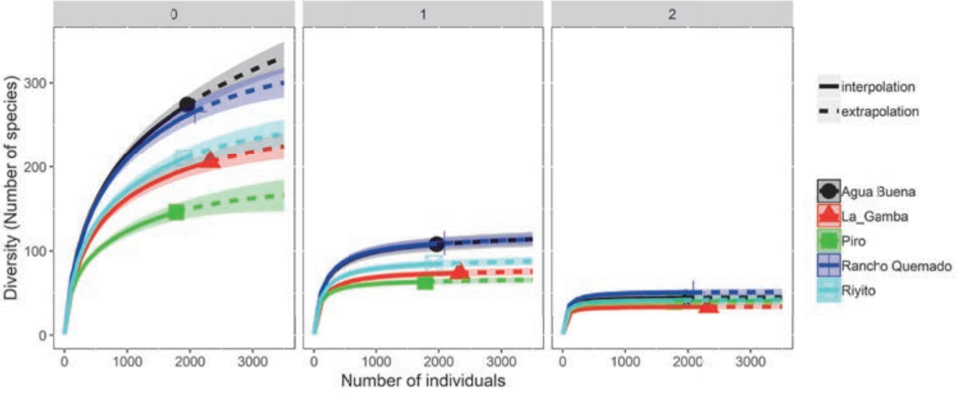

Fig. 2: Rarefaction analysis using Hill's numbers (0, 1, and 2) of the tree species by locality. The Hill's number 0 represents the species richness, the Hill's number 1 represents the effective number of rare species and the Hill's number 2 represents the effective number of abundant species. The solid lines represent the interpolation with the observed data and the dotted lines represent the extrapolation to 3,500 individuals. Shaded areas represent 95 % confidence intervals. – Abb. 2: Rarefaction-Analyse unter Verwendung der Hill-Zahlen (0, 1 und 2). Die Zahl 0 steht für den Artenreichtum der Baumarten nach Lokalität, die Zahl 1 für die effektive Anzahl seltener Arten und die Zahl 2 für die effektive Anzahl häufig vorkommender Arten. Die durchgezogenen Linien repräsentieren die Interpolation mit den beobachteten Daten und die gepunkteten Linien repräsentieren die Extrapolation auf 3.500 Individuen. Schattierte Bereiche repräsentieren 95 % Konfidenzintervalle.

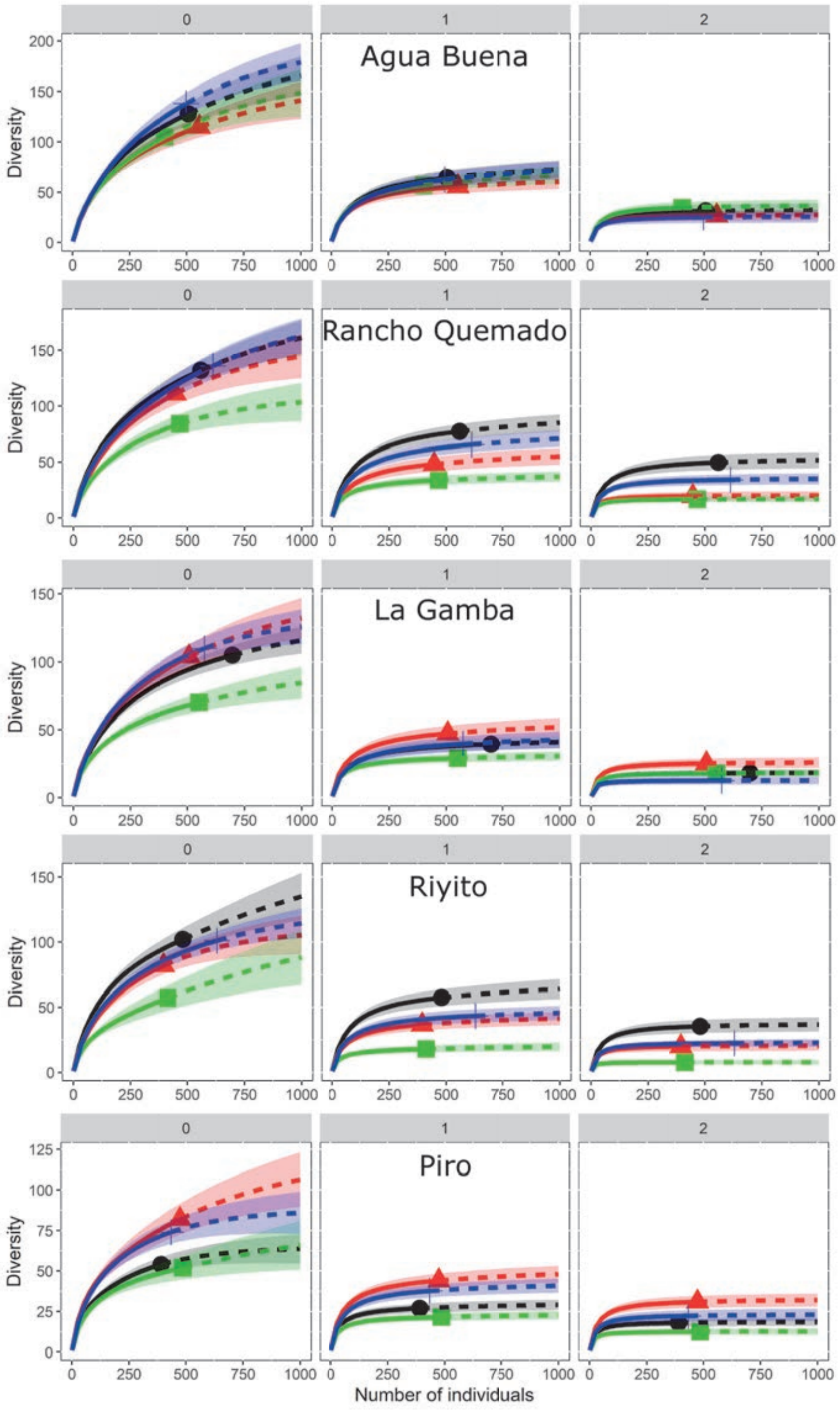

Fig. 3: Rarefaction analysis using Hill's numbers (0, 1, and 2) of the tree species by forest habitat type and locality. The Hill's number 0 represents the species richness, the Hill's number 1 represents the effective number of rare species and the Hill's number 2 represents the effective number of abundant species. The solid lines represent the interpolation with the observed data and the dotted lines represent the extrapolation to 3,500 individuals. Shaded areas represent 95 % confidence intervals. – Abb. 3: Rarefaction-Analyse unter Verwendung der Hill-Zahlen (0, 1 und 2). Die Zahl 0 steht für den Artenreichtum der Baumarten nach Lebensraumtyp und Standort, die Zahl 1 für die effektive Anzahl seltener Arten und die Zahl 2 für die effektive Anzahl häufig vorkommender Arten. Die durchgezogenen Linien repräsentieren die Interpolation mit den beobachteten Daten und die gepunkteten Linien repräsentieren die Extrapolation auf 3.500 Individuen. Schattierte Bereiche repräsentieren 95 % Konfidenzintervalle.

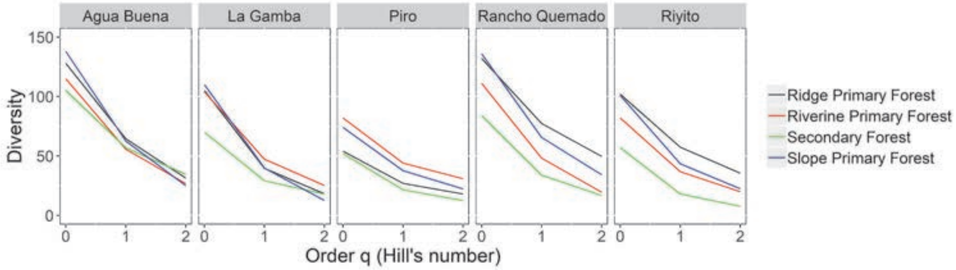

Fig. 4: Diversity of tree species observed for each Hill‘s number (0, 1, 2) in each locality and for each forest habitat type. The Hill‘s number 0 represents the species richness, the Hill‘s number 1 represents the effective number of rare species and the Hill‘s number 2 represents the effective number of abundant species. – Abb. 4: Diversität der Baumarten für die jeweilige Hill-Zahl (0, 1, und 2). Die Zahl 0 steht für den Artenreichtum der am jeweiligen Ort und für den jeweiligen Lebensraumtyp beobachtet wurden, die Zahl 1 für die effektive Anzahl seltener Arten und die Zahl 2 für die effektive Anzahl häufig vorkommender Arten.

secondary forest stands exhibited lower species diversity compared to old-growth forest plots (Fig. 3). However, this difference was less pronounced when only abundant species were considered (Fig. 3). Among old-growth forest stands within each region, there was no clear pattern of a particular forest habitat type association; however, the contribution of rare species to overall species diversity was higher for old-growth forests in slope position and lower for secondary forest stands (Fig. 4). The low alpha diversity and low contribution of rare species in more disturbed re-growth forest stands is likely a result of high colonization rates of opportunistic fast-growing species, taking advantage of high-light environments and thus attaining relatively high abundances. In contrast, high contribution of rare species to overall diversity in old-growth forests (especially in slope position) likely results from the fact that slope forests are situated between uphill ridges and downslope ravines, thus differing in edaphic properties and taxonomic species composition. Similar associations between rare species and edaphic conditions have been previously reported for other tropical regions (Laurance et al. 2010). Hence, our results indicate that the contribution of rare and abundant species to alpha diversity is affected by geological, topographic, and edaphic factors, as well as natural and anthropogenic disturbance regimes that in turn shape the distribution of species and determine the heterogeneity of habitats in the Golfo Dulce region.

Species composition (beta diversity) among forest plots

In tropical forest ecosystems the diversity of species assemblages across space and time has been explained by various theories (Hubbell 1979, Tilman 1999). Nonetheless, due to the spatial dependence of factors controlling plant species composition along environmental gradients, it might be impossible to separate contrasting theories explaining the high level of species co-existence in tropical forests (Wright 2002, Chave 2004, Chisholm et al. 2013, Chase 2014). While on a larger spatial scale intraspecific variability and functional equivalence of tropical plant species would indicate that species richness is maintained as a balance between immigration and extinction of species (Hubbell 1979, Garzon-Lopez et al. 2014), on a smaller local-scale specialization of species to particular environmental conditions may better explain the co-existence and distribution of tropical tree communities (Brown et al. 2013). Despite the fact that these theories emphasize different processes contributing to the maintenance of species diversity at the landscape-scale (Qiao et al. 2015), we found both significant effects of spatial distance and environmental factors (i.e. climate, parent material, soil type) on plant species composition across nearby lowland forest plots (Fig. 5). Our results suggest that both biotic and abiotic factors shape floristic community composition at the landscape-scale. Although residual variation was high (66 % unexplained variance), environmental factors, such as climate (4 %), soil type (6 %), parent material (11 %), and spatial autocorrelation (13 %) explained up to one third of the variation in species composition among lowland forest stands (Fig. 5). Similar levels of spatial autocorrelation have been observed by other authors in foregoing studies and have been attributed to multiple processes regulating forest structure and plant species composition across environmental gradients at the landscape-scale (e.g. Taylor et al. 2015). Furthermore, Prada & Stevenson (2016) reported that distinct floristic communities emerged on different parent materials and soil types, such that taxonomic species composition of tropical tree communities differed in association with topoedaphic factors. Based on differences in geological substrates and soil types described across the Golfo Dulce region, we hypothesized that floristic community composition varies in relation to regional differences in geomorphology, topography and climate. Indeed, we found that each region, i.e. La Gamba, Riyito, Agua Buena, Rancho Quemado and Piro, exhibited its unique floristic community composition. For instance, whereas La Gamba was characterized by a high abundance of palms (33 % Arecaceae), Piro exhibited only a very small proportion of palm species (4 % Arecaceae) but much higher percentage of tree species (Fig. 6). Hence, our findings of regionally variable taxonomic composition might indicate that both large-scale abiotic factors (i.e. geomorphology, topography, climate) as well as local biotic processes (i.e. competition, dispersal, stochastic events of colonization and extinction) influence taxonomic community composition at the landscape-scale (Condit et al. 2002) and thus contribute to the extraordinary plant species richness of the Golfo Dulce region.

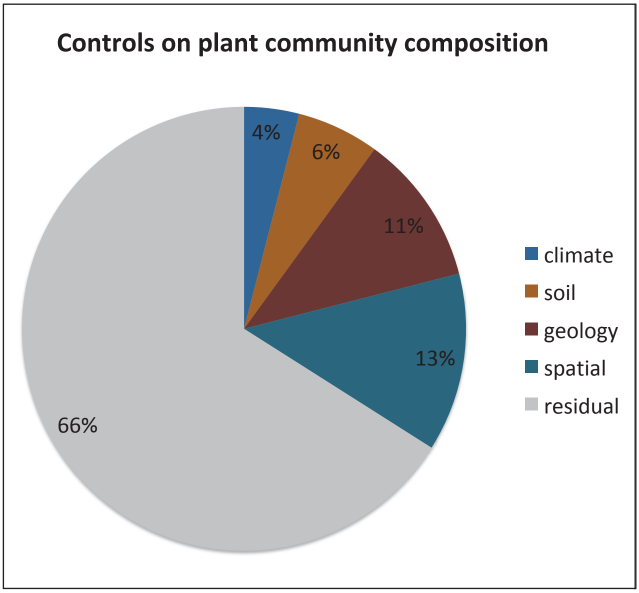

Fig. 5: Factors controlling beta diversity (i.e. variation of plant community composition) among permanent inventory plots established across the Golfo Dulce region, Costa Rica. Note the high amount of residual unexplained variation (66 %). – Abb. 5: Varianzanalyse der Faktoren, welche die Beta-Diversität (d. H. die Variation der Zusammensetzung der Pflanzengemeinschaft) in der Golfo Dulce-Region, Costa Rica steuern. Beachten Sie den hohen Anteil an ungeklärter Varianz (66 %).

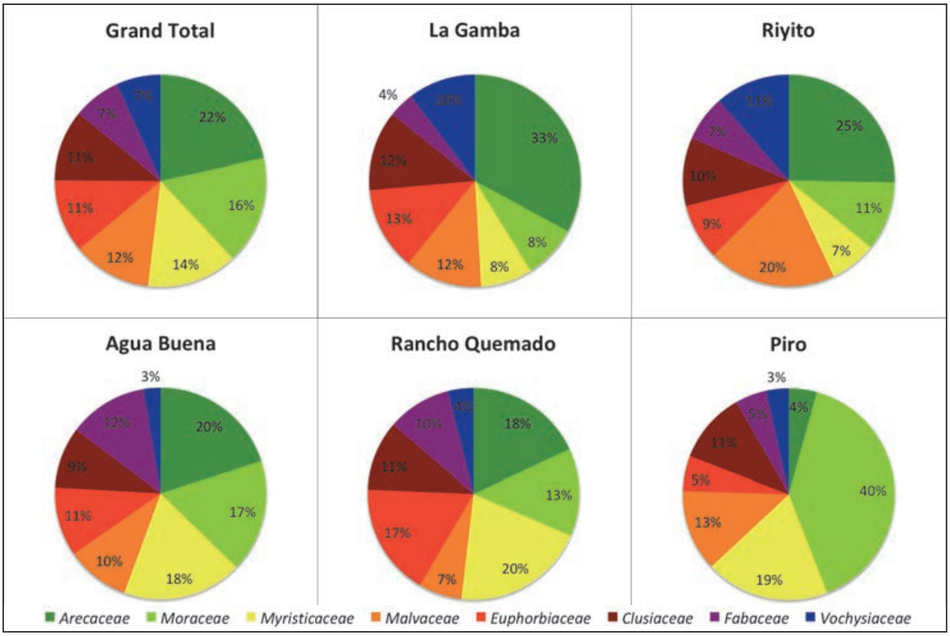

Fig. 6: Relative distribution (%) of dominant plant families (> 500 individuals) across different forest sites surveyed across the study region, i.e. for all forest sites (Grand Total), as well as for local clusters surveyed in La Gamba, Riyito, Agua Buena, Rancho Quemado and Piro, respectively. Note that most sites are dominated by palms (Arecaceae), but that every cluster exhibits a unique composition of tropical plant families (e.g. La Gamba dominated by Arecaceae; Piro by Moraceae, etc.). – Abb. 6: Relative Verteilung (%) der dominanten Pflanzenfamilien (> 500 Individuen) auf verschiedene Waldstandorte in der Golfo Dulce-Region, dh über alle Waldstandorte (Grand Total) sowie auf den lokalen Standorten La Gamba, Riyito. Agua Buena, Rancho Quemado und Piro. Beachten Sie, dass die meisten Standorte von Palmen (Arecaceae) dominiert werden, während jeder Cluster eine einzigartige Zusammensetzung tropischer Pflanzenfamilien aufweist (z. B. La Gamba, dominiert von Arecaceae; Piro von Moraceae usw.).

Species composition and relative abundance of plant functional groups

We found that local small-scale heterogeneity of environmental and topo-edaphic factors promotes tropical plant species richness in the Golfo Dulce region. Previous studies reported that variation in tree species composition across forest plots was highly correlated to gradients in resource availability (Prada & Stevenson 2016), due to feedbacks between edaphic properties, disturbance and floristic community composition (Prada et al. 2017). Indeed, we found that topographic forest habitat types, i.e. forest plots established in hilltop (ridge), slope and valley bottom (ravine) positions, showed a distinct pattern in plant species composition and relative abundance. We found that ridge and slope forests exhibited a higher abundance of palms (i.e. *Iriartea*, *Welfia*) and certain tree species (e.g. *Mabea*, *Compsoneura*), while ravine forests were dominated by other taxa (e.g. *Otoba*), and that secondary re-growth forests were characterized by a distinct taxonomic species composition (Fig. 7). The latter finding for re-growth forest stands indicates differences in successional stage and disturbance regime between forest habitat types, whereas the variation among old-growth forest stands points to variability in edaphic properties, in turn affecting species diversity and community composition between forest stands. It has been proposed that physical and chemical properties of forest soils determine local community composition via positive feedback mechanisms (Quesada et al. 2012). According to this theory, in relatively stable environments on flat terrain, low clay content and nutrient availability favor a rather slow-growing tree community, whereas in highly disturbed systems on steep terrain, high clay content and nutrient availability support a fast-growing community in competition for resources (Quesada & Lloyd 2016). This would suggest that local resource availability affects forest structure and function, such that distinct floristic communities emerge in different topographic forest habitat types. Accordingly, we found that *I. deltoidea*, a fast growing palm, was especially abundant in slope forests, as its characteristic stilt roots allow for early exploitation of light gaps without loss of stability on steep slopes (Huber 2005, Huber et al. 2008). In contrast, *O. novogranatensis*, a late-successional mid-canopy tree, was associated with moist but well-drained soils in ravine forests (Lieberman et al. 1996) but can be successfully introduced into early stages of succession (Cole et al. 2011). *Apeiba tibourbou* and *Goethalsia meiantha* were present in high densities in tree inventories of secondary re-growth forest stands, with juvenile stages associated with canopy gaps (Clark & Clark 2001). In contrast, *Mabea occidentalis* and *Compsoneura excelsa* were almost exclusively found at ridges and slopes in moderate to high densities, with the endemic *C. excelsa* being restricted to the very humid forests of Costa Rica and west Panamá (Cornejo et al. 2012). As a result, such differences in habitat preference between co-occurring plant species and associated differences in abundance across environmental gradients caused differences in floristic dominance between forest habitat types in the Golfo Dulce region (Fig. 7).

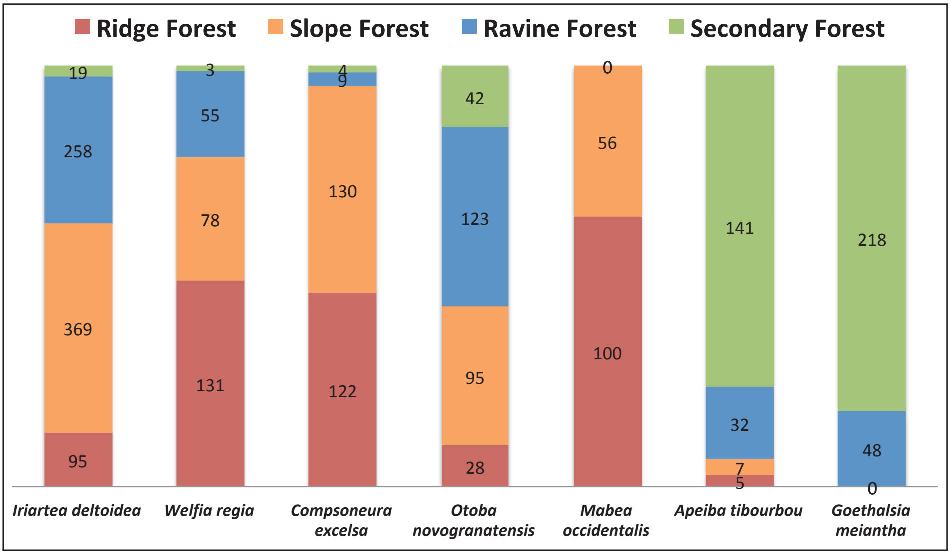

Fig. 7: Most abundant (> 100 individuals) plant species per habitat type, i.e. ridge forest, slope forest, ravine forest and secondary forest surveyed across the Golfo Dulce region, SW Costa Rica. – Abb. 7: Die am häufigsten vorkommenden Pflanzenarten (> 100 Individuen) pro Lebensraumtyp, d. H. Kammwald, Hangwald, Schluchtenwald und Sekundärwald, in der Golfo Dulce-Region, Costa Rica.

Hyperdominance in the Costa Rican plant flora

Tropical forests are characterized by extraordinary species richness and scientists still debate how many tree species might occur in the Amazon or how many will go extinct in the course of climate change (Hubbell et al. 2008). However, it may not be so important to know the exact number of species, as it has been proposed that only a few abundant tropical plant species make up a large proportion of tree stems found in the Amazon (ter Steege et al. 2013). Accordingly, it was reported that only about 1 % of Amazon tree species are responsible for 50 % of carbon storage and productivity (Fauset et al. 2015). Hence, knowledge about patterns in floristic dominance might be crucial for understanding ecosystem functioning and associated responses of tropical forests to changing climatic signals. Previous studies on nearby forest plots in the Golfo Dulce region found that the climate sensitivity of tropical forest productivity was affected by local topography and disturbance history (Hofhansl et al. 2014). This suggests that ecosystem resistance to projected global changes might be strongly related to local site characteristics, while ecosystem resilience might depend on the potential of the local species pool to adapt to these novel conditions. Hence, hyperdominant plant species could determine local responses of tropical forest ecosystems due to their abundance in certain habitat types. Nonetheless, the most species-rich plant families in a given region must not necessarily coincide with the most abundant plant families. For instance, while Fabaceae, amounting to approx. 7 % of flowering plant species, represent the most diverse plant family found in tropical rainforests, the palm *I. deltoidea* is reportedly one of the most abundant plant species in the Neotropics (Pitman et al. 2001, ter Steege et al. 2013, Arellano et al. 2014). Accordingly, we found that although Fabaceae represented the most species-rich plant family, Arecaceae was the most abundant plant family in the Golfo Dulce region, followed by Moraceae, Myristicaceae and Malvaceae (Fig. 8), which is a pattern reported similarly for other forests in the Neotropics (Gentry 1990). Nonetheless, our study further highlighted that floristic plant species composition and diversity was related to landscape heterogeneity and local topoedaphic properties, thus allowing for characterization of hyperdiverse forest communities and potentially improving our understanding of tropical ecosystem processes and associated crucial ecosystem services, such as carbon storage in tropical forest ecosystems

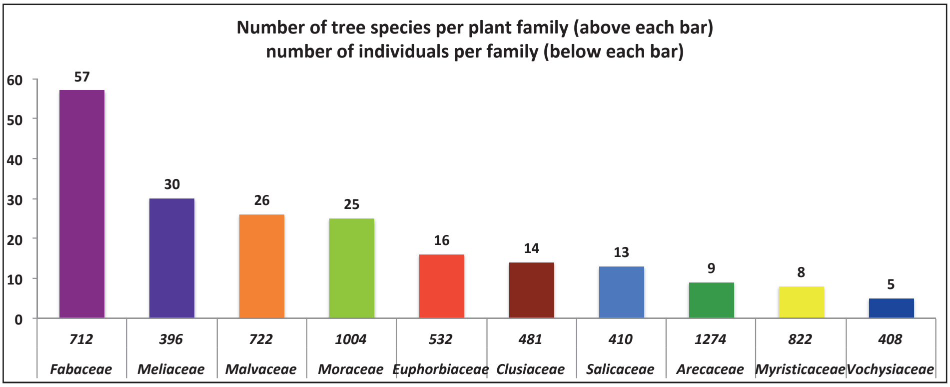

Fig. 8: Most species-rich plant families in the region (N above each bar). Most abundant plant families in the region (N below each bar). – Abb. 8: Die artenreichsten Pflanzenfamilien der Golfo Dulce-Region (N über jedem Balken), sowie am häufigsten vorkommende Pflanzenfamilien in der Region (N unter jedem Balken).

(ter Steege et al. 2013, Fauset et al. 2015). Eventually, we conclude that our findings of local hyperdominance in the Costa Rican plant flora could provide useful information for biodiversity conservation and management strategies by focusing on a subset of the most abundant plant species in the Golfo Dulce region (Tab. 1, Tab. 2).

Tab. 1: List of the 20 most abundant plant species in the Golfo Dulce region, SW Costa Rica. – Tab. 1: Liste der 20 häufigsten Pflanzenarten in der Golfo Dulce Region, SW Costa Rica.

Species	Family	Distribution	% of recorded individuals DBH>10cm	Mean density ha-1		Standard error	Max. density
Iriartea deltoidea	Arecaceae	Widespread in Tropical America	6,97	40,15	±	8,84	148
Otoba novogranatensis	Myristicaceae	Mesoamerica/ NW South America	2,51	14,45	±	3,39	46
Compsoneura excelsa	Myristicaceae	Costa Rica/ Panama	2,3	13,25	±	3,99	60
Tetrathylacium macrophyllum	Salicaceae	Widespread in Tropical America	2,08	12	±	2,09	36
Symphonia globulifera	Clusiaceae	Pantropical	1,97	11,35	±	2,01	24
Carapa nicaraguensis	Meliaceae	Mesoamerica/ NW South America	1,93	11,1	±	2,08	32
Tapirira guianensis	Anacardiaceae	Widespread in Tropical America	1,72	9,9	±	2,66	41
Apeiba tibourbou	Malvaceae	Widespread in Tropical America	1,61	9,25	±	3,95	76
Castilla tunu	Moraceae	Mesoamerica/ NW South America	1,44	8,3	±	4,42	85
Perebea hispidula	Moraceae	Mesoamerica	1,22	7	±	1,4	24
Vochysia ferruginea	Vochysiaceae	Widespread in Tropical America	1,02	5,85	±	2,43	39
Socratea exorrhiza	Arecaceae	Widespread in Tropical America	0,99	5,7	±	1,43	24
Brosimum guianense	Moraceae	Widespread in Tropical America	0,96	5,55	±	0,87	13
Tetragastris panamensis	Burseraceae	Widespread in Tropical America	0,92	5,3	±	1,86	31
Sorocea pubivena	Moraceae	Widespread in Tropical America	0,9	5,2	±	1,59	27
Brosimum lactescens	Moraceae	Widespread in Tropical America	0,86	4,95	±	1,5	23
Cecropia insignis	Urticaceae	Mesoamerica/ NW South America	0,86	4,95	±	1,64	29
Chimarrhis parviflora	Rubiaceae	Costa Rica/ Panama	0,83	4,75	±	1,45	21
Virola sebifera	Myristicaceae	Widespread in Tropical America	0,82	4,7	±	1,93	33
Chimarrhis latifolia	Rubiaceae	Mesoamerica	0,8	4,6	±	2,93	45
Pourouma bicolor	Urticaceae	Widespread in Tropical America	0,8	4,6	±	3,23	51
Pleuranthodendron lindenii	Salicaceae	Mesoamerica/ NW South America	0,77	4,45	±	1,98	35
Marila pluricostata	Calophyllaceae	Mesoamerica/ NW South America	0,68	3,9	±	1,18	15
Virola surinamensis	Myristicaceae	Widespread in Tropical America	0,68	3,9	±	0,62	10
Lacmellea panamensis	Apocynaceae	Mesoamerica/ NW South America	0,64	3,7	±	0,82	14
Virola koschnyi	Myristicaceae	Mesoamerica/ NW South America	0,58	3,35	±	0,57	9
Vochysia gentryi	Vochysiaceae	Mesoamerica/ NW South America	0,56	3,2	±	0,92	10

Tab. 2: Top 30 species (O: oligarch/ C: common/ R: rare; total number of individuals) per forest habitat type, i.e. ridge forest, slope forest, ravine forest and secondary forest surveyed across the Golfo Dulce region, SW Costa Rica. – Tab. 2: Die 30 häufigsten Arten (O: oligarchen / C: häufig / R: selten; Gesamtzahl der Individuen) nach Waldlebensraumtyp, d.H. Kammwald, Hangwald, Schluchtwald und Sekundärwald, der Untersuchungsflächen in der Golfo Dulce-Region, Costa Rica.

Ridge Forest	Slope Forest	Ravine Forest	Secondary Forest
Welfia regia (C; 131)	Iriartea deltoidea (O; 369)	Iriartea deltoidea (O; 258)	Goethalsia meiantha (C; 218)
Compsoneura excelsa (O; 122)	Compsoneura excelsa (O; 130)	Otoba novogranatensis (O; 123)	Apeiba tibourbou (O; 141)
Mabea occidentalis (C; 100)	Otoba novogranatensis (O; 95)	Tetrathylacium macrophyllum (O; 71)	Hieronyma alchorneoides (C; 91)

Tab. 2 continued

Ridge Forest	Slope Forest	Ravine Forest	Secondary Forest
Symphonia globulifera (O; 99)	Symphonia globulifera (O; 81)	Welfia regia (C; 55)	Castilla tunu (O; 90)
Iriartea deltoidea (O; 95)	Welfia regia (C; 78)	Pleuranthodendron lindenii (O, 51)	Alchornea costaricensis (C; 88)
Qualea paraensis (C; 86)	Tetrathylacium macrophyllum (O; 70)	Carapa nicaraguensis (O; 50)	Tetrathylacium macrophyllum (O; 85)
Tapirira guianensis (O; 72)	Carapa nicaraguensis (O; 64)	Goethalsia meiantha (C; 48)	Iriartea deltoidea (O; 81)
Vochysia ferruginea (O; 64)	Chimarrhis latifolia (O; 58)	Sorocea pubivena (O; 41)	Spondias radlkoferi (C; 81)
Carapa nicaraguensis (O;62)	Tapirira guianensis (O; 56)	Symphonia globulifera (O; 40)	Gmelina arborea (R; 67)
Pourouma bicolor (O; 61)	Mabea occidentalis (C;56)	Socratea exorrhiza (O; 40)	Guatteria chiriquiensis (C; 51)
Perebea hispidula (O; 58)	Sorocea pubivena (O; 49)	Chimarrhis parviflora (O; 40)	Carapa nicaraguensis (O; 46)
Marila laxiflora (C; 56)	Qualea paraensis (C; 45)	Virola sebifera (O; 39)	Miconia trinervia (C; 44)
Pausandra trianae (C; 52)	Castilla tunu (O; 43)	Calatola costaricensis (C; 36)	Otoba novogranatensis (O; 43)
Socratea exorrhiza (O; 48)	Marila pluricostata (O; 35)	Eschweilera biflava (C; 35)	Luehea seemannii (C; 43)
Brosimum guianense (O; 44)	Pausandra trianae (C; 33)	Apeiba tibourbou (O; 32)	Cecropia insignis (O; 42)
Tetragastris panamensis (O; 44)	Brosimum guianense (O; 31)	Cleidion castaneifolium (C; 31)	Tapirira guianensis (O; 40)
Brosimum lactescens (O; 41)	Tetragastris panamensis (O; 30)	Tapirira guianensis (O; 30)	Ficus tonduzii (C; 40)
Lacmellea panamensis (O; 33)	Perebea hispidula (O; 29)	Perebea hispidula (O; 30)	Hampea appendiculata (C; 39)
Guarea pterorhachis (C; 32)	Brosimum lactescens (O; 29)	Chrysochlamys glauca (C; 30)	Vochysia ferruginea (O; 36)
Euterpe precatoria (C; 30)	Cecropia insignis (O; 26)	Ocotea rivularis (C; 30)	Platymiscium curuense (C; 31)
Otoba novogranatensis (O; 28)	Lacmellea panamensis (O; 26)	Tetragastris panamensis (O; 29)	Inga oerstediana (C; 30)
Calophyllum brasiliense (C; 28)	Chimarrhis parviflora (O; 25)	Brosimum utile (C; 28)	Jacaranda copaia (C; 28)
Vochysia gentryi (O; 25)	Batocarpus costaricensis (C; 24)	Virola surinamensis (O; 26)	Chimarrhis parviflora (O; 25)
Virola sebifera (O; 21)	Marila laxiflora (C; 23)	Brosimum lactescens (O; 25)	Terminalia amazonia (C; 25)
Marila pluricostata (O; 21)	Virola surinamensis (O; 23)	Cecropia insignis (O; 25)	Trattinnickia aspera (C; 24)
Cassipourea elliptica (C; 21)	Guarea pterorhachis (C; 23)	Peltogyne purpurea (C; 23)	Perebea hispidula (O; 23)
Garcinia madruno (C; 20)	Pourouma bicolor (O; 21)	Cryosophila guagara (C; 23)	Vochysia allenii (C; 23)
Calophyllum longifolium (C; 20)	Protium pecuniosum (C; 20)	Brosimum guianense (O; 21)	Terminalia oblonga (C; 22)
Castilla tunu (O; 18)	Aspidosperma spruceanum (C; 20)	Marila pluricostata (O; 21)	Virola sebifera (O; 19)
Aspidosperma spruceanum (C; 18)	Protium glabrum (C; 19)	Virola koschnyi (O; 21)	Virola koschnyi (O; 19)

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Forest conservation and restoration in southwestern Costa Rica: The biological corridors COBIGA and AMISTOSA

Anton Weissenhofer, Alvaro Picado Zuñiga, Wendy Barrrantes Ramírez, Heiner Acevedo Mairena & Werner Huber

The southwestern parts of Costa Rica, especially the area around the Golfo Dulce and adjacent mountain areas such as the Fila Cal are hotspots of biodiversity and still boast a high proportion of forest cover. However, the natural ecosystems are increasingly affected by fragmentation and climate change. These have a strong negative impact on habitat diversity and biodiversity of flora and fauna. In order to improve this situation, the idea of biological corridors was developed: remaining patches of primary and secondary forest are protected and connected by reforestation of the pastures and wastelands in between. This allows plants and animals to disperse and migrate again over long distances, and promotes genetic exchange. An important aspect is to involve the local people in these projects. This article describes the organization and implementation of the COBIGA (Biological Corridor La Gamba) and AMISTOSA (Biological Corridor Amistad-Osa) corridor projects in southwestern Costa Rica.

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Die Golfo Dulce Region und die angrenzenden Berggebiete im Südwesten Costa Ricas sind nach wie vor größtenteils bewaldet und weisen eine sehr hohe Artenvielfalt an Tieren und Pflanzen auf. Dennoch sind diese Ökosysteme durch Fragmentierung und Klimawandel sehr gefährdet, was eine negative Auswirkung auf die Diversität der Flora und Fauna hat. Die Etablierung von Biologischen Korridoren sollen der Fragmentierung entgegenwirken und primäre und sekundäre Wälder wieder verbinden. Dadurch wird Pflanzen und Tieren ein genetischer Austausch über größere Distanzen ermöglicht. Ein wichtiger Aspekt von Biologischen Korridoren ist auch die Einbeziehung des Menschen.

Keywords: Biological corridor, sustainability, biodiversity, conservation, reforestation, forest restoration, AMISTOSA, COBIGA.

Introduction

Human development has deeply changed and often destroyed the natural coverage of the planet. The natural ecosystems have been drastically reduced in area and are heavily fragmented. Consequently, the landscape has been transformed into a mosaic of human settlements and agricultural land, often interspersed with tiny and isolated fragments of natural vegetation (e.g., Bennet 1998, Morera et al. 2007, Moreno & Guerrero-Jimenez 2019).

Before this human impact, Costa Rica was covered – with the exception of a narrow band of sandy beaches and the highest mountainous regions – by a continuous layer of forests. According to elevation and regional climatic conditions, this forest cover could be differentiated into a variety of forest types (e.g., tropical lowland forest, hill forest, montane forest, subalpine forest), but together they formed an unbroken continuum.

Nowadays, natural vegetation remains only in the form of larger or smaller patches ('islands'). These patches are ± strongly isolated from each other, with distances ranging from just a few to many hundreds of kilometers.

Forest fragmentation poses a great problem to the animals and plants living in these habitat remnants. Many animals are bound to the forest and avoid crossing open land to reach another patch of forest. This not only holds true for ground-dwelling animals such as mammals, snakes, amphibians etc., but also for flightless birds and insects. In consequence, these animals are captured in small areas that are often too small for long-term survival. They cannot migrate to find appropriate food and proper sexual partners. Finding only relatives for reproduction, inbreeding is inevitable. Some animals such as tigers, leopards, jaguars etc. are in need of huge forest areas for survival and reproduction. They die out when their territories are scaled down.

It is not as obvious that this also holds true for plants, e.g. the forest trees. In a tropical forest, the individuals of many species often grow kilometers apart. Their survival and reproduction is only ensured if the area is large enough to contain many individuals, and if the appropriate pollen vectors (insects, birds, bats, etc.) and seed dispersers are present. Even species whose seeds are transported by birds over large distances are threatened. If they do not fall (with the faeces) on forest ground, they are lost. The seeds either do not germinate or the seedlings dry out in the sun.

In summary, human-caused habitat fragmentation is a significant threat to biodiversity. The more that plant and animal populations decrease and become increasingly isolated, the greater the threat.

How to counteract these problems? One way to diminish the effect of genetic degeneration is the establishment of biological corridors. A biological corridor is a connection between two or more forest patches, or more generally, between patches of similar natural vegetation. It allows for migration, expansion and genetic exchange of animals and plants and enables an exchange of individuals between different populations. It thus helps to maintain the genetic diversity and to prevent the negative effects of inbreeding. Corridors may also facilitate the re-establishment of animal/plant populations that have been reduced or eliminated due to events such as fires, animal/plant diseases or humans. Thus, they contribute to alleviating the worst effects of habitat fragmentation.

During the past few years, the establishment of biological corridors has received great acceptance among experts. A large number of associations and institutions, such as NGOs and universities, cooperate together in initiatives such as the Mesoamerican Biological Corridor Project (MBS). The vision of this specific project is a green corridor belt between North and South America, with the aim of re-establishing or increasing biological exchange between the two continents. The crucial meeting point is Central America. The countries here are challenged to establish corridors with different degrees of protection and restrictions of land use.

In Costa Rica, biological corridors are among the most important conservation strategies in terms of territory size and scope. They are promoted by the National Program of Biological Corridors (created on 30 May 2006, executive decree N°33106-MINAE), with local stakeholders and platforms forming so-called Local Committees of Biological Corridors (Consejos locales). So far, Costa Rica has 44 biological corridors, which represents about 33 % of the continental territory (Fig. 1).

In 2018, a management plan was established for the Biological Corridors in Costa Rica, entitled Plan Estratégico 2018–2025 del Programa Nacional de Corredores Biológicos de Costa Rica, Informe Final (Sistema Nacional de Áreas de Conservación 2018).

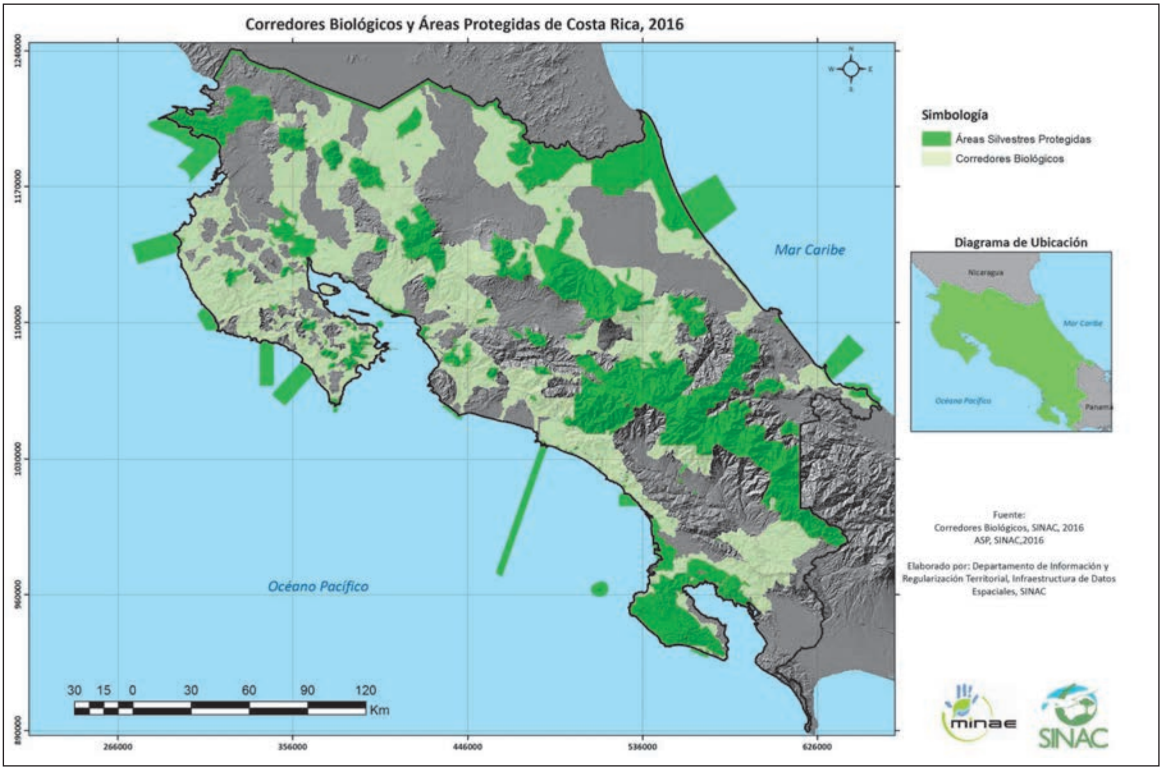

Fig. 1: Costa Rica's biological corridors (pale green) and National Parks (dark green). – Abb. 1: Biologische Korridore Costa Ricas (hellgrün) und Nationalparks (dunkelgrün).

Biological Corridors in the Golfo Dulce Region

The pristine forests of the Golfo Dulce region in southwestern Costa Rica harbor the most diverse ecosystems and encompass the most significant remaining areas of lowland Pacific tropical forest in Central America (see Weber et al. 2001 and literature cited). The extraordinary level of biodiversity and endemism in the region resulted in one of the highest conservation priorities in Central America (Ankersen et al. 2006). The Piedras Blancas National Park (approx. 150 km²) and the Corcovado National Park (424 km²) includes protected lowland rainforests and is surrounded by agricultural land and unprotected forests.

The Biological Technical Coalition Biologica Corridor OSA (CTCBO), founded in 2001 by the National System of Conservation Areas (SINAC) and NGOs, aims at (1) generating and transferring technical and scientific information, (2) implementing conservation strategies and consolidating local capacities, and (3) setting up sustainable development and management in the region (García 2008). As an example, the OSA Biological Corridor links different rainforest ecosystems including mangroves and cloud forests. Equally, it forms a connection between the Osa peninsula and the La Amistad International Park (PILA) in the Cordillera de Talamanca.

Fig. 2: A typical tree nursery run by Elias Padilla at the Finca Alexis, 400 m a.s.l. – Abb. 2: Typische Baumschule die von Elias Padilla auf der Finca Alexis auf 400 m Seehöhe aufgebaut wurde.

Fig. 3: View from the lowland forest near La Gamba up to the mountain forests of Fila Cal (1,700 m). Foto: Dennis Kollarits. – Abb. 3: Blick vom Tieflandregenwald Richtung Bergregenwald der Fila Cal in der Nähe von La Gamba (1.700 m). Foto: Dennis Kollarits.

The COBIGA project (Biological Corridor La Gamba)

The COBIGA project was initiated in 2006 by the first author and is guided by the Tropical Station La Gamba. The focus of the project is mainly on the connection of the lowland forests of the Piedras Blancas National Park with the 'Fila Cal', a largely unprotected area covered with montane rainforests. Local people in the villages of La Gamba, San Miguel and La Virgen are integrated in this project (Fig. 2 and 3).

The exchange of species of the lowland and mountain forests is thus facilitated and contributes to an enrichment of the flora and fauna in both ecosystems. Furthermore, the Fila Cal is an important transition corridor zone to the Talamanca mountains in the north.

On the basis of aerial photographs taken in 2003 (CARTA 2003), particularly important prospective corridor areas were identified (Fig. 4). Special importance was attached to (1) closing forest gaps in order to create – as far as possible – a continuous forest area, and (2) reforesting or restoring pastures and/or river banks for water protection and the formation of ± compact corridors. MORERA & ROMERO (2008) analyzed the vegetation types in an area of 117.8 km^2 between the Piedras Blancas National Park and the Fila Cal based on aerial photographs taken in 1998. Around 54 % of the area proved to be covered with forest. The dominant use of the remaining area was for agriculture, particularly pastures (24 %) and timber plantations (9 %).

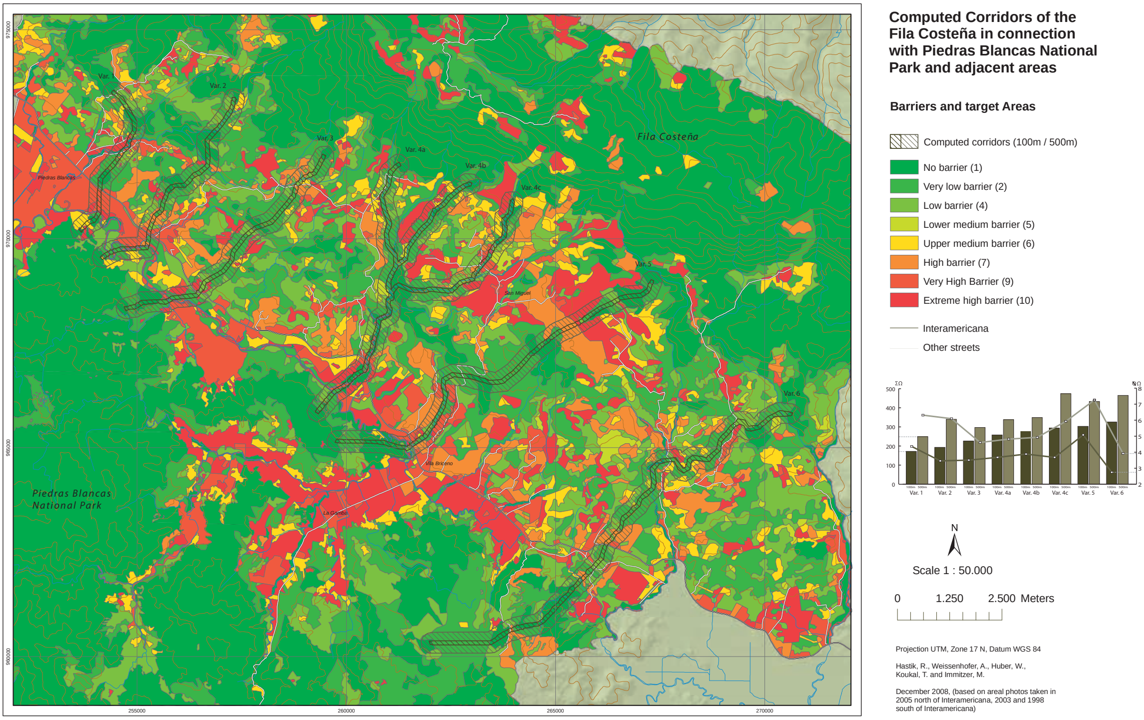

Fig. 4: Computed corridors in the COBIGA region around La Gamba and Fila Cal with different levels of barrier effects. – Abb. 4: Errechnete Korriodorvarianten aufgrund von Barrierestufen in der COBIGA Region von La Gamba und Fila Cal.

At present, most of the sites envisaged for corridors are in private hands. The farmers are the first people that have to be convinced of the corridor idea. Since 2010, the Austrian association 'Rainforest of the Austrians' has taken an active part in the COBIGA project and engages mainly in purchasing suitable land areas, and in reforesting them. Examples that have been successfully reforested or are presently in the process of reforestation or natural succession are the Finca Amable (12.5 ha), Finca La Bolsa (16.5 ha), Finca Alexis (137 ha), and several smaller areas. The Fincas are then included into the FONAFIFO projects (Fondo Nacional de Financiamiento Forestal) for better visualization and protection of the forest areas.

Since 2015 the association Rainforest Luxemburg is a major sponsor. In 2017, the COBIGA project and Rainforest Luxemburg started working intensively together on the AMISTOSA biological corridor. The Tropical Station La Gamba is main actor in the local committee (consejo local) of La Gamba. It takes the lead in planning, managing and organizing local activities (see below), in association with national organizations (SINAC), NGOS and the involvement of the local people.

Reforestation of agricultural land and restoration of forests in COBIGA

Reforestation of agricultural land and restoration of forests with native tree species is an important step in the establishment of biological corridors, particularly in the COBIGA project. Moreover, agroforestry systems consisting of a mix of timber trees, fruit trees, short-lived crops and vegetables can be well utilized by the local people.

It is important to emphasize that reforestation with native tree species plays a fundamental role in environmental conservation, because it helps to recover certain species that face a particularly high risk of extinction, either because they are endemic or have very slow growth rates.

There is no doubt that trees play an important role in the history and economy of southern Costa Rica. One of the main problems is the over-exploitation of valuable species such as Cachimbo (*Platymiscium curuense*), Manú Negro (*Minquartia guianensis*) and Chirraco (*Caryodaphnopsis burgeri*). These species have been largely exploited without control and nowadays it is difficult to find trees of such species in their natural habitat.

There are many benefits of reforestation projects such as the COBIGA project. Along with the reforestation of areas, forest carbon storage areas are enlarged and safe zones for the dispersal of fauna and flora species are created. Such areas become central reservoirs both for common and endangered species. In addition, this allows study areas to be created for future research. Reforestation projects provide opportunities for collaboration in solving social issues, such as providing jobs directly and indirectly for the community of La Gamba and adjacent areas.

In total, between 2006 and 2019, more than 60 ha of pasture were reforested. More than 44,000 trees of 200 species were planted in the region of La Gamba, San Miguel and La Virgen. At present, the Finca Luis (La Gamba; approx. 1,000 trees, reforestation in patches, see Fig. 5) and the Finca Alexis 3 (San Miguel; species enrichment, 2,000 trees) are in the process of reforestation.

Fig. 5: Día de acción – Day of reforestation at Finca Luis, with students from the University of Vienna, volunteers of La Gamba and workers involved in the COBIGA project. – Abb. 5: Typischer *Aktionstag* – Wiederbewaldung auf der Fica Luis mit Studierenden der Universität Wien, Volontären aus La Gamba und Mitarbeitern von COBIGA.

Reforestation, forest restoration and species enrichment: explanation of terms and concepts

Reforestation projects are mainly conducted by or in cooperation with private farmers. In the case of the COBIGA project, up to 50 species of selected timber trees and species of high ecological value are used for reforestation. The private owners are allowed to use the wood after a certain period of time and have to replant the trees after cutting. In this way, a simple and sustainable forest management is established.

In contrast, restoration of forests means that there is no intention of using the planted trees after the forest has developed. In this case, one has to attach great importance to species selection for each individual site. Up to 200 different species have to be planted per Finca (e.g. Finca Amable). Such projects can only be realized in cooperation with national institutions, and only rarely with private owners. Restoration of forests is only practicable at sites where natural succession is hampered, due to soil conditions and/or lack of natural vegetation, e.g. pastures without contact to natural forest.

At sites where the seed bank is not disturbed and natural succession can take place successfully, we generally do not plant trees but rather promote growth of the spontaneous vegetation. In rare circumstances (e.g. Finca Alexis) we perform species enrichment. That means that we select rare tree species of the region (e.g. species that were overexploited) and plant them in selected patches in the shade of the spontaneous vegetation. This is to avoid complete extinction of those species.

Tab. 1: List of the most important tree species used in the reforestation project. – Tab. 1: Liste der wichtigsten im Wiederbewaldungsprojekt verwendeten Baumarten.

Nr.	**Family**	***Genus***	***species***	**Vernacular name**
1	Anacardiaceae	*Anacardium*	*excelsum*	Espavel
2	Anacardiaceae	*Astronium*	*graveolens*	Ron ron
3	Anacardiaceae	*Spondias*	*mombin*	Jobo
4	Apocynaceae	*Aspidosperma*	*myristicifolium*	cara tigre
5	Apocynaceae	*Aspidosperma*	*spruceanum*	Manglillo
6	Bignoniaceae	*Tabebuia*	*guayacan*	Corteza
7	Malvaceae	*Ceiba*	*pentandra*	Ceiba
8	Clusiaceae	*Calophyllum*	*brasiliense*	Maria
9	Combretaceae	*Terminalia*	*amazonica*	Amarillon
10	Euphorbiaceae	*Hyeronima*	*alchorneoides*	Pilon, zapatero
11	Euphorbiaceae	*Croton*	*schiedeanus*	Colpachí
12	Fabaceae - Casalpinioideae	*Schizolobium*	*parahyba*	Gallinazo
13	Fabaceae - Casalpinioideae	*Copaifera*	*camibar*	Camibar
14	Fabaceae - Casalpinioideae	*Cynometra*	*hemitomophylla*	Cativo, guapinol negro
15	Fabaceae - Casalpinioideae	*Peltogyne*	*purpurea*	Nazareno
16	Fabaceae - Faboideae	*Platymiscium*	*curuense*	Cristobal, Cachimbo
17	Fabaceae - Faboideae	*Dussia*	*discolor*	Sangregao, targuayugo
18	Fabaceae - Mimosoideae	*Inga*	*oerstedtiana*	Cuajiniquil
19	Fabaceae - Mimosoideae	*Inga*	*spp.*	Guaba
20	Fabaceae - Mimosoideae	*Parkia*	*pendula*	Tamarindo, tamarindo gigante
21	Fabaceae - Mimosoideae	*Zygia*	*longifolia*	Sotocaballo
22	Humiriaceae	*Humiriastrum*	*diguense*	Chiricano alegre, lorito, nispero
23	Lauraceae	*Ocotea*	*sp.*	Ira
24	Lecythidaceae	*Couratari*	*guianensis*	Copo hediondo
25	Malvaceae	*Apeiba*	*membranacea*	Peine de mico
26	Malvaceae	*Apeiba*	*tibourbou*	Peine de mico
27	Malvaceae	*Luehea*	*semanii*	Guacimo colorado
28	Malvaceae	*Mortoniodendron*	*anisophyllum*	Cuero de vieja
29	Meliacae	*Carapa*	*guianensis*	Cedro bateo
30	Meliaceae	*Cedrela*	*odorata*	Cedro amargo
31	Meliaceae	*Guarea*	*grandifolia*	Caobilla
32	Moraceae	*Brosimum*	*utile*	Lechoso
33	Moraceae	*Brosimum*	*alicastrum*	Ojoche
34	Moraceae	*Ficus*	*insipida*	Chilamate
35	Myristicaceae	*Virola*	*koschnyi*	Fruta dorada
36	Olacaceae	*Minquartia*	*guianensis*	Manu, manu negro, palo de piedra
37	Salicaceae	*Tetrathylacium*	*macrophyllum*	Lengua de vaca, zapote
38	Verbenaceae	*Vitex*	*cooperi*	Manu platano
39	Vochysiaceae	*Vochysia*	*ferruginea*	Mayo
40	Vochysiaceae	*Vochysia*	*alleniii*	Mayo

Species selection

In order to make a suitable selection of species for a given locality, it is necessary to know about factors such as soil type (acidity, prior use, fertility, presence of compacted layers etc.), weather conditions (precipitation) and topography, as well as the ecological characteristics of each species that is considered for planting. Unfortunately, very little information exists for most species of the area. The lack of knowledge about the ecological factors, paired with improper handling, led to the failure of many reforestation initiatives in the past. The selection of appropriate species is an indispensable prerequisite for successful reforestation. This step can best be achieved in close cooperation between forest engineers, botanists and local people with good knowledge of forest trees. Species of prime importance are those with a high ecological value. A selection of the most important species used for reforestation is given in Table 1. Practical information on reforestation and suitable tree species is presented in the book *Creating a forest – trees for biological corridors in the Golfo Dulce region, Costa Rica* (Weissenhofer et al. 2012).

The Finca Modelo

The Finca Modelo (*Escuela vieja*) is a model farm situated close to the Tropical Station La Gamba. It plays an important part in the COBIGA project. It is based at the site of a former primary school which was converted into a tree nursery for the permaculture pro-

Fig. 6: Seed exhibition at Finca Modelo: this is held once a year during the dry season. – Abb. 6: Die Samenmesse auf der Finca Modelo wird einmal jährlich in der Trockenzeit veranstaltet.

ject (agroforest system) in 2006. It has become an important communication and education center.

Since 2014, plant exhibitions (Feria de semillas, Fig. 6) take place annually in the dry season (February/March). People come from far away to exchange seed and plant material and learn about organic gardening. Courses on permaculture, forestry and cultivation of different plant species are held at the Finca Modelo as well. In 2019 the Finca Modelo was awarded – as the first Finca in southern Costa Rica – the so-called *Bandera Azul* by the Ministerio de Agricultura y Ganadería de Costa Rica MAG.

Practical and theoretical knowledge and experience in growing and planting trees, producing compost, and selection of tree species was collected during the years and compiled in the book *Creating a forest* mentioned above (Weissenhofer et al. 2012).

The AMISTOSA biological corridor

The aim of the AMISTOSA biological corridor is to integrate both the enlargement of forest areas and the connection between the Osa Peninsula and La Amistad International Park (PILA) in the Cordillera de Talamanca. Moreover, sustainable land management through projects of reforestation, agriculture and sustainable development is promoted.

For several years, various international, national and private organizations, such as the OTS (Organization for Tropical Studies), Tropical Research Station La Gamba (COBIGA Project), Association Rainforest Luxemburg, FUNDAOSA, SINAC, CATIE, local groups, etc. have sought to formalize the biological corridor AMISTOSA. In December 2018, the AMISTOSA project was accredited in the Programa Nacional de Corredores Biológicos (SINAC), which is a further step in the environmental conservation of the Golfo Dulce area.

The AMISTOSA biological corridor covers an area of 929 km^2 and is located in the Brunca region, in the cantons of Buenos Aires (5 %), Corredores (9 %), Golfito (39 %) and Coto Brus (48 %) (SINAC 2018). The AMISTOSA is a geographical and altitudinal bridge that connects the Parque Nacional Piedras Blancas and the Refugio Nacional de Vida Silvestre Mixto Golfito (ACOSA) with the Zona Protectora Las Tablas and the Parque Internacional La Amistad (ACLAP).

In total, 43 % of the AMISTOSA biological corridor are covered by forests (398 km^2), while 32 % are dedicated to pasture cultivation (294 km^2) and 21 % (194 km^2) to annual and permanent crops, such as coffee and oil palm (Fig. 7). The three major problems of AMISTOSA are (1) the fragmentation of forests, (2) the lack of knowledge about the biological diversity of the corridor, and (3) the impact of climate change (SINAC 2018).

There are 1,017 forest fragments in the AMISTOSA biological corridor, of which 37 % have an area of less than 2 hectares. Because of their small size, these fragments are not legally recognized as forests by the Ley Forestal de Costa Rica (N° 7575). There are only four forest fragments with an extension of more than 1,000 hectares. These represent approx. 68 % of all AMISTOSA forests. The largest fragment covers an area of 18,186 hectares and is located in the southwest of the biological corridor, between the communities of La Gamba-Bajo, Cedros-Santo and Domingo-Kilómetro 29. The northern sector of AMISTOSA is the most fragmented one. Unfortunately, there is – as yet – a lack of concrete biological studies outlining the functional connectivity of AMISTOSA. Information from local management elements or indicator species that would allow assessment of the current state of health of the biological corridor is also missing. Regarding climate change, the AMISTOSA biological corridor contains areas that act as 'climatic shelters', which were identified during formation of the Estrategia Nacional del Sector Biodiversidad ante el Cambio Climático (ENASB-CC).

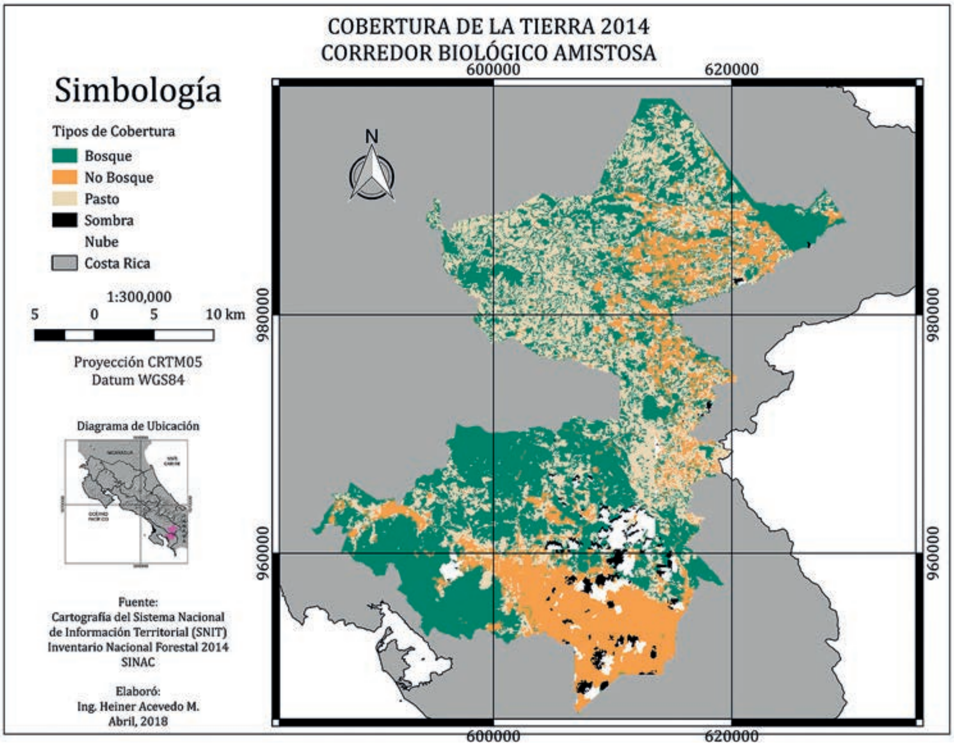

Fig. 7: Map of the AMISTOSA Biological Corridor. – Abb. 7: Karte des Biologischen Korridors AMISTOSA.

For this reason, the AMISTOSA management plan *Viveros Cuna del Corredor Biológico Amistosa* 2018–2027 (Acevedo 2018) identifies 'promoting ecological connectivity and social articulation for conservation, recuperation and sustainable use of natural resources for the wellbeing of mankind' as their primary mission goals. The management plan consists of seven strategical concepts: (1) planning, monitoring and evaluation; (2) governance; 3) connectivity, forests and natural spaces; (4) sustainable production; 5) climate change; 6) generation of economic resources and (7) communication and management of knowledge (SINAC 2018). This management plan has 18 goals that are stated for development within ten years. These goals are in line with at least five of the national goals outlined in the Estrategia Nacional de Biodiversidad 2016–2025 (ENB2).

The areas of highest priority for reforestation, production and restoration are areas containing water resources for the association of rural water providers ASADAS, routes of structural connectivity between select forest fragments, living fences or hedges of fruit trees.

Scientific work in the Biological Corridors COBIGA and AMISTOSA

The region around La Gamba is ideal for studying natural, semi-natural and anthropogenic habitats and organisms. Since the COBIGA project was established, many scientists have conducted their research on effects and impacts of biological corridors.

In 2013, the Association Rainforest of the Austrians bought a 13.7 ha Finca, called Finca Amable, in the vicinity of the village La Gamba. The Finca was used for banana production since the 1940s and was converted into a rice field in the 1980s. In the last years it was used for cattle breeding. When we purchased the Finca in 2013, vegetation was dominated by the introduced grass *Paspalum fasciculatum*. Many Fincas in the region have quite a similar history and Finca Amable is therefore an ideal model site for a long term study concerning reforestation and regeneration on agricultural land. A scientific design with 80 permanent plot replicates and different combinations of trees according to wood density and inclusion of legumes was established. The planted trees in the plots are monitored 1–2 times per year and should give exact data on growth and CO2 sequestration (see Hietz et al., this volume).

Other reforestation sites such as the Finca La Bolsa, Finca Amable, Finca Mundo, and Finca Alexis are ideal areas for studying regeneration as well as population ecology. Christian Schulze and his students have carried out scientific work on bats, birds, mammals and reptiles in natural habitats and in oil palm plantations (Freudmann et al. 2015, Gallmetzer et al. 2015, Seaman & Schulze 2010). Macroinvertebrates as indicators in tropical streams with different land-use are studied by Leopold Füreder and collaborators, University of Innsbruck (see Duschek et al., this volume).

To get information on the diversity and migration of big mammals, Randy Teal and Christian Schulze, University of Vienna, established a research project using camera traps (Fig. 8). In cooperation with the University of Natural Resources and Life Science, Vienna (BOKU), another new project was started by Ramon Enguidanos Requena and Chris-

Fig. 8: Photo traps are crucial to study the success and routes of animal migration. Photo of *Puma concolor* taken near the Tropical Station La Gamba. – Abb. 8: Mit Hilfe von Kamerafallen kann der Erfolg von Biologischen Korridoren und Migrationsrouten der Tiere erforscht werden. Das Foto von *Puma concolor* wurde in der Nähe der Tropenstation La Gamba aufgenommen.

tian Vogl, dealing with the use of organic fertilizers under tropical climatic conditions. In total, around 20 master theses were conducted in the COBIGA area. They are referenced in the 'Scientific report' of the Tropical Station La Gamba (www.lagamba.at).

Research in the AMISTOSA biological corridor started only recently. The main focus is on migration of larger animals (Wendy Barrantes, pers. comm.).

Conclusions

The establishment of biological corridors is important to connect isolated forest patches, thus enabling migration and genetic exchange of animals and plants. Nature conservation is impossible without involvement and support of the local people. Habitat protection or reforestation should be accompanied by educational programs to sensitize the local people. To guarantee attendance and success, the projects must be long-term. Success depends on the effective accomplishment of the guidelines, the monitoring processes and the time available to react and to correct mistakes. Scientific research is important and inevitable to gain data and information about migration, population ecology, carbon sequestration, etc.

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Without the help of many locals and international volunteers the COBIGA project could no be realized. Pura vida!

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Biomass accumulation and carbon sequestration in a reforestation project in La Gamba, Costa Rica

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Tropical reforestation is increasingly seen as important to provide ecosystem services, including climate regulation, carbon dioxide sequestration, and biodiversity conservation. To optimize the management of active reforestation projects, it is important to be clear about the goals and to compare the effect of reforestation on ecosystem services as well as the efficiency of the project in terms of costs, land area or other limiting resources. These effects will depend on many factors, including species selection. In the Finca Amable reforestation project in La Gamba, Costa Rica, different combinations of trees were planted in replicated plots. We monitored the growth of trees during the first six years and present here the results on biomass accumulation and carbon sequestration. We tested the effect of using different allometric models to predict biomass, and use a global model that includes tree diameter and height. After approximately five years, above-ground biomass of the planted trees had reached 13.5–59.6 t ha^{-1} (mean 36.4), which corresponds to a CO_2 sequestration of 64 t ha^{-1}. This is high compared to natural regeneration, but the comparison is limited by the few comparable data on very young secondary forests in the region and the effect of site factors. Considerable uncertainties also remain in the best allometric models, which could be improved by more detailed measurements of tree allometries.

Hietz P., Kleinschmidt S., Mala B., West Z., Schwarzfurtner K., 2019: Biomasseakkumulation und Kohlenstoff-Sequestrierung in einem Wiederbewaldungsprojekt in La Gamba, Costa Rica.
Die Wiederbewaldung in den Tropen wird zunehmend als wichtiger Beitrag zu Ökosystemdienstleistungen wie Klimaregulation, CO_2-Sequestrierung und Erhaltung der Biodiversität anerkannt. Um eine Wiederbewaldung optimal durchzuführen sollten zunächst deren Ziele klar definiert und müssen im Verlauf sowohl der Effekt auf Ökosystemdienstleistungen als auch die Effizienz in Bezug auf Kosten, Landnutzung und anderen Ressourcen quantifiziert werden. Diese Effekte hängen von verschiedenen Faktoren einschließlich der Auswahl der Baumarten ab. Im Wiederbewaldungsprojekt Finca Amable bei La Gamba in Costa Rica wurden verschiedene Kombinationen von Bäumen in replizierten Versuchsflächen gepflanzt, das Wachstum in den ersten sechs Jahren gemessen und daraus Biomasseakkumulation und CO_2-Sequestrierung berechnet. Nach einem Vergleich verschiedener allometrischer Modelle zur Berechnung der Biomasse wurden ein generelles Modell, das Baumdurchmesser und –höhe einschließt, als das geeignetste ausgewählt. Etwa fünf Jahren nach dem Auspflanzen der Bäume hatte die oberirdische Biomasse in den einzelnen Flächen 13.5–59.6 (Mittel: 36.4) t ha^{-1} erreicht, was einer CO_2 Sequestrierung von durchschnittlich 64 t ha^{-1} entspricht. Dies ist hoch im Vergleich zu einer natürlichen Regeneration ohne aktivem Setzen von Bäumen, allerdings ist der Vergleich limitiert weil es wenige vergleichbaren Daten von jungen Sekundärwäldern in der Region gibt. Auch beim besten allometrischen Modell bleiben beträchtliche Unsicherheiten, die durch detailliertere Messungen von Bäumen reduziert werden könnten.

Keywords: Costa Rica, reforestation, functional diversity, biomass growth, allometric biomass model.

Introduction

Tropical forests store about 37 % of the global terrestrial carbon pool (Dixon et al. 1994), account for approx. 33 % of net primary production (Bonan 2008) and 60 % of gross photosynthesis (Beer et al. 2010), and tropical rainforests are the most biodiverse biome on earth (Barlow et al. 2018). In addition, they provide many vital ecosystem services including climate regulation and resources that sustain the livelihoods of millions of people (Millenium_Ecosystem_Assessment 2005). Increasing human impact on tropical forests via hunting, degradation, conversion to other land uses, fragmentation and impacts of climate change threaten this diversity as well as ecosystem functions and services (Lewis et al. 2015). Tropical forest degradation is a major source of carbon emissions contributing to the increasing atmospheric CO_2 concentration and thereby global warming. Emissions from tropical forest loss and degradation were estimated at approx. 2.9 Pg (or billion tons) of carbon per year at the beginning of this century (Mitchard 2018). Currently, the effect of tropical forest areas on atmospheric CO_2 thus appears to be approximately neutral. Without the sinks, the increase of atmospheric CO_2 would be faster still, but if forest degradation were reduced or forest recovery were increased, tropical forests could become important net carbon sinks.

The need to restore deforested and degraded land for reasons that include biodiversity conservation, climate mitigation, water protection and security of livelihoods has gained increasing support. International initiatives such as REDD+ (Reducing Emissions from Deforestation and Forest Degradation, Alexander et al. 2011) and the Convention on Biological Diversity (www.cbd.int) focus global efforts to restore degraded tropical land. For instance by the end of 2018, 57 countries and private organizations have pledged to bring 170 million hectares of degraded and deforested land into restoration with the goal of reaching 350 million hectares by 2030 (www.bonnchallenge.org). Costa Rica alone has committed to restore 1 million hectares by 2020. Landowners in Costa Rica are encouraged to contribute to this goal through a program of payments for ecosystem services (including carbon sequestration, biodiversity and water protection) managed by the Fondo Nacional de Financiamiento Forestal (Fonafifo, www.fonafifo.go.cr).

When disturbance stops or agricultural land is abandoned, the forests will eventually regrow. These secondary forests that are re-growing on previously deforested land were neglected for a long time as ecosystems that do not match primary forests in terms of biodiversity and complexity. They consequently received limited scientific interest, but are recognized today as important refuges for many species where primary forests have been lost or are strongly reduced and fragmented, and regrowing forests are the most important terrestrial carbon sink (Chazdon 2014). In 2008 second-growth tropical forest covered 2.4 million km^2 in Latin America. These lands could sequester 31 Pg CO_2 over 40 years through natural succession or assisted regeneration, and permitting forest regeneration on 40 % of often low-productivity pastures could capture an additional 7 Pg CO_2 (Chazdon et al. 2016).

Left alone, tropical forests will eventually regrow under most circumstances, but how fast they recover their biomass, diversity, and other ecosystem functions and services depends on many factors. Soil degradation, the loss of the soil seedbank, absence of seed sources, the harsh microclimate or very competitive herbaceous vegetation may hinder regrowth and arrest succession (Walker 1994; Slocum et al. 2004; Elgar et al. 2014). A study in the Australian wet tropics found that compared to natural regeneration of rainforests, actively reforested sites increased substantially faster in wood volume (and thus biomass) and woody plant diversity, the latter largely independent of the dispersal mode (Shoo et al. 2016). By contrast, the diversity of non-arboreal plants (vines, epiphytes and ferns) increased only slowly with stand age in the actively reforested as well as the naturally regrowing sites. In situations where natural regrowth is slow or when there is a strong interest to restore the ecosystem services and biodiversity of the forest rapidly, actively planting trees may be the strategy of choice.

Since actively planting a forest is more costly than natural forest recovery, the advantages need to be weighed against the costs. Avoiding failure requires detailed information on obtaining seeds or seedlings, raising seedlings in nurseries, and planting and caring for the trees during the first years. For a number of tropical trees of commercial value, this information is readily available, but for many others our knowledge on how to manage them is scant or absent (but see Román et al. 2012 and Barquero Palma et al. 2012 for Central American trees). Many reforestation projects have contributed important insights into the practical management of tree species and human-assisted forest recovery (Lamb 2011; van Breugel et al. 2011a). For most regions, however, only a fraction of the local forest species has been tested and the trees planted are mostly from relatively few species that are easily available and known to grow well, which results in a forest with a strongly selective species-composition that is often not representative of a natural old-growth forest. Apart from the successful management of a reforestation, decisions about the aim of the project also need to be taken when selecting trees. The purpose of reforestation could, for instance, be carbon sequestration, protection of biodiversity, creating a biological corridor, scientific studies, commercial timber and non-timber products for local or non-local markets, or non-commercial forest products and services (Lamb 2011). While many forests will provide all of these functions to some degree, there is no forest that maximizes all. For instance, a young forest composed of a few fast-growing species will initially sequester more carbon but will be less diverse than a forest that includes many fast- and slow-growing species.

Overall, higher tree diversity tends to result in higher productivity through complementarity in resource use (Morin et al. 2011). This has recently been confirmed from a subtropical reforestation project, where tree diversity per plot ranged between 1 and 16 species (Huang et al. 2018). However, a global study of mature tropical forests found that the relationship between diversity and productivity depends on the spatial scale, and at a scale of 1 ha negative relationships were more common (Chisholm et al. 2013). Whether diversity is positively or negatively related to growth depends on the selection of individual species in a comparison (which may have intrinsically higher or lower growth) and on the complementarity of species. When the species number is high but their requirements are very similar, they will likely compete with each other similarly to individuals of a single species. If, on the other hand, the species are functionally very diverse, the complementarity and thus the positive effect of diversity is likely greater.

To test the effect of functional diversity independent of species diversity, trees were planted in plots with the same number of species per plot but with different numbers of functional groups in a reforestation project on Finca Amable close to La Gamba, Costa Rica. We do not evaluate growth performance of individual tree species here, but ask if functional types of trees differed in their growth and if a combination of different functional types had an effect on plot-based tree biomass accumulation without changing the number of species. In addition, the analysis of the first five years of growth on Finca Amable presented here serves to estimate the carbon sequestration potential of this and other reforestation projects in the region, and provides a basis to compare growth and carbon sequestration with natural succession or other land management strategies.

Material and Methods

The reforestation site Finca Amable (Fig. 1) is located in the vicinity of the village La Gamba, close to the La Gamba field station in the Puntarenas province, SW Costa Rica (8°42′03.78″N 83°12′06.14″W). It is part of the local reforestation project COBIGA (Corredor Biológico La Gamba), whose main purpose is to establish a network of reforested areas connecting the protected lowland rainforest of the Golfo Dulce region with the lower montane rainforests in the Fila Cal mountain range. Finca Amable was previously a cattle pasture dominated by the introduced pasture grass *Paspalum fasciculatum*. The site is flat at approximately 70 m above sea level. Average annual temperature is 28.3° C and annual rainfall is 5930 mm (Weissenhofer et al. 2008). December to April is less rainy, but there is no month with < 100 mm of rainfall (which is often used to characterize a dry season) and most months receive > 200 mm. The natural vegetation is a humid lowland

Fig. 1: Aerial photograph of Finca Amable, the reforestation sites is outlined in red. Photo: A. Weissenhofer, March 2018. – Abb. 1: Luftbild der Finca Amable, die Wiederbewaldungsfläche ist rot umrandet. Foto: A. Weissenhofer, März 2018.

rainforest. Soils are plastic loamy clays and can be flooded during the rainy season. Because of the stagnant water resulting in partly anoxic soil, drainage of the area was improved by digging several parallel ditches with a distance of approx. 50 m and some smaller ditches perpendicular to these to drain towards a river.

Trees were planted on approx. 13.7 ha in a rectangular pattern with a spacing of 3.5 × 4 m in plots of 6 × 6 trees separated by at least one tree row between plots. Seeds or seedlings had been collected in local forests and were grown in a nursery for several weeks. Planting was done mostly during the wetter season with trees 30–100 cm tall. For planting and the first 2–3 years afterwards, grass and other competing vegetation including vines and lianas were manually cut around each tree several times per year. Trees were first planted between 2012 and early 2014, but trees that had died were replaced until 2015.

We classified species into three functional groups. Legumes (Caesalpiniaceae, Fabaceae and Mimosaceae, "LEG"), non-legume trees with high wood density (> 0.5 g / cm^3, "HWD"), and non-legumes with low wood density (<0.5 g / cm^3, "LWD"). Wood density data were obtained from the wood density database (Zanne et al. 2009) with some own local measurements for species not included in the database. In each plot of 6 × 6 trees, nine species with four individuals each were planted. While the number of species was therefore constant, we modified the number of functional groups by planting nine spe-

Fig. 2: Finca Amable in La Gamba before tree planting (2012, note the drainage ditch) and in 2015, 2016 and 2018. Note the low-branching trees in 2016 and 2018 (*Inga* sp.), which made diameter measurement at breast height impractical. – Abb. 2: Die Finca Amable in La Gamba vor der Pflanzung 2012 (mit Drainagegraben), 2015, 2016 und 2018. Bei den tief verzweigenden Bäume 2016 und 2018 (*Inga* sp.) wurde die Messung der Durchmesser nicht in Brusthöhe durchgeführt.

cies of only LEG, HWD or LWD, any combination of two functional groups with four or five species per group, or all three functional groups with three species per group. These seven combinations of functional groups were planted in a total of 56 plots. Originally, each combination should have been planted with eight replicates but due to tree mortality and species not available at the time of planting, the number of replicates is variable. Trees that had died in the first two years were replanted with the same species as far as possible, or another species from the same group if the same species was not available or had a high mortality rate and was therefore considered unsuitable for the local conditions. Trees that died later or where the replacement also died were not replaced again.

Trees in plots were measured at or soon after planting and again in 2013, 2015, 2016, 2017 and 2018 (Fig. 2). Due to time constraints, ten of the younger plots could not be re-measured in 2017, as goes for nine plots in 2018. Tree diameter was generally measured at breast height (1.3 m above soil surface), except when trees were branching below that height or when they were very irregular at 1.3 m, in which case the diameter was mostly measured below the lower branches and where the stem was terete. Tree height was measured with a marked pole to a height of approximately 4 m and with a laser rangefinder (Vertex IV, Haglöf, Sweden or TruPulse 350R, Laser Technology, Centennial CO) for taller trees.

Biomass allometric models

Biomass was estimated using published allometric models. Chave et al. (Chave et al. 2014) present global models based on 4004 tropical trees from old-growth or secondary forests whose biomass was measured (usually by measuring the volume or fresh weight and drying subsamples of the tree) and related to height, diameter at breast height and wood specific gravity of individual trees. Their best pantropical model is:

Equ. 1: $AGB_{dh} = 0.0673 \text{ x } (\rho\ D^2\ H)^{0.976}$

with ρ: wood specific gravity, D: diameter at breast height (dbh, in cm) and H: tree height (in m).

When tree height is not available, which is challenging to measure in closed tall forests, an alternative model uses dbh and a factor scaling for height, which depends on the climate:

Equ. 2: $AGB_d = \exp(-1.803 - 0.976{*}E + 0.976{*}\log(\rho) + 2.673{*}\log(D) - 0.0299{*}(\log(D))^2)$

For the climate in La Gamba, $E = -0.0959$.

This global dataset from 58 sites spans from trees > 5 cm dbh to large forest trees but is certainly biased towards smaller size classes. This likely makes biomass estimates for large trees less reliable, but the trees we studied were comfortably within the range covered by the dataset.

Allometric models for 26 species in a Panamanian reforestation project were presented by van Breugel et al. 2011b. Given that the trees were also growing in an open reforestation site (in contrast to trees from the Chave *et al.* dataset) and included some of our species or genera, the allometric model derived from the Panama data may be more appropriate for the trees in La Gamba. The sampled trees of these 26 species had a maximum dbh between 3.8 and 26.5 cm, whereas the largest tree in the plots sampled in La Gamba measured 53 cm dbh and 78 trees (3 % of stems but 23 % of basal area) had reached a dbh > 26 cm in

2018 (Fig. 3). Species-specific models differ substantially among species (Fig. 4). However, hardly any of their species-specific models can be used for our species, and we therefore used the overall model from van Breugel *et al.* 2011b.

Equ. 3: $AGB_{vB} = \exp(-1.13 + 2.267*\log(dbh) + 1.186*\log(WSG))$

and compared it to models Equ. 1 and 2. The van Breugel model does not include tree height and all models include wood density, which scales nearly linearly with biomass.

To compare the effect of using different models, we calculated biomass for each tree using the Chave models with and without height (Equ. 1 and 2) and the van Breugel model (Equ. 3).

For trees with buttresses or a broader lower stem, diameter for growth or biomass models is generally measured above the buttresses. The young trees we measured never had buttresses above 1.3 m, but branching at lower heights or irregular stems at 1.3 m was common, which forced a diameter measurement below 1.3 m (Fig. 2). This was frequently the case in *Inga* spp., *Zygia longifolia*, *Croton schiedeanus*, and *Vitex cooperi*. Given that the diameter will be somewhat greater than if measured at 1.3 m in a regular stem, this might result in an over-estimate of the biomass. Since *Inga* spp. were planted very often in the reforestation and have high biomass, we explored the potential implication of non-standard diameter measurements on biomass estimates. Stem taper, the gradual decrease of stem diameter with height above ground, has been measured in many trees and follows a rather uniform pattern (Burkhart & Tomé 2012), but for measurements below 1.3 m, where stems can broaden substantially more than described by standard taper functions, this is not feasible without specific measurements.

AGB of the trees within individual plots was scaled to 1 ha by dividing by 36 (6 × 6 trees) and multiplying by the number of trees per ha using the spacing between trees (10,000 m^2 / (3.5 m * 4 m) = 714). Biomass was converted to CO_2 absorbed using a carbon content in biomass of 0.48, typical for tropical wood (Martin & Thomas 2011), and the carbon to

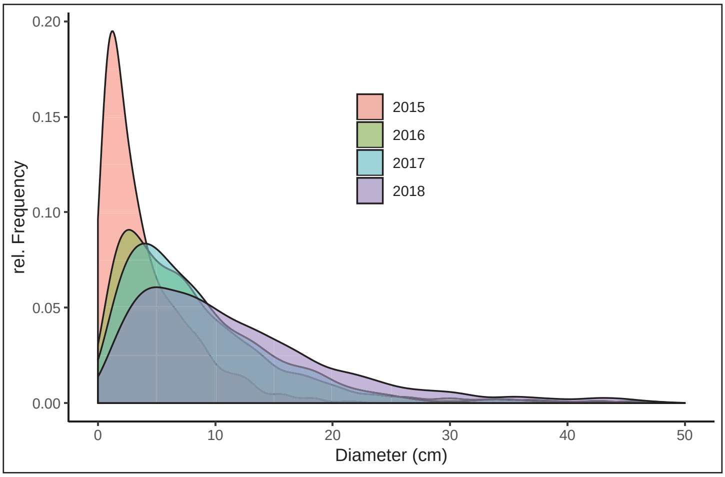

Fig. 3: Breast height diameter distributions in years 2015–2018 of trees on Finca Amable planted between 2012 and 2015. – Abb. 3: Verteilung der Brusthöhendurchmesser der auf der Finca Amable gepflanzten Bäume in den Jahren 2015–2018.

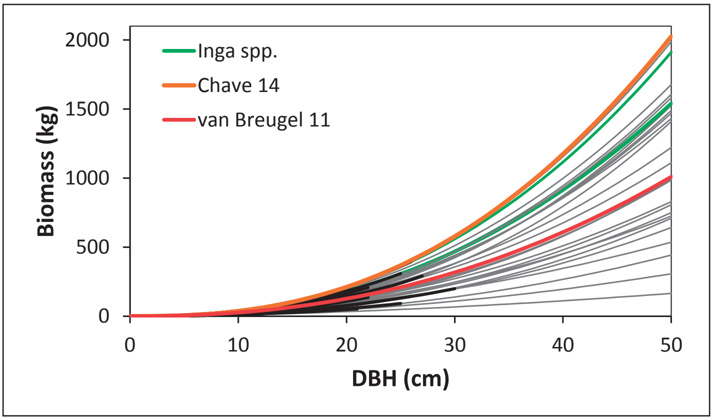

Fig. 4: General allometric models of Chave et al. (2014, Equ. 2) and the mixed-species models by van Breugel et al. (2011b). The other lines are allometric regressions for individual species (green for two *Inga* spp.) by van Breugel. Black lines show the diameter range of the data covered by van Breugel and thin grey lines extend the regressions beyond the data. – Abb. 4: Allgemeines allometrisches Modell nach Chave et al. (2014, Equ. 2) und das Modell von van Breugel et al. (2011b). Die anderen Linien entsprechen allometrischen Regressionen einzelner Arten (grün für zwei *Inga* spp.) nach van Breugel. Schwarze Linien entsprechen den Regression im Datenbereich, der von van Breugel abgedeckt wird, graue Linen gehen über diesen Datensatz hinaus.

CO_2 conversion factor of 3.66. One ton of biomass thus equates to 1.76 t CO_2 absorbed. Change in root biomass and soil carbon is difficult to measure and was ignored here. The age of the plot was the average time since the individual trees comprising each plot were planted.

To test if plot type (i.e. the composition of various functional groups) had an effect on AGB growth, we calculated a mixed effect model (LME) with AGB as the predicted variable, plot type and age as fixed factors, and age nested within plot-ID as random factor. Age and AGB were scaled by dividing by the root mean square because variables strongly differing in magnitude can bias LMEs. The LME was calculated using the R-function lmer and has the form: AGB ~ plot-type * age.s + (1 + age |plot-ID).

Results

We first tested the differences between the global biomass model (Chave et al. 2014) with or without using tree height, and the local model for a young reforestation site (van Breugel et al. 2011b). Given that *Inga* spp. had become large trees that contribute substantially to biomass and have distinctly non-linear diameter: height relationships, we also visualized if the biomass estimates in *Inga* are more affected by including height or not. Over most of the size-range of the trees measured, the global biomass model without height yielded higher biomass estimates than the model including height (grey dots are above the 1:1 line in Fig. 5, note that the axes are on log-scales, so a relatively small deviation from the 1:1 line translates into substantial differences in biomass estimates as shown in the insert of Fig. 5). *Inga* spp. did not differ from the other trees in this respect. The global model based on dbh accounts for the variation in the height:

diameter relationship by using a factor related to the local climate. The comparison with the model including height suggests that this factor is not perfectly suited for our dataset, i.e. would overestimate tree height. This is plausible because trees in the young reforestation site likely invested less in height growth than trees in closed forests, which were mostly used for the global biomass model. The van Breugel model gave higher biomass estimates for small trees than the global model with height, while for larger trees the estimates were quite similar (along the 1:1 line). The van Breugel allometries are based on smaller trees (mostly < 20 cm dbh, Fig. 4), and extrapolating biomass estimates beyond the data range of the model is problematic. Although only a small proportion of trees we measured were > 20 cm dbh, these do account for a substantial proportion of biomass. For these reasons we use the global model with height to assess biomass accumulation per plot.

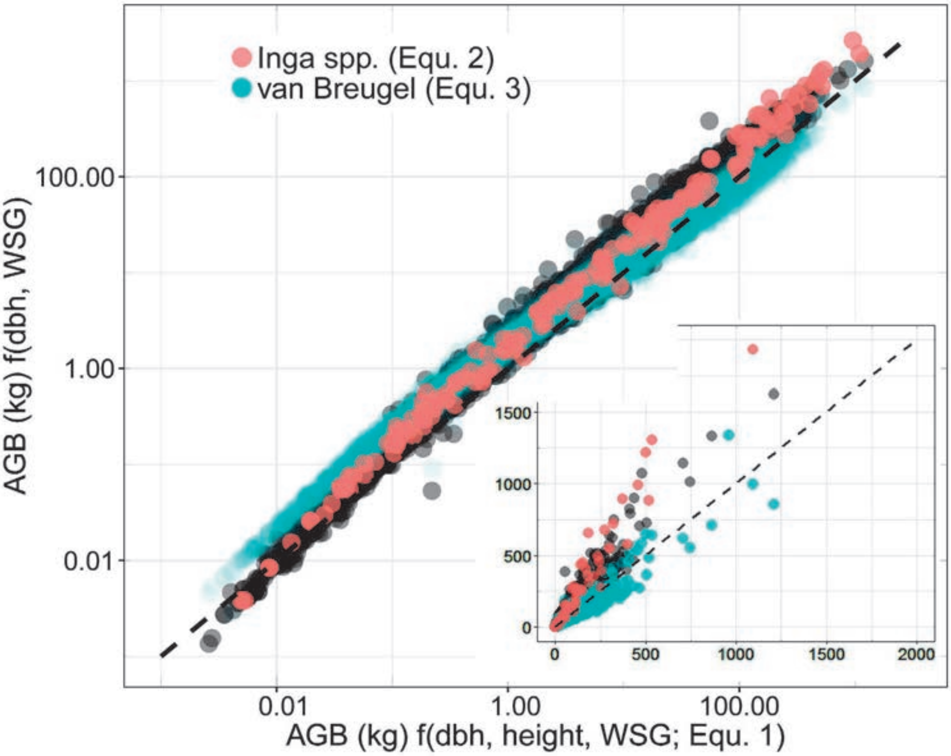

Fig. 5: Comparison between above-ground biomass (AGB) estimates based on diameter, wood specific gravity (WSG) and height (Equ. 1) against estimates based on diameter and WSG only (Equ. 2; grey symbols). Blue symbols compare the Equ. 1 with the van Breugel model for all trees (Equ. 3) and red symbols represent Equ. 2 for *Inga* spp. only. The insert shows the same data on a linear scale to highlight potential absolute errors. – Abb. 5: Oberirdischen Biomasse (AGB) berechnet aus Durchmesser, spezifischer Holzdichte (WSG) und Baumhöhe (Equ. 1) im Vergleich zur Berechnung, die nur auf Durchmesser und WSG basiert (Equ. 2, graue Symbole). Blaue Symbole vergleichen Equ. 1 mit dem van Breugel Modell für alle Bäume (Equ. 3), rote Symbole entsprechen Equ. 2 für *Inga* spp. Das Insert zeigt die gleichen Daten auf einer linearen Skala, um die absolute Größe der Fehler darzustellen.

Trees of some species tend to branch at low height (Fig. 2) so they were measured below 1.3 m, where the diameter is larger. To correct for this bias, we first compared the diameter: height correlation of trees measured at 1.3 m with the correlation of trees measured below 1.3 m. As expected, the diameter < 1.3 m was higher for a tree of the same height than the dbh (Fig. 6). We therefore corrected the diameter for trees not measured at 1.3 m by assuming that the diameter linearly decreased from ground level (0 m) to breast height (1.3 m) by 40 %. This is the best estimate so that the relationship between height and the estimated diameter is similar to the one between height and diameter measured at breast height (Fig. 6), which is the basis for all biomass calculations. This is not a perfect solution, but appears to be a viable way around the difficulty of measuring diameter in these trees, at least with the data available.

In April 2018, the average time since planting of the trees of each plot was between 3 and 5.5 years (Fig. 7). Biomass accumulation was low in the first two years, but afterwards increased substantially. Plots that were between 4.5 and 5.5 years old had reached an AGB of 12.5–52.2 t / ha (mean 35.7). The variation in biomass accumulation within any plot type (composition of functional types) was substantial and appeared to be greater than the variation between plot types. Indeed, in the mixed effect model, plot type (i.e., the func-

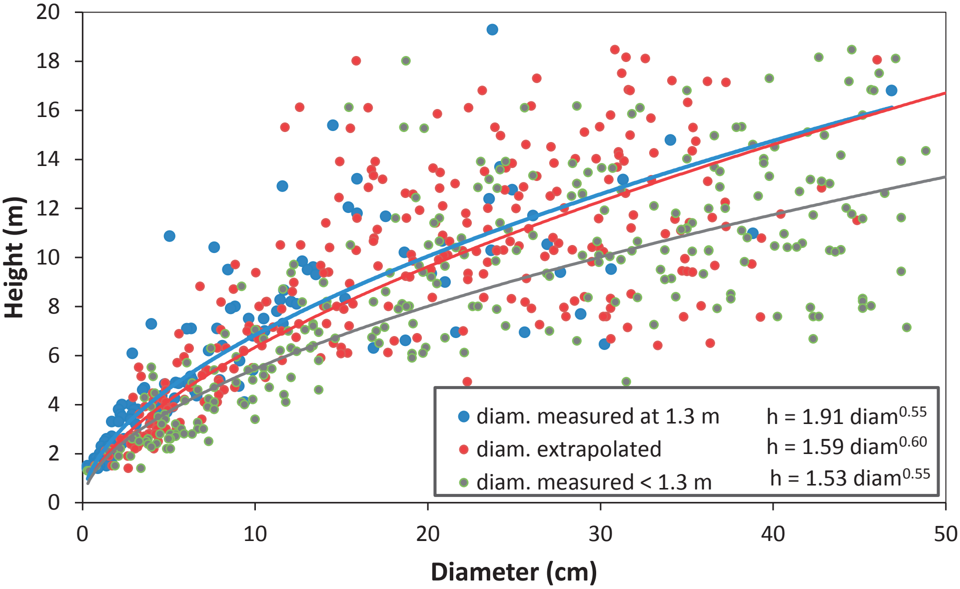

Fig. 6: Diameter: height relationships of *Inga* trees at the Finca Amable reforestation trial, measured 2016–2018, to illustrate the dbh-estimate for trees where the diameter was not measured at 1.3 m. Blue symbols and regression line show the diameter : height correlation for trees that were measured at 1.3 m; green symbols are diameter : height for trees measured below 1.3 m and red symbols are the same trees with corrected diameters (see text for details). – Abb. 6: Durchmesser: Höhen-Verhältnis für *Inga* auf der Finca Amable, gemessen 2016–2018, zur Illustration der Abschätzung des Brusthöhendurchmessers für die Bäume, deren Durchmesser nicht in 1.3 m gemessen wurde. Blaue Symbole und Regressionslinie: Bäume, die in 1.3 m gemessen wurden, grüne Symbole: Durchmesser : Höhen-Verhältnis für Bäume, die unter 1.3 m gemessen wurden, und rote Symbole entsprechen den selben Bäumen mit korrigiertem Durchmesser (Details im Text).

tional composition of trees) did not affect biomass accumulation (p > 0.4). Surprisingly, plots with low wood density (LWD) species and with LWD plus legumes were increasing biomass particularly slowly, but only two plots of each variant had been planted successfully, these were rather young because they were planted somewhat later and their biomass also lay within the large biomass variation of the other plot types (Fig. 7). In plots where *Inga* was planted, these trees accounted for a large part of total AGB (compare to small symbols in Fig 7 for biomass without *Inga*), although the biomass of some plots without legumes was similar.

Remarkably, biomass in a few of the plots with high biomass in 2017 decreased somewhat or had not increased in 2018. Inspection of the original data showed that a few very large trees, mainly *Inga*, had decreased in estimated height and biomass. Although their diameter continued to increase, the height was substantially reduced in 2018 because, as noted in the field, large parts of the crown had broken off. This would not have been seen by us-

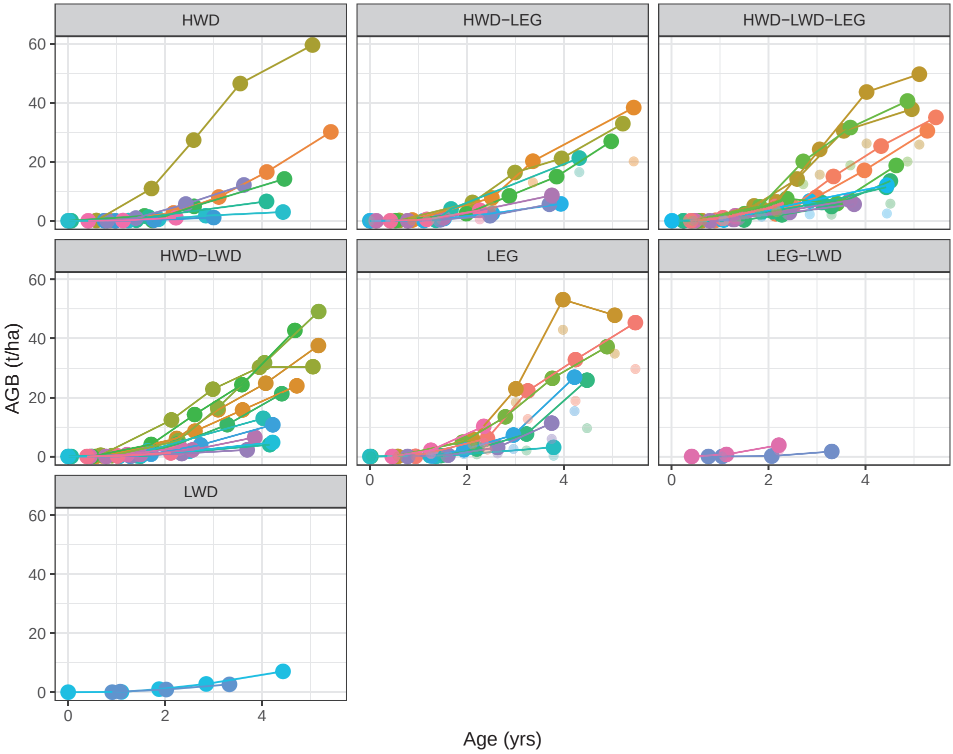

Fig. 7: Biomass accumulation over the first five years after planting in plots with different combinations of three functional groups (HWD: high wood density, LWD: low wood density, LEG: legumes). Individual plots within the panels are distinguished by symbol color and connecting lines. Small opaque symbols represent biomass excluding *Inga* spp. – Abb. 7: Biomasseakkumulation während ersten fünf Jahre nach der Pflanzung auf Versuchsflächen mit unterschiedlichen Kombinationen von drei funktionellen Gruppen von Bäumen (HWD: hohe Holzdichte, LWD: niedrige Holzdichte, LEG: Leguminosen). Die einzelnen Versuchsflächen innerhalb einer Teilgrafik sind durch unterschiedliche Farben und durchgezogene Linien gekennzeichnet. Kleine, semi-transparente Symbole entsprechen der Biomasse ohne *Inga* spp.

ing the diameter measurements alone. Similarly, a number of larger trees had broken off or been uprooted and had re-sprouted by 2018. In these cases, diameter and / or height were not useful to estimate their (remaining) biomass and these individuals were omitted from biomass estimates (i.e. treated as completely dead trees). Future surveys will show if and how damaged trees recover.

Discussion

A biomass of 35.7 t ha^{-1} corresponds to a sequestration of 61.8 t CO_2 ha^{-1}. In 2018, all planted and surviving trees in approximately 2/3 of the total area were measured (3834 trees), which includes trees in the plots as well as trees between plots. Their combined biomass was estimated at 194.8 t, which corresponds to a CO_2 sequestration of 342.2 t. This does not include trees < 1.3 m and all spontaneously regenerating vegetation. The small trees would contribute very little to biomass and in 2018 the spontaneous regeneration included very few larger trees, so the underestimate in the above-ground biomass would be very minor. Also not accounted for is below-ground biomass, which is likely to contribute more but is hard to measure and mostly not included in biomass or carbon sink estimates. If we apply the average root: shoot ratio of 0.2 recorded in four young tropical moist forest plantations (Mokany et al. 2006), the biomass and C sequestration would increase by 20 %. This is likely a conservative estimate and other compilations report higher root: shoot ratios for tropical trees (Waring & Powers 2017). The few studies looking at below-ground biomass during secondary succession of tropical forests show that it increases similarly to above-ground biomass and may reach 50 % of mature forests after 30–40 years (Martin et al. 2012). By contrast, soil carbon is affected by forest disturbance much less than AGB and hardly changes during succession (Martin et al. 2012).

How does the AGB accumulation of the active reforestation compare to natural succession and tree monocultures?

A large compilation of AGB in tropical secondary forests found a rapid accumulation of biomass, particularly during the first 40 years, after which forests had recovered more than 50 % of the biomass of old-growth forests (Poorter et al. 2016, using Equ. 2). This dataset includes a number of very young plots that can be compared to the age of trees and plots we measured. Average AGB of forests reported to be 5 yrs old was 46.6 t / ha, somewhat larger than Finca Amable plots of the same age. However, the Poorter et al. data includes sites where large remnant trees were likely present at age 0 (L. Poorter, pers. comm.) and thus show an unrealistically high biomass at a very young age. Two local secondary forest plots around La Gamba of 5–6 years and 12 years had estimated AGB of 16 and 41 t / ha, respectively, which is low compared to the reforested plots, though the age estimate may also not be very accurate (Oberleitner 2016, using Equ. 2). From the rather few comparable data of secondary forests of the same age as Finca Amable plots, it thus appears that growth is greater than or at least in the upper range of natural regeneration. If the plots are re-measured in a few years, it should become clearer if and how the biomass growth compares to secondary forests in the region and elsewhere.

Monocultures of fast-growing species might increase biomass faster than mixed-species plantations that focus on biodiversity. Trial plantations with monocultures of six tree species close to Buenos Aires (province Puntarenas, Costa Rica) had produced AGB up to 147.3 t / ha (Arias et al. 2011) after six years. While monocultures of the two exotic species tested (*Gmelina arborea* with 147.3 t and *Pinus caribea* with 85.7 t) clearly grow faster than the multi-species reforestation plots we studied, biomass growth of the other (native) species (31.8–76 t after six years) was comparable to several species combinations in Finca Amable (up to 60 t after five years, Fig. 7). To achieve the goal of high carbon sequestration it is thus not necessary to use monocultures when a more diverse tree community can sequester as much. Of course, differences between tree growth in the reforestation at Finca Amable and other tree plantations or also natural regeneration sites will also be due to differences in soil, local climate, or management of the plantation and not only the selection of tree species. To analyze these factors requires comparing multiple sites, which should be done in the future but was beyond the scope of our work.

Improving biomass estimates

Tree biomass estimates strongly depend on the allometric model (Letcher & Chazdon 2009; van Breugel et al. 2011b), thus the potential error in plot-level AGB propagated via the choice of the model is substantial. Establishing species-wise models for each region is not feasible and nearly all forest biomass estimates in diverse tropical forests therefore rely on general models. Using the same model for different stands to be compared at least reduces the bias when comparing plots, even if the absolute values may be biased. Short of cutting and weighing trees, the models we used may be improved somewhat by more detailed measurements of individual trees. Diameter is the minimum measure taken and wood density data are available for many species and are also rather easy to measure. In light of the large discrepancy between the Chave 2014 model with and without tree height (Fig. 5), it may be worthwhile to adjust the model to be more suitable for trees growing in open sites or to use models based on biomass measurements of such trees, particularly when height measurements are not available. Tree height is the next most common parameter to measure and will improve the accuracy of our estimates. More detailed measurements of tree allometries, including crown radius or stem taper, might improve models still more. Since the stem and crown shape of *Inga* differs from most other species planted and since these are large and common trees, such detailed but also time-consuming measurements should start with this genus. Airborne or terrestrial laser scanning might further improve the accuracy of data on forest structure and tree allometries substantially in a very time-efficient way and, if available, could greatly improve the accuracy of biomass estimates in the future (Taylor et al. 2015; Palace et al. 2016).

Dead trees on Finca Amable are no longer replaced and more will eventually die. Concurrently, spontaneous regeneration adds species and biomass. In the young reforestation site, the contribution of spontaneous regeneration is rather insignificant, at least for biomass. With time, the contribution of these plants will increase and spontaneous regrowth should be monitored in addition to the planted trees to characterize the effect of reforestation management on ecosystem recovery.

This first evaluation of the development of the young forest has been looking at forest biomass only. Carbon sequestration is one, but not the main aim of the reforestation project COBIGA, whose focus is mainly the protection of biodiversity. By planting more than 100 different tree species, the tree diversity is certainly much higher than in secondary forests of similar ages and comparable to that of old-growth forests. To understand the effect of high tree diversity on the diversity of non-arboreal vegetation and fauna, it would be worthwhile to monitor the change in these groups with time and in comparison with natural succession and old-growth forests.

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The importance of reforested and naturally regenerating young forest patches as secondary habitats for forest birds in the Biological Corridor La Gamba, Costa Rica

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Bernhard Paces & Andrés F. Reyes Páez

Although the protection of tropical primary forests is of primary importance for conservation, secondary forests may help to reduce increasing forest fragmentation and related biodiversity loss. This study from the Pacific lowlands of Costa Rica evaluates the potential of secondary forests to provide additional habitat and to act as stepping stones for dispersal movements of forest species. Forest understory bird assemblages were assessed at five mature and five young forest sites using mist-netting. To identify important vegetation structures for forest birds utilizing disturbed habitats (including secondary forests), point counts were used to survey birds across a habitat gradient ranging from pastures to secondary forest of different successional stages. Furthermore, bird surveys were conducted at one reforestation site to document the colonization by forest birds over a short time span of five years after the implementation of the reforestation measures. Species composition differed significantly between mist-netting sites in mature and young secondary forest, nevertheless 66.1 % of the more abundant species could be recorded in both forest types. Richness of forest specialists assessed along the gradient of habitat disturbance increased significantly with increasing number of large trees, while other habitat variables were of no or only minor importance. At our studied reforestation site, species composition changed almost continuously with progressing forest succession over the period of five years, related to an increase of the relative richness of forest birds, while the total number of species per survey remained similar. Besides potentially representing important stepping stones for forest birds by facilitating crossings of open areas, our results indicate that secondary forest patches embedded with the human-modified landscape can represent important secondary habitats for a substantial proportion of forest birds. Considering the close relationship between the number of large trees and the richness of forest specialists, further research has to evaluate the potential of using fast-growing trees to more rapidly increase the conservation value of actively restored secondary forests for the recovery of forest birds.

Schulze C.H., Leidinger P., Paces B. & Reyes Páez A.F., 2019: Die Bedeutung von wiederbewaldeten und natürlich regenerierenden jungen Waldflächen als Sekundärhabitat für Waldvögel im Biologischen Korridor La Gamba, Costa Rica.

Obwohl der Schutz tropischer Primärwälder nach wie vor das naturschutzfachliche Hauptanliegen sein muss, können Sekundärwälder dazu beitragen, der zunehmenden Fragmentierung von Wäldern und den damit verbundenen Verlust an biologischer Vielfalt entgegenzuwirken. Diese Studie aus dem pazifischen Tiefland Costa Ricas untersuchte das Potenzial von Sekundärwäldern, als zusätzliche Lebensräume und als Trittsteine für die Ausbreitung von Waldarten zu fungieren. An jeweils fünf Standorten in weitgehend ungestörten Wäldern und jungen Sekundärwäldern wurden mittels Japannetzen Unterwuchsvögel erfasst. Um für Waldvögel wichtige Vegetationsstrukturen in gestörten Lebensräumen (einschließlich Sekundärwäldern) zu identifizieren, erfolgten Punktzählungen entlang eines Habitatgradienten von Weiden bis hin zu Sekundärwäldern verschiedener Sukzessionsstadien. Darüber hinaus wurden auf einer Wiederbewaldungsfläche Vogelerhebungen über einen kurzen Zeitraum von fünf Jahren durchgeführt, um die Besiedlung durch Waldvögel nach Umsetzung der Wiederbewaldungsmaßnahmen zu dokumentieren. Die Artenzusammensetzung unterschied sich deutlich zwischen den Netznetzstandorten in alten Wäldern und jungen Sekundärwäldern. Dennoch konnten 66,1 % der häufigeren Arten in beiden Waldtypen nachgewiesen werden. Der entlang des Habitatstörungsgradienten erfasste Artenreichtum an Waldvögeln nahm mit zunehmender Anzahl großer Bäume signifikant zu, während anderen Habitatvariablen keine oder eine nur geringe Bedeutung zukam. Auf der untersuchten Wiederbewaldungsfläche änderte sich die Artenzusammensetzung über den Zeitraum von fünf Jahren mit fortschreitender Sukzession mehr oder weniger kontinuierlich, einhergehend mit einer Zunahme des relativen Artenreichtums an Waldvögeln, wohingegen die Gesamtartenzahl pro Erhebung ähnlich blieb. Unsere Ergebnisse zeigen, dass die in der anthropogen stark veränderten Landschaft eingebetteten Sekundärwälder wichtige Sekundärlebensräume für einen erheblichen Teil der Waldvögel darstellen können. In Anbetracht des engen Zusammenhangs zwischen der Anzahl großer Bäume und dem Reichtum an Waldvogelarten sollte in weiteren Untersuchungen geprüft werden, inwieweit schnell wachsende Bäume den Wert von Wiederbewaldungsflächen für Waldvögel positiv beeinflussen können.

Keywords: secondary forest, reforestation, large trees, species composition, forest birds.

Introduction

Deforestation and land use have been identified as the main drivers for reducing the primary forest areas in the world's tropical regions (Turner 1996, Lamb 1998, Laurance 1999, Debinski & Holt 2000, FAO 2010). The resulting fragmentation of tropical forests negatively affects population dynamics of forest species and contributes to the ongoing biodiversity loss (Rosero-Bixby & Palloni 1998, Sala et al. 2000, Sodhi & Smith 2007, Harvey et al. 2008, Sodhi et al. 2008).

Remaining old-growth forests proved to be of prime importance especially for species with specific habitat dependencies, restricted geographical ranges and little or no tolerance to habitat fragmentation and landscape change (Stotz et al. 1996, Soh et al. 2006). This has been especially well documented for some tropical bird species (Kofron & Chapman 1995, Estrada et al. 1997, Fjeldså 1999, Blake & Loiselle 2001, Naidoo 2004, Waltert et al. 2004, Sodhi et al. 2005, Arriaga-Weiss et al. 2008, Sodhi et al. 2008, Maas et al. 2009). Although the conservation of tropical primary forests is still the main concern, due to their irreplaceable characteristics and high value for many tropical species (Dirzo & Raven 2003), secondary forests may help to reduce increasing forest fragmentation and related biodiversity loss (Chazdon et al. 2009, Seaman & Schulze 2010, Fahrig et al. 2011).

Nowadays, it is almost impossible to find pristine, undisturbed forests (Willis et al. 2004), and the small remaining forest areas are still in the focus of commercial or illegal logging activities (Bawa & Dayanandan 1997, Vitousek et al. 1997). Thus, in an attempt to compensate this loss, forest restoration and renaturation measures are implemented on degraded land (Lamb 1998, Rey Benayas 2000, Petit & Montagnini 2006). Despite efforts to mitigate the damage to nature, there are many limitations, especially of a monetary nature, which is why most forest regeneration takes place by passive restoration (natural regeneration) (Rey Benayas 2000), resulting in various types of secondary forests (Aide et al. 2000, Wright 2005, Guerrero & da Rocha 2010). Problems for natural regeneration can be a lack of a remaining seed bank or seed dispersal (Reid et al. 2008, Reid et al. 2012).

Regenerated secondary forests are classified as being suitable to support a certain fraction of biodiversity (Sodhi et al. 2004) with a positive species recovery over time (Grau et al. 2003, Dunn 2004). They maintain more forest-dependent species of higher conservation concern than extensive plantations (Peh et al. 2006, Edwards et al. 2010, 2011). Likewise, some studies suggested that forests with secondary growth are potential reservoirs of biodiversity (Chazdon 1998, 2008, Brook et al. 2006, Wright & Muller-Landau

2006a, 2006b, Barlow et al. 2007a, 2007b), and could also serve as temporal refuges, foraging areas and – most importantly – as landscape structures connecting remaining forest fragments (Myers 1997, Blake & Loiselle 2001, Chazdon 2003, Fischer et al. 2006, Höbinger et al. 2012). Therefore, even though they differ in structural and floristic composition from primary forests, they can contribute to the creation of biological corridors for forest species. Also, due to their relatively high productivity, these areas should be incorporated into agroecosystems, which consequently may benefit from indirect services (e.g. pollination and pest control) provided by forest species interacting with the surrounding human-dominated landscape matrix (Finegan 1992, Chazdon 2008).

Secondary forests are rapidly expanding in the tropics (Wright 2005). They are especially emerging in abandoned areas which are not further used for agricultural purposes (Thomlinson et al. 1996). Hence, they should be considered in approaches improving biodiversity conservation on a landscape scale.

In this study from the Pacific lowlands of Costa Rica, birds were used to study the capability of secondary forests to facilitate forest species at the margin of protected rainforest areas. We were particularly interested in the potential of these secondary forests to increase landscape permeability and to act as corridor habitats for forest birds. In particular, the following hypotheses were tested: (1) Although secondary forests can support a relatively high bird species richness, they are characterized by a distinct species composition. (2) Species richness and composition of forest species assemblages in disturbed habitats depend on larger trees representing a key habitat structure for forest species. (3) Species composition of secondary forests changes with progressing vegetation succession, (4) due to an increasing richness and abundance of forest-dependent species with progressing forest succession age. To test these hypotheses, we conducted mist-netting in order to compare understory bird assemblages of old-growth forests and young secondary forests created by reforestation measures. We further conducted bird surveys in a variety of habitats mimicking a forest succession sequence, and monitored changes of the bird assemblage of a young secondary forest over a time period of six years after reforestation measures were implemented.

Methods

Study area

The study area is located in the "Golfo Dulce" region of southwestern Costa Rica, between the "Piedras Blancas" National Park (including "Esquinas" Forest, Valley "Río Esquinas" and Valley "Río Bonito") and the "Fila Gamba". The Golfo Dulce region is classified as an important Endemic Bird Area (EBA 021: South Central American Pacific slope) hosting several range-restricted bird species (Stattersfield et al. 1998, Birdlife International 2012) and belonging to one of the most diverse regions in terms of faunistic and floristic richness within the tropical region of Central and South America (Hammel et al. 2004, Lobo Segura & Bolaños 2005). So far, more than 300 bird species (including several range-restricted species and subspecies of high conservation value) (Tebb 2008) have been recorded in our study area in the vicinity of the Tropical Research Station La Gamba (N 08°42.063′, W 083°12.102′).

The study area has an annual precipitation of 6,000 mm with a rainfall peak between August and November and lowest precipitation between January and March; the annual mean temperature is 28.5° C (Weissenhofer & Huber 2008). The natural vegetation is mainly represented by tropical lowland wet forest (Weissenhofer et al. 2008a). The surroundings of the village "La Gamba", which is located in the center of our study area, are characterized by pastures with small and elongated forest patches, strips of gallery forests, few big plantations (e.g. oil palm), old (and mostly abandoned) agroforests (cacao) and annual cultures (e.g. rice) (Seaman & Schulze 2010, Höbinger et al. 2012).

Assessing understory bird assemblages in old-growth forest and secondary forest

To assess the understory bird assemblages of old-growth forest and young secondary forests, birds were mist-netted at five replicate sites in each forest type (Fig. 1). A one-way ANOSIM was used to test for differences in spatial distances between mist-netting sites belonging to the same habitat type. It did not indicate that sites belonging to the same habitat type were closer situated to each other than sites belonging to different forest types (Global $R = 0.002$, $p = 0.357$). Hence, we do not expect spatial autocorrelation to affect our analyses. At each site, six 12 m mist nests (2 with 16 mm mesh size, 2 with 30 mm mesh size, 2 with 45 mm mesh size) were used to trap birds during four mist-netting events. During each mist-netting event it was aimed to trap birds for a total of 15 hours, between 5:30–15:00 on the first day and between 5:30–11:00 on the second day. Hence, the total mist-netting effort per site should have been 60 hours. However, due to bad weather conditions (mist-nets were not operated during strong rain), the total mist-netting duration per site

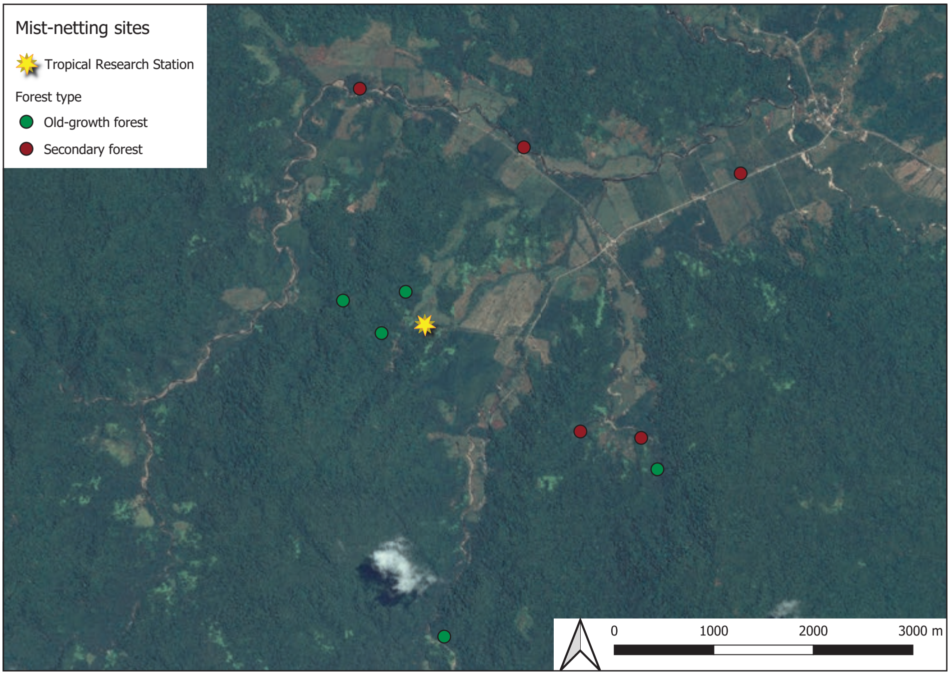

Fig. 1: Map indicating the mist-netting sites used to assess the composition of understory bird assemblages in young secondary forests and old-growth forests. – Abb. 1: Karte der Netzstandorte für die Untersuchung der Artenzusammensetzung von Unterwuchsvogelgemeinschaften in jungen Sekundärwäldern und alten Wäldern.

(± SD) was only 59.70 (± 0.67) and 59.75 (± 0.35) hours in old-growth and secondary forest, respectively. As the difference was extremely small, we assume that understory birds were sampled with an identical sampling effort in both forest types. As the focus was on diurnal understory forest birds, waterbirds (American Pygmy Kingfisher: 1 bird mist-netted) and nocturnal birds (Common Pauraque: 2 birds mist-netted) were excluded from all analyses.

Assessing bird assemblages across a gradient of vegetation succession

To study how forest bird assemblages change with progressing vegetation succession, birds were assessed using point counts at study sites representing a chronosequence of forest succession. Hence, five different habitat types were sampled, ranging from pastures with scattered shrubs and small trees, to planted young secondary forests, naturally regenerated young secondary forests, old cacao agroforestry systems and naturally regenerated old secondary forests (Fig. 2). Four replicate sites were selected per habitat type. Sites were located between N 08°41.112'– N 08°43.308' and W 083°11.828'–W 083°12.277' at altitudes between 67–146 m a.s.l. The size of each surveyed habitat patch was >1.5 ha with homogeneous vegetation and similar biotic characteristics among the habitat categories. The minimum distance between studied sites was 200 m, which is reported to make survey points statistically independent (Gutzwiller 1991, Whitman et al. 1998, Barlow et al. 2007a, 2007b, Edwards et al. 2011). All census points were located on private farm areas; some of these areas are part of a forest restoration project developed by the Tropical Research Station "La Gamba" in collaboration with the local community. Other sites were situated on abandoned land once used for agricultural purposes. All sites had a similar distance to areas of old-growth forest (<300 m to forest margin). A one-way ANOSIM testing for differences in spatial distances between census points belonging to the same habitat type did not indicate a significant effect, thus indicating that spatial autocorrelation should not weaken our results (Global $R = 0.005$, $p = 0.439$).

To assess the bird assemblages, point counts were conducted between 22 November 2010 and 27 January 2011. Each census point was visited ten times between dawn and 10:00 a.m., which should allow for recording of a large proportion of the sites' bird species (Ralph et al. 1995). Each point count lasted for 20 minutes. The order of surveyed census points was random to avoid a sampling bias that can be caused by a reduction in bird activity during the course of the day (Blake 1992, Blake & Loiselle 2001). Bird species were recorded visually and acoustically within a radius of 30 m. Unfamiliar bird songs, or songs from birds that could not be visually identified (Parker 1991), were recorded using a Telinga Pro7 StereoDat-Microphone and a Foster FR-2 recorder. Bird identifications were facilitated using Garrigues & Dean (2007). Recorded bird songs were then identified with the help of Isabell Riedl (University of Vienna), who had two years of experience with bird identification in this region. Additionally, the xeno-canto online-database (http://www.xeno-canto.org/), CD recordings by Ross Jr. & Whitney (1995), Ross Jr. (2000), and Boesman (2006) were used for identification.

Birds that were observed flying over the census-point, birds without obvious direct habitat affiliation (swifts, swallows), all freshwater birds (ducks, herons, kingfishers, waders etc.), birds with a nocturnal life mode (nightjars, owls) and those bird species associated with open land habitats were not considered in any statistical analysis (Borges & Stouffer 1999, Schulze & Riedl 2008). Birds were classified according to their habitat affinity as forest specialists (restricted to old-growth forest), forest generalist and open country bird species (Stiles & Skutch 1989, Schulze & Riedl 2008, Tebb 2008, Seaman & Schulze 2010). Nomenclature and taxonomy refer to Gill & Donsker (2012).

The vegetation structure of all study sites was characterized by measuring six variables within a radius of 30 m around census points: density of large trees, density of small trees, maximum tree height, canopy closure, understory density and herb cover. These variables have been shown to be important in similar studies (e.g. Thiollay 1999, Barlow et al. 2007b, Abrahamczyk et al. 2008, Reid et al. 2012). Large trees were defined as trees with a diameter at breast height (DBH) greater than 10 cm. Small trees were defined as trees with a height not larger than 10 m. These two measurements are important to quantify the structural diversity of woody vegetation in secondary forests (Guariguata et al. 1997, Guariguata & Ostertag 2001). Height of the tallest tree was measured with a range finder (Nikon Laser 1200S). To quantify canopy closure, a photograph of the canopy was taken directly above the census point. This was then digitalized and processed with a free software photo editor ImageJ 1.44p (Abramoff et al. 2004). The editing process involved changing the photos to binary mode in which pixels will have values for black/white colours. Then it is possible to analyze canopy closure in terms of percentage of pixels. Un-

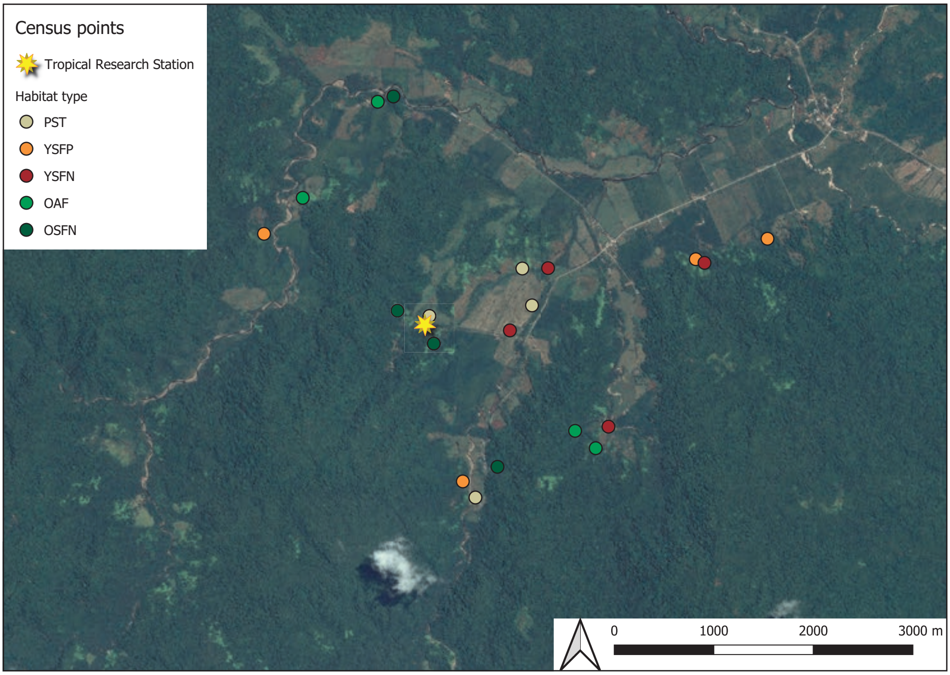

Fig. 2: Map indicating census points at which bird counts were conducted to assess bird assemblages in five different habitat types ranging from pastures with scattered shrubs and small trees (PST), to planted young secondary forests (YSFP), naturally regenerated young secondary forests (YSFN), old cacao agroforestry systems (OAF) and naturally regenerated old secondary forests (OSFN). – Abb. 2: Karte der Zählpunkte, an denen Vogelzählungen zur Ermittlung der Vogelgemeinschaften in fünf verschiedenen Habitattypen durchgeführt wurden: Weide mit vereinzelten Büschen und niedrigen Bäumen (PST), gepflanztem jungen Sekundärwald (YSFP), natürlich regeneriertem jungen Sekundärwald (YSFN), altem Kakao Agroforstsystem (OAF), und natürlich regeneriertem alten Sekundärwald (OSFN).

derstory density was determined by taking twenty different distance measurements to the nearest tree stem or bush around the census point above 1.5 m height. The twenty measurements were averaged into a single estimate value; this was done using a laser distance measurement device (Leica DISTO™ D2). Herb cover was estimated as percentage cover, taking into account all herbaceous species, and some low woody plants less than 15 cm tall.

Assessing temporal succession of birds at Finca Amable

To assess changes in species richness and composition of bird assemblages at the reforestation site Finca Amable, a total of seven bird surveys were conducted between July 2013 and November 2018. Every survey unit consisted of two zig-zag transects covering the entire area of the reforestation area (4 ha). The two surveys per unit were on average 13 days apart (min.-max. = 5–19 days). Average survey time was 204 min (max.-min = 182–243 min). During each survey unit, all acoustically and visually detected birds were counted, trying to avoid double counts.

Data analysis

Recorded species richness of bird assemblages recorded by mist-netting at the five old-growth and the five secondary forest sites was compared using a t-test. We further calculated species accumulation curves for both groups of forest sites using the iNEXT package (Hsieh et al. 2014). Similarity relationships of species assemblages assessed by mist-netting at the 10 forest sites were assessed using Bray-Curtis similarities (based on square-root transformed abundances, not considering recaptures). Subsequently, similarity relationships between sites were visualized using a non-metric multidimensional scaling (NMDS) ordination. The ordination was considered to reliably visualize similarity relationships when the associated *stress* value was <0.2 (Clarke 1993). A one-way ANOSIM was calculated to test for differences in species composition between the two forest types.

To identify relationships between the number of recorded species of forest generalist as well as forest specialist birds and the habitat variables, Pearson correlations were calculated. Afterwards, False Discovery Rate (FDR) transformations were applied to correct for bias caused by multiple testing (Pike 2011).

Changes in bird species composition between surveys over the time period 2013–2018 at the reforestation site Finca Amable were quantified using Bray-Curtis similarities (using square root transformed abundances) and visualized in a NMDS ordination. Since we expected species composition to change with progressing forest succession, extracted Dimension 1 and 2 values of the resulting NMDS ordination were correlated with the survey period order using a Spearman rank correlation. A Spearman rank correlation was also used to test if the total number of recorded bird species and in particular the relative richness of forest bird species changed with progressing forest succession.

Results

Species richness and species composition in old-growth and secondary forests

Species assemblages assessed by mist-netting were very similar in terms of species richness in both forest types, with a mean number of recorded species (± Std.dev.) of 31.6 (± 7.37) and 32 (± 7.81) species recorded per site in old-growth and secondary forest, respectively

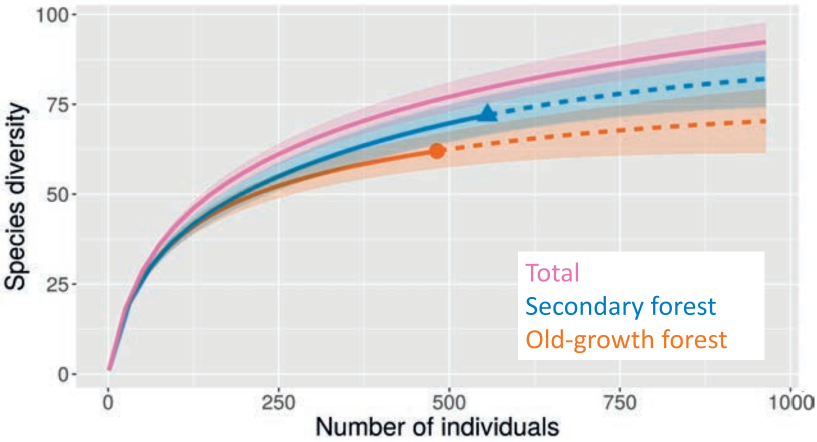

Fig. 3: Species accumulation curves with 95 % confidence intervals for the two forest types old-growth and secondary forest. Additionally, the curve for all sites combined is provided. – Abb. 3: Artenakkumulationskurven mit 95 % Konfidenzintervallen für alten Wald und Sekundärwald. Zusätzlich wird die Kurve für die Gesamtheit aller Standorte gezeigt.

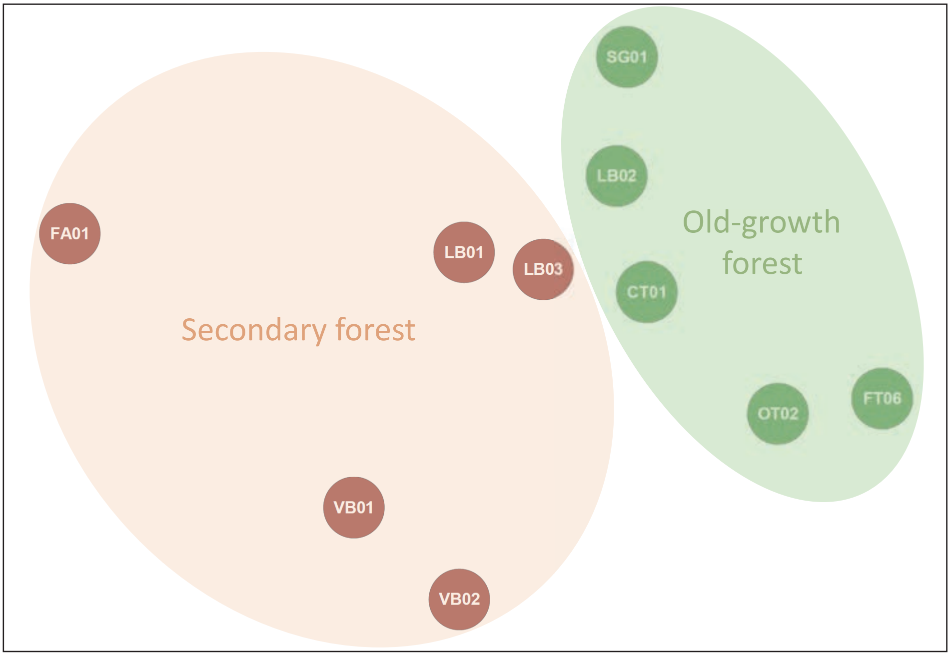

Fig. 4: NMDS ordination based on Bray-Curtis similarities (with square root transformed abundances) visualizing similarity relationships of understory bird assemblages assessed by mist-netting at five secondary and five old-growth forest sites. *Stress* = 0.04. – Abb. 4: NMDS-Ordination basierend auf Bray-Curtis-Ähnlichkeiten (mit wurzeltransformierten Abundanzen). Visualisiert werden die Ähnlichkeitsbeziehungen von Unterwuchsvogelartengemeinschaften, die an fünf Sekundärwaldstandorten und fünf Standorten in altem Wald mittels Japannetzfang untersucht wurden. *Stress* = 0,04.

(t-test: t = 0.08, p = 0.9357). Species accumulation curves calculated for both groups of forest sites did indicate a slightly lower richness of old-growth understory birds (Fig. 3). In contrast, species composition differed clearly between both forest types as indicated by the NMDS ordination (Fig. 4) and by a calculated one-way ANOSIM (Global R = 0.456, p = 0.008). However, two of the secondary forest sites (LB01 and LB03) were characterized by species assemblages which closely resembled old-growth forest ones (Fig. 4).

Of the 95 mist-netted understory bird species, 42.5 % were recorded in both forest types, while 23.4 % and 34.0 % of the species were unique to old-growth and secondary forest, respectively. When excluding species which were only mist-netted once or twice as they

Tab. 1: Results of Pearson correlations between habitat variables and species richness of forest specialists and forest generalists. Results printed in bold remained significant after calculating the False Discovery Rate. – Tab. 1: Ergebnisse von Pearson-Korrelationen zwischen Habitatvariablen und Artenreichtum von Waldvogelspezialisten und -generalisten. Fettgedruckte Ergebnisse waren auch nach Berechnung der Falscherkennungsrate signifikant.

Habitat variables	**Forest specialists**		**Forest generalists**	
	R	**FDR-adjusted *p***	***R***	**FDR-adjusted *p***
Herb cover	-0.55	**0.023**	-0.01	0.953
Maximal tree height	0.60	**0.014**	0.51	**0.038**
Large trees	0.85	**<0.001**	-0.02	0.953
Understory density	-0.61	**0.014**	-0.09	0.787
Small trees	0.37	0.143	0.50	**0.038**
Canopy closure	0.59	**0.014**	0.30	0.252

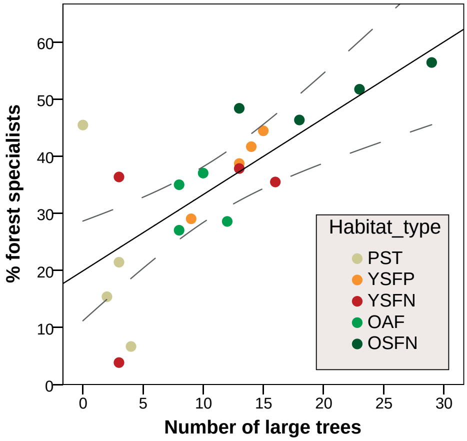

Fig. 5: Relationships between percentage of forest specialists at census points and the number of large trees. Different symbol colours indicate the habitat types: PST – Pastures with scattered shrubs and small trees, YSFP – planted young secondary forests, YSFN – naturally regenerated young secondary forests, OAF – old agroforestry systems and OSFN – naturally regenerated old secondary forests. – Abb. 5: Zusammenhang zwischen Anteil an spezialisierten Waldvögeln an den Zählpunkten und der Zahl großer Bäume. Die Symbolfarben zeigen unterschiedliche Habitattypen: PST – Weide mit vereinzelten Büschen und niedrigen Bäumen, YSFP – gepflanztem jungen Sekundärwald, YSFN – natürlich regeneriertem jungen Sekundärwald, OAF – altem Agroforstsystem, und OSFN – natürlich regeneriertem alten Sekundärwald.

are very rare or difficult to trap, figures change substantially. Of the remaining species, as many as 66.1 % could be recorded in both forest types, while only 15.3 % were unique to old-growth forest and 18.5 % were only mist-netted in secondary forest.

Species richness and species composition across a chronosequence of vegetational succession

A total of 115 forest bird species were recorded during the point counts, including 61 species classified as forest generalists and 54 classified as forest specialists. Richness of forest specialists was most strongly related to the number of large trees in that the number of specialist species increased significantly with the increasing number of large trees. Other variables were only weakly (herb cover, maximum tree height, canopy closure, understory density) or not significantly related to the richness of forest specialists. Variance in species numbers of forest generalists was only weakly positively related to the maximum tree height and the density of small trees (Tab. 1). While the total number of bird species recorded at census points was not related to the number of large trees ($r = 0.036$, $p = 0.8793$), the percentage of forest specialists increased significantly ($r = 0.702$, $p = 0.0006$; Fig. 5).

Colonization of a reforested area by forest birds

Bird species composition of Finca Amable changed almost continuously with progressing forest succession as Dimension 1 values of the resulting NMDS ordination (Fig. 6) are correlated with the survey period order ($r_s = 0.942$, $p = 0.0028$). This change in species composition can be attributed to an increase of the relative richness of forest birds ($r_s = 0.893$,

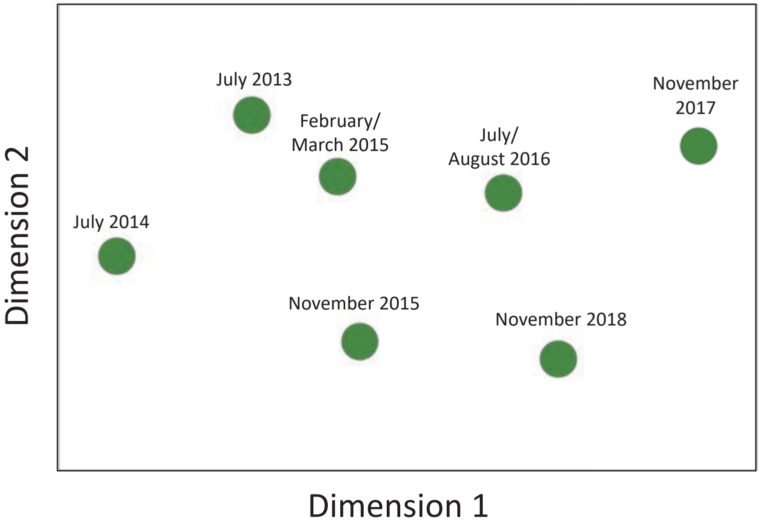

Fig. 6: NMDS ordination based on Bray-Curtis similarities (with square root transformed abundances) visualizing similarity relationships of understory bird assemblages surveyed at the reforestation area Finca Amable over a period of six years after the planting of the first trees. Stress = 0.01. – Abb. 6: NMDS-Ordination basierend auf Bray-Curtis-Ähnlichkeiten (mit wurzeltransformierten Abundanzen). Visualisiert werden die Ähnlichkeitsbeziehungen von Unterwuchsvogelartengemeinschaften, die auf der Wiederbewaldungsfläche Finca Amable über einen Zeitraum von sechs Jahren nach Beginn der Baumpflanzungen untersucht wurden. Stress = 0,01.

p = 0.007; Fig. 7), while the total number of recorded species per survey did not change within the period of five years (r_s = 0.206, p = 0.658).

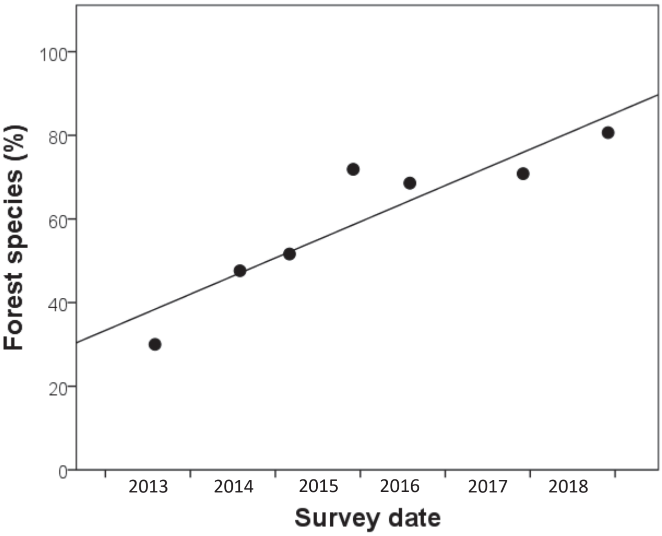

Fig. 7: Change in the relative richness of forest bird species surveys at the reforestation area Finca Amable over a period of six years after the planting of the first trees. – Abb. 7: Veränderung der relativen Artenvielfalt von Waldvogelarten am Wiederbewaldungsstandort Finca Amable über einen Zeitraum von sechs Jahren nach Beginn der Baumpflanzungen.

Discussion

The importance of secondary forest for forest birds

Although understory species assemblages assessed by mist-netting at old-growth forest and young secondary forest sites showed a similar species richness, both forest types differed clearly in species composition. However, a substantial proportion of old-growth forest species could also be found in young secondary forest. It must also be emphasized that, in the latter forest type, several forest species were also recorded which appear to prefer disturbed forest, underlining that such secondary forest patches also contribute to the local forest species richness. For example, the 21 individuals of Cherrie's Tanager (*Ramphocelus costaricensis*), an endemic of the Pacific slope of Costa Rica and Panama, were all mist-netted in young secondary forests. Other common (>8 trapped individuals) resident breeding birds exclusively trapped in secondary forests were Buff-throated Saltator (*Saltator maximus*) with 10 individuals, Clay-colored Thrush (*Turdus grayi*) with 22 individuals, Northern Royal Flycatcher (*Onychorhynchus mexicanus*) with 9 individuals, Stripe-throated Hermit (*Phaethornis striigularis*) with 10 individuals and White-tipped Dove (*Leptotila verreauxi*) with 18 individuals. Only two of the more common resident birds were exclusively trapped in old-growth forest, the Rufous Mourner (*Rhytipterna holerythra*) with 11 individuals and the Sulphur-rumped Flycatcher (*Myiobius sulphureipygius*) with 12 individuals. Our results also underline the importance of secondary forest for northern migrants, as all of the 15 individuals of the Northern Waterthrush (*Parkesia noveboracensis*) were mist-netted in young secondary forests. The importance of secondary forest for northern migrants was already reported by other studies from the Neotropics (Karr 1976, Martin 1985, Reid et al. 2008). It was even assumed that several of the Nearctic migrants can play an important role as seed dispersers and, hence, contribute substantially to the regeneration of young secondary forests (Blake & Loiselle 1992, Reid et al. 2008).

Secondary forests predominantly support generalists (Brook et al. 2003) and probably act as reproductive "sinks" for some forest species (Battin 2004, Peh et al. 2005, Aubrecht & Schulze 2008). However, the total of 115 species of forest-depending birds (with 47 % classified as forest specialists) recorded in secondary forests of our study area by point counts underlines their substantial contribution to bird diversity on the landscape level. That secondary forest may result in an increase of species richness at a regional level was already emphasized by other studies (Graham & Blake 2001, Hughes et al. 2002).

Furthermore, the conservation value of secondary forest sites is highlighted by our records of several range-restricted forest species such as the Charming Hummingbird (*Amazilia decora)*, Baird's Trogon (*Trogon bairdii*), Black-hooded Antshrike (*Thamnophilus bridgesi*), Riverside Wren (*Cantorchilus semibadius*), Black-cheeked Ant-Tanager (*Habia atrimaxillaris*) and Spot-crowned Euphonia (*Euphonia imitans*), and the near threatened forest specialist species, the Golden-winged Warbler (*Vermivora chrysoptera*).

Large trees as key habitat structure for forest birds

Across our census point sites representing a gradient of vegetational complexity, large trees emerged as an important variable for the richness of forest-dependent species. Overstory tree density also proved to be an important explanatory variable for differences in the species richness of various functional groups of forest birds across a gradient from natural forest to secondary forest, agroforestry systems and annual cultures in Cameroon (Waltert et al. 2005). Some earlier studies highlighted canopy closure and structural complexity (i.e. number of vegetation layers) of secondary vegetation as important determinants for the species richness and the composition of tropical forest birds (Blake & Loiselle 2001, George & Zack 2001, Dunn 2004, Barlow 2007b, Borges 2007). The number of large trees contributing to the vertical complexity of secondary forests and the canopy closure seemed to influence the species richness of specialist birds also at our study sites. However, the effect of the number of large trees appeared to have a particularly important role for the occurrence of forest specialist bird species (but not forest generalists). The value of large trees as a key structural element for many forest birds, providing breeding niches and food resources, has already been emphasized by other studies (Sodhi et al. 2005, Van Bael et al. 2007, Abrahamczyk et al. 2008). However, the presence of large trees may only facilitate the colonization of young secondary forests by bird species that require living trees, because such young forests are mostly still lacking larger dead trees (Dewalt et al. 2003).

Although early stages of forest succession may not be appropriate breeding habitat for many forest specialists (MacArthur & MacArthur 1961, Bowen et al. 2007), at least some of them may infrequently use various resources provided by this forest type, e.g. when situated close to the margin of old-growth forest. For example, at one of our pastures with scattered shrubs and small trees, eight forest specialist species were reported. Most of these being omnivorous (i.e. *Attila spadiceus*, *Cyanocompsa cyanoides*, *Euphonia imitans*, *Ornithion semiflavum* and *Ramphastos swainsonii*), but also including one insectivore (*Pachyramphus aglaiae*), one granivorous (*Leptotila cassini*) and one frugivore species (*Penelope purpurascens*). The presence of such forest specialist bird species can be explained by the presence of single larger remnant trees (Guevara et al. 1986). Such scattered large trees are considered to be keystone structures in human-modified areas, offering benefits to forest birds, not only at a landscape level (e.g. connectivity) but also locally (e.g. structural complexity, nesting) (Manning et al. 2006).

Importance of secondary habitats as corridors for the conservation of forest birds

The relatively high number of forest-dependent species recorded in secondary forests indicate that they may also have a high potential to improve connectivity between remaining areas of old-growth forests in our study area. In fact, even smaller groups of trees embedded in the human-dominated landscape can already be utilized by at least some forest birds as stepping stones for dispersal movements between forest fragments (Gillies and Cassady St. Clair 2010). In contrary to the assumption that the majority of tropical forest birds have a very limited dispersal capability, a recent study from southeastern Brazil reported movements of forest birds between forest fragments across open areas of up to 650 m (Marini 2010). Although direct evidence (e.g. by recaptures of banded birds) is still missing, we can thus expect that most of the secondary patches in our study area can act as stepping stones for at least a certain proportion of forest birds, as the majority of them is located well within a distance of less than 500 m to the margin of larger forest areas. Besides their importance as stepping stones, secondary forests attached or adjacent to mature forest may increase the habitat quality for forest species in the buffer zone around mature forest, thus reducing negative edge effects (Fischer et al. 2006).

Rapid colonization of secondary forest patches by forest birds

A recent meta-analysis documented that species richness of different vertebrate groups, including birds, reached a level in secondary forests similar to that of mature reference forest sites within a couple of decades of natural succession. However, species compositional similarity still differed even between old secondary forests and mature or old growth forest. In birds, the main reason may be the delayed recovery of forest specialists and functional groups such as insectivorous birds (Acevedo-Charry & Aide 2019). This is also emphasized by our study on the recovery of forest species at the reforestation site Finca Amable. Although the relative contribution of forest species, including forest generalists able to utilize disturbed forests, dramatically increased from 30 to nearly 80 % during a period of only six years after the start of the reforestation, true forest specialists common in adjacent mature forest (e.g. Black-cheeked Ant-Tanager) are still missing. Another study on birds comparing data from 44 tropical secondary forest patches with nearby primary forest sites also indicated that the species richness of forest specialists increased continuously with secondary succession but reached similar species richness of forest specialists only after 100 years (Sayer et al. 2017).

Conclusions

Besides potentially representing important stepping stones for forest birds by facilitating crossings of open areas, our results indicate that secondary forest patches embedded with the human-modified landscape can represent important secondary habitats for a substantial proportion of forest birds. Additionally, when attached to the forest margin they may act as important buffer habitats at the border of protected forest areas. Hence, conservation efforts should aim to protect such emerging forest structures and additionally should target to increase the density of such forest patches to enhance the permeability of the human-modified landscape. Such measures will not only contribute to interconnecting remaining fragments of old-growth lowland rainforest but will improve the function of the biological corridor COBIGA as a link between the lowland forest zone and the adjacent mountain range further inland (Weissenhofer et al. 2008b). This may also help to maintain and perhaps partly re-establish elevational movements of forest birds between forests of the adjacent mountain range and the larger lowland forest blocks (e.g. Piedras Blancas National Park).

Although secondary forests cannot replace old-growth and undisturbed forest (Sayer et al. 2017, Acevedo-Charry & Aide 2019), they represent an important landscape structure contributing to maintaining biodiversity. This is particularly important as biodiversity is declining even in protected areas (Laurance et al. 2012). Consequently, young secondary forest should be protected, particularly in human-dominated landscapes where only small patches of mature forest remain (Sayer et al. 2017).

Reforested and naturally regenerating young forests proved to be of similar importance for forest birds. However, considering the close relationship between the number of large trees and the richness of forest specialists, further research has to evaluate the potential of using fast-growing trees to more rapidly increase the conservation value of actively restored secondary forests for the recovery of forest birds, particularly forest specialists. Potential candidate trees could be fast-growing native species such as *Terminalia amazonia* (Combretaceae) and *Vitex cooperi* (Verbenaceae) (Lechner et al. 2012).

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Macroinvertebrates as indicators in tropical streams with different land use in southern Costa Rica

Verena G. Duschek, Monika Springer, Georg H. Niedrist & Leopold Füreder

Costa Rica is one of the countries with the highest species richness worldwide and forms an important land bridge between North and South America. In the past, Costa Rica suffered from deforestation due to conversion of primary forests into plantations or pastures, which caused degradation and destruction of natural habitats. To evaluate the potential influence of anthropogenic land use on tropical river ecosystems, we compared macroinvertebrate assemblages of forested and impacted reaches of three rivers near the Tropical Field Station La Gamba. Water quality was evaluated with the BMWP-CR index, which resulted in excellent water quality for both forest sites and impacted sites. Nevertheless, the effects of land use and deforestation were apparent in the latter, as evidenced by high abundances of only few taxa, such as chironomids and oligochaete worms, and low macroinvertebrate diversity. Our results indicate that intact riparian vegetation reduces the impact of anthropogenic influence significantly and should be established to avoid ongoing stream degradation.

Duschek V.G., Springer M., Niedrist G.H. & Füreder L., 2019: Makroinvertebraten als Indikatoren in tropischen Bächen mit unterschiedlicher Landnutzung im Süden Costa Ricas.
Costa Rica ist eines der artenreichsten Länder der Welt und bildet eine wichtige Landbrücke zwischen Nord- und Südamerika. In der Vergangenheit litt Costa Rica unter der Abholzung und Umwandlung von Primärwäldern in Plantagen oder Weiden, was zu einem Abbau und Zerstörung der natürlichen Lebensräume führte. Um den potenziellen Einfluss der anthropogenen Landnutzung auf tropische Flussökosysteme zu bewerten, haben wir Makroinvertebraten Gemeinschaften von bewaldeten und beeinflussten Bereichen von drei Flüssen nahe der Tropenfeldstation La Gamba erfasst und verglichen. Die Wasserqualität wurde mit dem BMWP-CR-Index evaluiert und zeigte hervorragende Wasserqualität sowohl für Waldstandorte als auch für beeinflusste Standorte. Dennoch waren Auswirkungen der Landnutzung und der Entwaldung an anthropogen beeinflussten Standorten deutlich erkennbar, und zeigten sich in hohen Abundanzen weniger Taxa wie etwa Chironomiden und oligochaeten Würmern, sowie in der geringeren Makroinvertebraten- Vielfalt. Unsere Ergebnisse deuten darauf hin, dass eine intakte Ufervegetation die Auswirkungen anthropogener Einflüsse erheblich verringert und etabliert werde sollte, um zunehmende Verschlechterung des Flusses zu vermeiden.

Keywords: Golfo Dulce region, freshwater habitats, human impact, water quality index, Central America.

Introduction

Costa Rica is an acknowledged hotspot of biodiversity and features a high variety of forests, wetlands, rivers, estuaries and marine environments. In some of its natural landscapes, it supports one of the highest known species densities and diversities on earth (Obando 2002). Nevertheless, due to the tropical climate conditions, it is also a well-known source for agricultural products, such as coffee, bananas, sugar cane, palm oil and pineapples (Kohlmann et al. 2015), often produced in monoculture plantations. Along with these impacts, the expansion of pastures for meat production in the past has resulted in the destruction of primary habitats and severe fragmentation of the remaining forest. Human land use has caused changes, degradation and destruction of natural landscapes, which has a significant effect on the diversity and distribution of the tropical flora and fauna.

Deforestation not only causes the disappearance or diminishing of rainforest area but also a strong fragmentation of habitats. As forest patches become increasingly smaller and more and more separated from each other, the dispersal and behaviour of flora and fauna is strongly impacted.

Under natural conditions, river ecosystems and riparian and floodplain areas are strongly interlinked (e.g. Vannote et al. 1980) and riparian vegetation can strongly influence the aquatic conditions and habitats. Riparian forests can provide coarse and fine organic material to the instream habitats and organisms, eliminate solar radiation, and consequently may have a strong effect on the structure and function of the aquatic communities. Conversely, deforested floodplains show an altered ecological connectivity compared to riparian forests.

Since 2010, efforts of the La Gamba Field Station in the rainforest of the Austrians have focused on the establishment of the Biological Corridor La Gamba (COBIGA) with two principal goals: the preservation of biodiversity by connecting isolated patches of forest, and the reduction of atmospheric carbon dioxide by converting it into biomass. As river

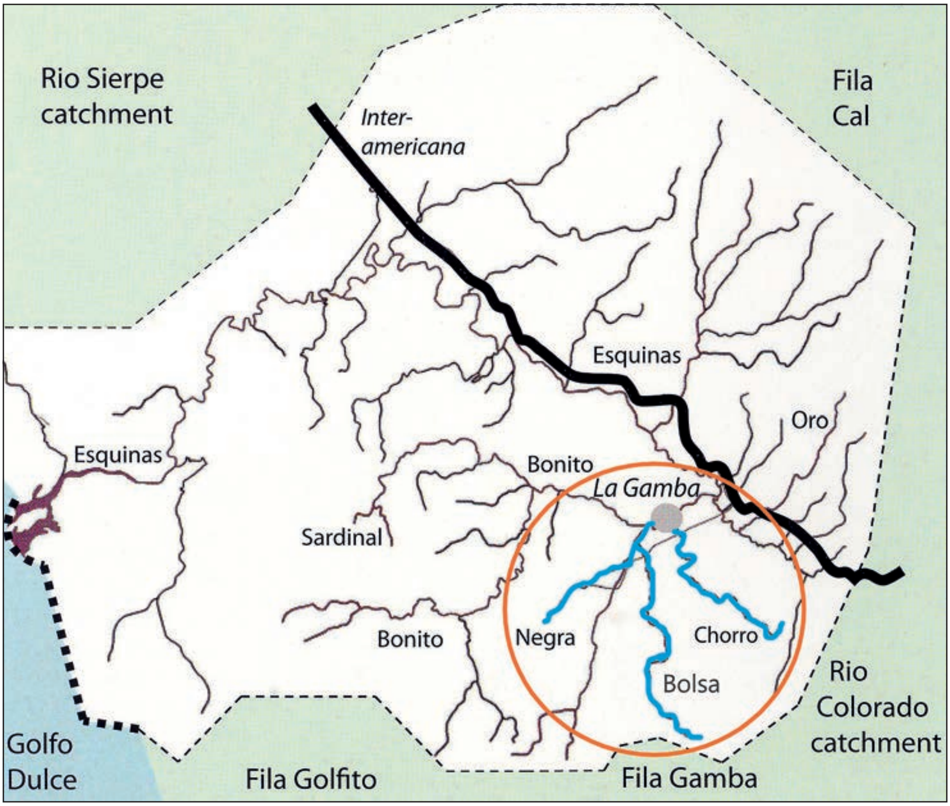

Fig. 1: The catchment of the Esquinas River system with the three studied rivers. The bold line shows the Interamerican highway. Adapted from Stream Ecosystems of Costa Rica, (Schiemer et al. 2010, p. 30). – Abb. 1: Das Flusseinzugsgebiet des Esquinas Flusses mit den drei untersuchten Flüssen. Die fettgedruckte Linie zeigt die Interamericana. Adaptiert aus Stream Ecosystems of Costa Rica (Schiemer et al. 2010, p. 30).

ecosystems are strongly interlinked with riparian and floodplain habitats, we can expect that reforested areas along rivers will have an effect on aquatic habitats and organisms.

As the survey area currently lacks reforested riparian vegetation along rivers, we compared natural with impacted stream sites in order to evaluate (i) the potential importance of primary/secondary riparian vegetation for the macrozoobenthic assemblages and (ii) the potential effect of riparian land use activities on freshwater ecosystems. Our results should provide an indication of what to expect when riparian conditions are improved by reforestation, and evidence of how anthropogenic land use influences the typical stream macroinvertebrate assemblage and diversity.

Materials and Methods

Study site: The study was conducted in the Golfo Dulce region, Golfito in the South of Costa Rica (Puntarenas province), near the tropical research station "La Gamba". The region receives annual precipitation of 5,836 mm and belongs to the wet lowland forests. The annual average temperature is 28,2° C (Weissenhofer & Huber 2008). Research was carried out from December 2018 to February 2019 during the dry season. The streams studied are tributaries of the Rio Esquinas Catchment, which drains into the Golfo Dulce. In total, six sampling sites were selected in three small streams (Fig. 1), two in each. The sites in the upstream reaches show almost no signs of anthropogenic disturbance and have

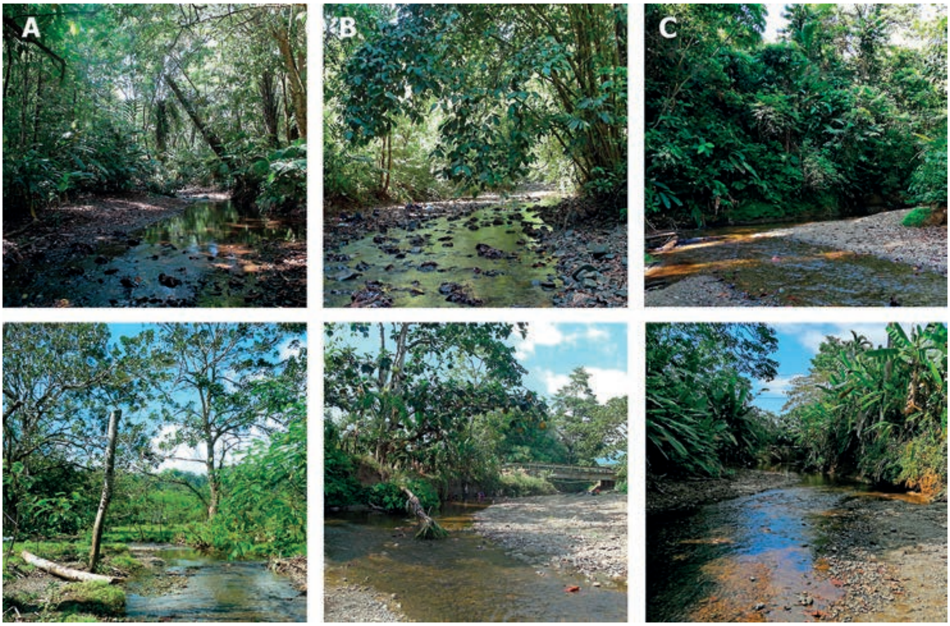

Fig. 2: Study sites at Quebrada Negra (a), Quebrada Bolsa (b) and Quebrada Chorro (c). Reference sites (above) show a high forest cover. Impacted sites (below) include pasture, residential area and oil palm plantation. Photos: V. Duschek. – Abb. 2: Untersuchungsgebiete am Quebrada Negra (a), Quebrada Bolsa (b) and Quebrada Chorro (c). Referenzstandorte (oben) weisen eine hohe Waldbedeckung auf. Beeinflusste Standorte (unten) beinhalten Weideland, Siedlungsgebiet und Ölpalmplantage. Fotos: V. Duschek.

an extensive forest cover (reference sites), whereas the downstream sites were located in between a pasture (Quebrada Negra), an oil palm plantation (Quebrada Chorro) and next to a settlement (Quebrada Bolsa), all of them almost entirely without riparian vegetation (impacted sites) (Fig. 2). The sampled stream sections reached from 15 to 30 meters depending on the river morphology.

Macroinvertebrate sampling

At the Quebrada Negra and Quebrada Bolsa sites, quantitative sampling was carried out between December 2018 and January 2019. An additional qualitative sampling took place in February 2019 at all six study sites (Quebrada Negra, Quebrada Bolsa and Quebrada Chorro). For quantitative sampling, a kick sampler with a size of 30 × 30 cm and a mesh size of 250 µm was used. Macroinvertebrates were collected by kick sampling for approximately 1 minute. Big stones were cleaned with an ordinary dish brush. Three samples were taken randomly at each site, including two riffles and one pool. All material was preserved in 80 % ethanol and transported to the laboratory, where macroinvertebrates were separated from organic matter using a stereoscopic microscope.

The qualitative sampling was done by catching drifting macroinvertebrates with a smaller net after disturbing upstream substrate, as well as by scooping organic material such as leaf litter packs into a tray. This sampling was carried out by four persons trying to cover as many different micro-habitats as possible and lasted for 30 minutes including the time for collection and separation. Individuals were handpicked and fixed in 80 % ethanol for later identification.

All macroinvertebrates collected were identified to family and in some groups to genus level, using the taxonomic keys of Springer et al. (2010) and Roldán- Perez (1996).

The biotic index BMWP-CR and statistical analyses

The Biological Monitoring Working Party (BMWP) was created in 1978 for evaluating rivers of Great Britain. Later, it was adapted for the Iberian Peninsula (Alba-Tercedor & Sanchez-Ortega 1988), and in 2007 modified for Costa Rica (MINAE-S 2007). This index is based on the presence of macroinvertebrate families and their assigned sensitivity score, and its calculation does not take into account the number of collected genera or individuals (Springer 2010). The index value of each family is listed in the Executive Decree No. 33903-MINAE-S (2007), as well as the six categories of water quality, obtained by adding the sensitivity score of each family found at a given site.

Tab. 1: Classification of water quality up to the total score of macroinvertebrates families. – Tab. 1: Klassifizierung der Wasserqualität anhand der Gesamtpunktezahl der Makrozoobenthos- Familien.

Level of Quality	Index value BMWP'-CR	Representative Colour
Excellent water quality	>120	🟦
Good water quality	101–120	🟦
Regular water quality, moderately contaminated	61–100	🟩
Bad water quality, contaminated	36–60	🟨
Very bad water quality, very contaminated	16–35	🟧
Extremely bad water quality, extremely contaminated	<15	🟥

To test for differences between community compositions and to quantify the influence of intact riparian vegetation, we applied a non-metric multidimensional scaling (NMDS) and a PERMANOVA with 999 permutations based on a Bray-Curtis dissimilarity matrix out of the community data (taxa x abundance matrix), using the *vegan* package (Oksanen et al. 2019) in R v3.6.1 (R Core Team 2018).

Pairwise differences of specific indices (abundance, taxa diversity, abundance of key groups in the benthic community such as Ephemeroptera, Trichoptera, Diptera without Chironomidae, and Chironomidae) between reference and impacted reaches were examined using estimation statistics (using bootstrapped samples) together with null hypothesis tests (providing p-values of differences). We provide group means, standard deviations, and also effect sizes (differences) with 95 % confidence intervals (CI) computed from 5000 bootstrapped samples using the *dabestr* package (Ho et al. 2018).

Results

Macroinvertebrate abundance and diversity

In total, 11,313 individuals were collected at all six study sites. Of these, 10,714 were collected from the quantitative samples from Quebrada Negra and Quebrada Bolsa and 599 from qualitative sampling at all three rivers. The highest number of individuals per site was found downstream at the Quebrada Negra (pasture) with a total of 5,735 individuals, of which 4,454 belonged to the dipteran family Chironomidae (non-biting

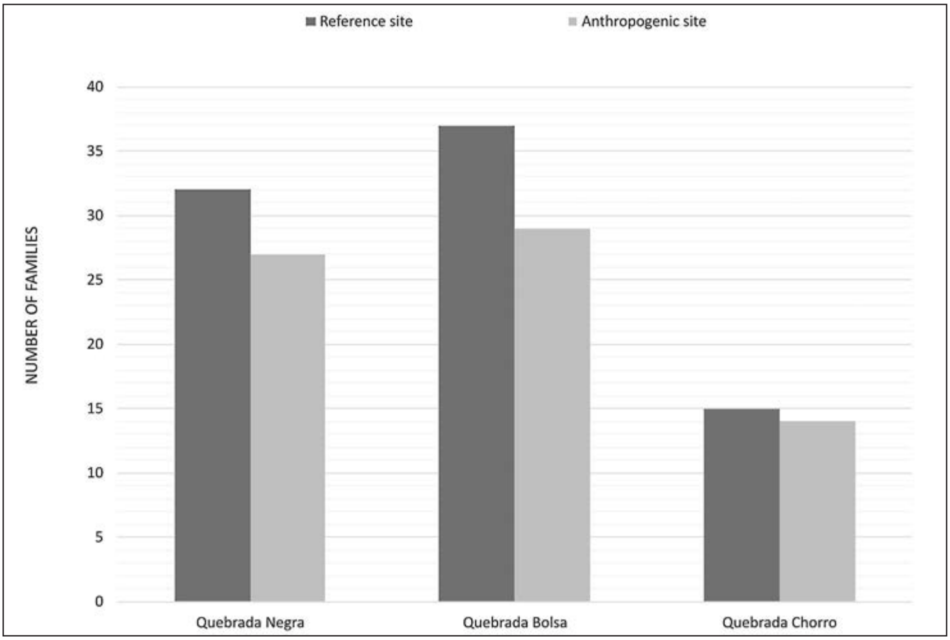

Fig. 3: Family richness found at every site. Quebrada Negra and Quebrada Bolsa include qualitative and quantitative sampling, Quebrada Chorro just qualitative. – Abb. 3: Anzahl der Familien an jedem Standort. Quebrada Negra und Quebrada Bolsa umfassen qualitative und quantitative Probenahmen, Quebrada Chorro nur qualitative.

midges). The second highest number of individuals per site was recorded at the anthropogenically influenced site at Quebrada Bolsa with a total of 3,130, with the highest abundance of 792 individuals in the Oligochaeta, of which 684 were from a single pool sample. In the reference sites of Quebrada Bolsa and Quebrada Negra, we collected only 1,647 and 629 invertebrates, respectively. When comparing reference and impacted reaches of the same streams, we found that benthic communities in anthropogenically influenced reaches showed higher abundances than the corresponding reference stretches (mean difference in Q. Negra of 1,710 individuals sample^{-1}, 95 % CI = 523–2410 and in Q. Bolsa of 493 individuals sample^{-1}, 95 % CI = -82–983). In Quebrada Negra, taxa diversity was also higher in influenced reaches, with mean differences of 10 taxa (95 % CI 3–19 in Q. Negra), while the effect in Quebrada Bolsa was negligible. In contrast, reference sites had a higher family diversity compared to their adjacent sites downstream (Fig. 3).

A total of 67 macroinvertebrate taxa were identified in the study streams (Table 2), representing 44 insect families and seven other taxonomic groups including Hydrachnidia, Oligochaeta, Ostracoda, Platyhelminthes, Nematomorpha and Collembola. Taxa richness ranged from 36 taxa at the forest site of Quebrada Negra to 44 taxa at both impacted sites and the reference site of Quebrada Bolsa (data from Quebrada Chorro not included).

Tab. 2: Taxonomic diversity of aquatic macroinvertebrates collected in the forested and impacted sites of three streams. Data for Quebrada Chorro from qualitative sampling. (Gen. Indet. = Genus not identified; Gen. Var. = Various genera; Fam. Indet. = Family not identified). – Tab. 2: Taxonomische Vielfalt aquatischer Makroinvertebraten bewaldeter und betroffener Standorte von drei Bächen. Daten für Quebrada Chorro nur aus qualitativen Stichproben. (Gen. Indet. = Gattung nicht identifiziert; Gen. var. = Verschiedene Gattungen; Fam. Indet. = Familie nicht identifiziert).

Taxonomic Group	**Family**	**Genus**	**Quebrada Negra**		**Quebrada Bolsa**		**Quebrada Chorro**	
			Forest	Impacted	Forest	Impacted	Forest	Impacted
Ephemeroptera	Leptophlebiidae	*Farrodes*	11	34	9	25		
		Terpides	6	22		12		
		Thraulodes	23	57	20	17		
		Hagenulopsis	2					
		Gen. Indet.	16	10	41	20	6	10
	Leptohyphidae	*Allenhyphes*		7		18		
		Leptohyphes	7	60	36	278		
		Tricorythodes		26	18	273		
		Gen. Indet.	12	8		43	10	6
	Baetidae	*Americabaetis*	1	102	8	29		
		Baetodes	9	164	309	4		
		Camelobaetidius		10	13			
		Genus 1		26		19		
		Genus 2		47		11		
		Genus 3		14				
		Gen. Indet.	7	58	14	28	2	6
	Caenidae	*Caenis*	1	3	2	1		
Trichoptera	Hydropsychidae	*Leptonema*	18	74	17	200		
		Macronema		3	1			
		Smicridea		13	9	82		
	Hydroptilidae	*Hydroptila*				44		

Tab. 2 continued

Taxonomic Group	Family	Genus	Quebrada Negra		Quebrada Bolsa		Quebrada Chorro	
			Forest	Impacted	Forest	Impacted	Forest	Impacted
		Leucotrichia				1		
		Metrichia				10		
		Ochrotrichia		3				
		Oxyethira		31		7		
		Genus 1*		20	14	97		
		Gen. Indet.		2				
	Helicopsychidae	*Helicopsyche*				1		
	Calamoceratidae	*Phylloicus*		1	1			
	Philoptamidae	*Chimarra*	7		13	9	2	13
	Polycentropodidae	*Polycentropus*	1		2			
	Lepidostomatidae	*Lepidostoma*	3		1			
	Leptoceridae	*Nectopsyche*	9	1	5			2
	Xiphoncentronidae	Gen. Indet.	1					
	Glossosomatidae	Gen. Indet.	3	1				
	Hydrobiosidae	*Atopsyche*					2	
	Fam. Indet.	Gen. Indet.		5				
Plecoptera	Perlidae	*Anacroneuria*	20	3	25	16	17	9
Diptera	Ceratopogonidae	Gen. Var.	1	4	29	196		
	Chironomidae	Gen. Var.	338	4582	799	725	3	1
	Culicidae	Gen. Indet.			1	1		
	Dixidae	Gen. Var.	2		1			
	Dolichopodidae	Gen. Indet.				1		
	Empididae	Gen. Var.		13	3	5		
	Muscidae	Gen. Indet.	3					
	Psychodidae	Gen. Var.	1		9			
	Rhagionidae	Gen. Indet.	1					
	Simuliidae	Gen. Var.	21	98	97	11	8	
	Stratiomyidae	Gen. Indet.			3			
	Tabanidae	Gen. Indet.			1			
	Tipulidae	Gen. Var.	9		2	3		1
Coleoptera	Elmidae adult	Gen. Var.	5	6	4	4		
	Elmidae larvae	Gen. Var.	21	62	43	22	6	
	Dytiscidae	Gen. Indet.			3			
	Limnichidae	Gen. Indet.			1	1		
	Psephenidae	Gen. Var.		10	6	5	6	3
	Staphylinidae	Gen. Indet.				1		
	Ptilodactylidae	Gen. Var.	3	1			6	
Megaloptera	Corydalidae	Gen. Var.	4	1	6	2		3
Hemiptera	Gerridae	Gen. Var.			2			
	Naucoridae	Gen. Var.	3	14		16		
	Veliidae	Gen. Var.		3		1		
Zygoptera	Calopterygidae	*Hetaerina*		3	2			
	Coenagrionidae	Gen. indet.	1		1			1
	Polythoridae	*Cora*	1			1	1	
	Megapodagrionidae	Gen. Indet.	1					
	Fam. Indet.	Gen. Indet.	4	4	9			
Anisoptera	Aeshnidae	Gen. Indet.		2				
	Libellulidae	Gen. Var.		1	3		1	1

Tab. 2 continued

Taxonomic Group	**Family**	**Genus**	**Quebrada Negra**		**Quebrada Bolsa**		**Quebrada Chorro**	
			Forest	Impacted	Forest	Impacted	Forest	Impacted
	Gomphidae	Gen. Indet.			2			
	Fam. Indet.	Gen. Indet.				1		
Lepidoptera	Crambidae	*Petrophila*	4	11	2	8	2	2
Blattodea	Blaberidae	Gen. Indet.				1		
Hydrachnidia	Fam. Indet.	Gen. Indet.	1	31	25	17		
Oligochaeta	Fam. Indet.	Gen. Indet.	1	9	6	793		
Ostracoda	Fam. Indet.	Gen. Indet.	1			2		
Platyhelminthes	Fam. Indet.	Gen. Indet.		8				
Nematomorpha	Fam. Indet.	Gen. Indet.		1	2			
Unspecified			20					
TOTAL			**624**	**5696**	**1647**	**3130**	**100**	**90**

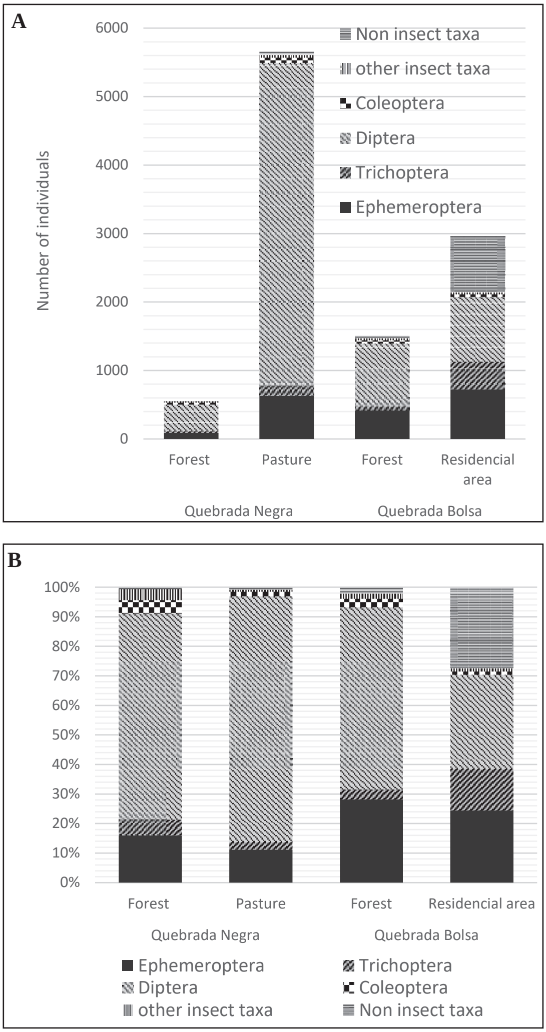

Fig. 4: Absolute (A) and relative contribution (B) of taxonomic groups of macroinvertebrates to the benthic community in forest and impacted sites of Quebrada Negra and Quebrada Bolsa. – Abb. 4: Absoluter (A) und relativer Anteil (B) taxonomischer Makroinvertebraten-Gruppen zur benthischen Gemeinschaft in Wäldern und beeinflussten Gebieten von Quebrada Negra und Quebrada Bolsa.

Macroinvertebrate community composition

Aquatic insects were the dominating group in all studied streams. Non-insect taxa, such as oligochaete worms, were most abundant in the anthropogenically influenced site in Quebrada Bolsa (settlement) with a mean difference of 262 individuals sample^{-1} compared to the reference site (95 % CI = 42–682), where this group made up more than 25 % of all individuals found in that reach. Diptera were the most abundant order at all studied sites (approx. 62 % of all sampled individuals), with the most numerous family being Chironomidae (approx. 57 % of all individuals). The contribution of Chironomidae to the overall benthic community, however, differed significantly between reference and impacted reaches of both streams (significant interaction effect, $p<0.05$). In contrast to the reference site, we found significantly higher densities of Chironomidae in the impacted reach of Quebrada Negra only (mean difference of 1,430 sample^{-1}, 95 % CI = 472–1990), where more than 80 % of all collected macroinvertebrates belonged to this family. Besides Diptera, Ephemeroptera was the most abundant order, followed by Trichoptera, Coleoptera and other insect taxa such as Odonata (Fig. 4).

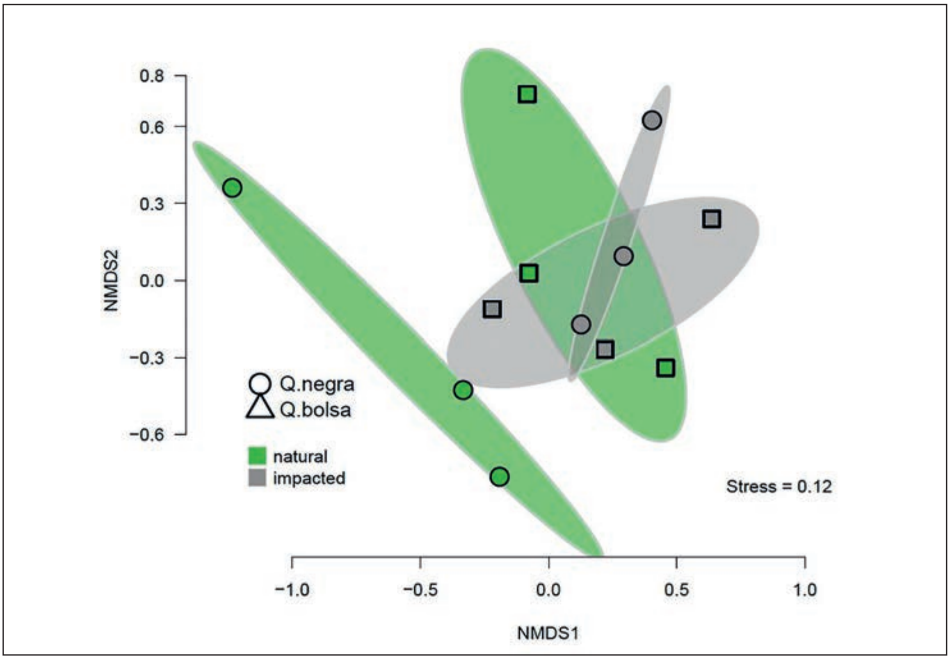

Fig. 5: Nonmetric multidimensional scaling (NMDS) of the overall benthic communities at all reaches (reference and impacted) in both streams (Quebrada Negra and Quebrada Bolsa). The distance of the two-dimensional ordination corresponds to differences in the community structure. Points represent samples, ellipse hulls border samples from same reaches, colours indicate the condition of the riparian vegetation (reference/natural vs. impacted). – Abb. 5: Nonmetric multidimensional scaling (NMDS) der gesamten benthischen Gemeinschaften an allen Standorten (Referenz- und beeinflussten) in beiden Flüssen (Quebrada Negra und Quebrada Bolsa). Der Abstand der zwei-dimensionalen Ordination entspricht Unterschieden in der Gemeinschaftsstruktur. Punkte repräsentieren Proben, Ellipsen umgrenzen Proben aus demselben Gebiet, Farben zeigen den Zustand der Ufervegetation an (Referenz/natürlich vs. beeinflusst).

Through ordination of benthic community structures and the comparison of reference and impacted sites, we revealed that the presence or absence of riparian vegetation is significantly linked to the composition of benthic communities (PERMANOVA, $p<0.05$, $F=3.0$, $R^2=0.15$), but also that the overall differences between reference and impacted reaches of the same stream were most pronounced in Quebrada Negra (Fig. 5).

BMWP-CR

Both Quebrada Negra and Quebrada Bolsa were considered to have excellent water quality according to the BMWP-CR (**Table 1**), although the index values varied greatly with the sampling method:

Tab. 3: Values of the BMWP-CR index at 6 sampling sites determined by the two methods of sampling, total value per site. Colours represent water quality categories as shown in Table 1. – Tab. 3: Werte des BMWP-CR-Index an 6 Probenahmestellen ermittelt anhand der beiden Probenahmemethoden, Gesamtwert pro Standort. Die Farben stellen die Wasserqualitätskategorien dar, die in Tabelle 1 gezeigt werden.

	Sampling method	Forest	Impacted sites
Quebrada Negra	quantitative	133	145
	qualitative	94	38
Total value		**166**	**160**
Quebrada Bolsa	quantitative	172	137
	qualitative	73	58
Total value		**185**	**146**
Quebrada Chorro	quantitative	X	X
	qualitative	93	81
Total value		**X**	**X**

Quantitative sampling resulted in higher values than qualitative sampling (Table 3) to such an extent that the level of quality differed from bad water quality to excellent water quality at the pasture site of Quebrada Negra, from regular to excellent water quality at the residential area of Quebrada Bolsa, and finally from good water quality to excellent water quality at the forest sites of Quebrada Bolsa and Quebrada Negra. Consistent with the difference in family richness, the scores of the BMWP-CR biotic index were higher at reference sites than at impacted sites, except for the quantitative samples of the Quebrada Negra (Fig. 3, Table 3). Comparing qualitative samples, the highest variation of index values was found at the Quebrada Negra with a difference of 56 between forest site and impacted site. The highest index value of qualitative sampling was reached at the forest site at Quebrada Negra (94) followed by the forest (93) and the impacted site (81) of Quebrada Chorro.

Discussion

Results obtained in this study demonstrated a clear difference in benthic communities between reference and impacted sites in all three streams, and provide evidence to assume that anthropogenic influence in riparian areas has direct and indirect influences on macroinvertebrate density, diversity and assemblage structure in small Costa Rican lowland streams. Considering the geographical proximity of the sampling sites and given that riverbanks of impacted reaches are not completely treeless, the differences in macroinvertebrate density, diversity and assemblage structures between the sites are remarkable. Impacted sites had lower diversity and different taxonomic composition compared to reference sites, but a higher number of total individuals, consistent with the effects of deforestation observed in Bojsen & Jacobsen (2003) in Ecuadorian streams. Similar observations for Costa Rican lowland streams were made by Lorion & Kennedy (2009) in the south-eastern part of the country. They compared 12 different sites, including reference sites, forested buffer sites and pasture sites. In this study, pasture reaches differed significantly from reference sites, but also from forested buffer sites.

In accordance with some studies (e.g. Hawkins et al. 1982, Bojsen & Jacobsen 2003), anthropogenically influenced sites showed higher total macroinvertebrate abundance due to increased primary production, but also showed excessive differences in the density of few families in contrast to the reference sites. In Quebrada Negra, for example, chironomid density was more than 10 times higher at the pasture site, which corresponded to 80 % of all invertebrates present. Similar results were found in African streams were chironomids made up more than half of the zoobenthos at cultivated sites (Dudgeon 2008). In contrast, the impacted site of Quebrada Bolsa showed a chironomid density comparable to its reference site. In this reach, however, individuals of Oligochaeta dominated the invertebrate community. While Lorion & Kennedy (2009) reported a generally higher abundance of both chironomids and oligochaetes, at pasture reaches, this study suggests that the colonisation by Oligochaeta is related to the manifold influences of human settlements on a stream ecosystem. As Oligochaeta were found to be highly tolerant against organic pollution (Finogenova 1996, Schenkova et al. 2001, Kazanci & Girgin 1998) and are able to increase their density with increasing organic matter (Schenkova & Helesic 2006), this could also be the reason why they were found to be highly abundant in that urban stretch of Quebrada Bolsa.

EPT (Ephemeroptera, Plecoptera and Trichoptera) taxa are commonly used as an indicator of stream degradation (e.g. García & Jimenez 2006, Dudgeon 2008, Lorion & Kennedy 2009, Bücker et al. 2010, Chará-Serna et al. 2015, Kohlmann et al. 2015). In our streams, some contrasts between impacted and forest reaches were evident for EPT taxa. The total number of Ephemeroptera and Trichoptera was higher at impacted sites, with significant differences in Quebrada Negra, but the populations were more diverse at forested sites. Plecoptera are considered especially sensitive to stream degradation (e.g. Mafla 2005, Springer 2010), which explains the lower abundance at the impacted site in Quebrada Negra. Their overall low density in all streams, however, limits the conclusions about influences on the stonefly populations in the sampled streams.

Regarding the BMWP-CR, the highest difference between two reaches was seen at Quebrada Bolsa, but still both sites were considered to have excellent water quality. A similar trend was seen at Quebrada Negra, although the difference of family richness and the BMWP-CR index value were not as clear as for Quebrada Bolsa. This could be due to the very short distance between the reference and the pasture site and the setting for sampling. In this study, Quebrada Chorro was sampled only qualitatively, and the total index value just reached regular water quality. We assume that water quality according to the BMWP-CR would be similar if quantitative samples had also been taken. Also, qualitative samples were taken through direct hand-picking by non-specialists, who might have overlooked those families that are more difficult to find (e.g. small body size) or recognise (e.g. well camouflaged shelters). Several authors discuss the effect of sampling method and intensity on the BMWP index (e.g. Maue & Springer 2008, Stein et al. 2008, Gutiérrez-Fonseca & Lorion 2014, Reyes- Morales & Springer 2014), pointing out that certain methods, such as hand-picking and lower sampling effort, may result in significantly lower scores and, consequently, lower water quality.

Riparian deforestation was present at all impacted sites and was related to changes in macroinvertebrate abundance and diversity, as it reduces shading and in turn leads to increased stream temperatures, light penetration and plant growth, decreases bank stability and inputs of litter and wood and reduces retention of nutrients and contaminants (e.g. Allan 2004, Springer et al. 2014). Indeed, all the studied streams were warmer at impacted sites compared to sites with canopy cover (1.2° C in Quebrada Negra and 2.5° C in Quebrada Bolsa). This difference could be partly related to observed changes in macroinvertebrate assemblages, as EPT taxa are sensitive to higher temperatures and altered thermal regimes (Lorion & Kennedy 2009). Furthermore, it is known that water temperature generally influences invertebrate growth, fecundity and survival (Sweeney 1993).

In Costa Rica, Lorion & Kennedy (2009) found that pastures with a riparian forest buffer of at least 15 m show similar macroinvertebrate abundance and assemblage structure as reference sites in the forest. Similar effects of riparian buffers are shown for oil palm plantation in Borneo (Chellaiah & Yule 2018) and Brazil (Juen et al. 2016). Also, Wantzen et al. (2006) suggested that planting buffer zones of native trees between the agricultural areas and riparian wetlands might reduce the degradation of streamside habitat in Brazil. Even if forest buffers cannot replace forests, they reduce the effects of deforestation on the streams and their biota. For those reasons we highly recommend to reforest the riparian zones of the anthropogenically influenced river stretches with native tree species to prevent ongoing stream degradation and maintain ecological balance. The commercial use of non-wood products from these trees could bring farmers an additional income and cover the costs of planting (Dudgeon 2008). Although Costa Rican environmental legislation prescribes that rivers and lakes must maintain a 15 m riparian buffer in rural areas, 10 m in urban areas and 50 m in areas of steep slopes, these regulations are often not respected or implemented as law enforcement is weak. Rather, education programs could help to include and convince local farmers and residents to protect the streams.

Conclusion

Although the values of the BMWP-CR were high in all sites and report excellent water quality, differences of macroinvertebrate assemblages and abundances between reference sites and impacted sites were apparent. Deforestation influenced the macroinvertebrate fauna by leading to higher overall density, lower taxa diversity and higher dominance of few families. Forested buffers can reduce these effects and help to establish a nearly natural macroinvertebrate assemblage structure.

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Ground-dwelling ant assemblages severely degrade in oil-palm plantations – a case study from the Golfo Dulce region, SW Costa Rica

Martin Falk, Christian H. Schulze & Konrad Fiedler

Due to the rising demand for bio-fuels and plant oils, and fostered by high economic benefits, the area of oil palm cultivation is tremendously increasing all over the tropics. This development has spilled over to Costa Rica during the past 20 years, with manifold potentially negative effects on biodiversity. We surveyed ground-dwelling ants in 5 oil palm plantations, in comparison to 5 old-growth and 5 secondary forest sites in the Golfo Dulce region (SW Costa Rica), to assess the magnitude of faunal impoverishment in these agro-ecosystems. By combining hand sampling with pitfall traps and the Winkler method, we observed 136 ant species from 44 genera and 9 subfamilies during two months in the year 2015: 34 species in oil palm plantations, 76 species in secondary forest, and 90 species in old-growth forest. On average, individual old-growth forest sites harbored more than twice as many ant species as plantations, with secondary forest taking an intermediate position. Litter samples from Winkler bags contributed a far lower proportion of ant species in plantations, compared to secondary and especially old-growth forest. Hence, the litter fauna is particularly severely hit by converting forest into plantations. Secondary forest showed the largest variation in species composition between individual sites, whereas ant assemblages of oil palm plantations were the most uniform. Our data show that assemblages of ground-dwelling ants are severely impoverished in oil palm plantations (ca 50 % reduction in species). Young regenerating secondary forest harbored distinctly fewer ant species than old-growth stands, but was far less impoverished than plantations and thus of substantial conservation value.

Falk M., Schulze C.H. & Fiedler K., 2019: Bodenlebende Ameisengemeinschaften verarmen in Ölpalmplantagen stark – eine Fallstudie aus der Golfo Dulce Region, SW Costa Rica.
Aufgrund ihrer hohen Rendite nimmt die Kultivierung der Ölpalme in allen Tropenregionen der Erde massiv zu. In den letzten 20 Jahren hat diese Entwicklung auch Costa Rica erreicht, mit vielfachen negativen Folgen für die Biodiversität. Wir haben die bodenlebenden Ameisen in 5 Ölpalmplantagen, 5 jungen Sekundärwäldern und an 5 Standorten im weitgehend naturnahen Tieflandregenwald in der Golfo-Dulce-Region (SW Costa Rica) erfasst, um das Ausmaß der Faunenveränderungen zu quantifizieren. Mittels Handaufsammlungen, Bodenfallen und Winkler-Extraktion wiesen wir in 2 Monaten 136 Ameisenarten aus 44 Gattungen und 9 Unterfamilien nach: 34 Arten in Plantagen, 76 Arten im Sekundärwald und 90 Arten im Regenwald. Im Mittel beherbergten Regenwaldstandorte etwa doppelt so viele Arten wie Plantagen, während Sekundärwälder intermediären Reichtum aufwiesen. Streuproben lieferten in den Plantagen einen besonders geringen Beitrag zur Artenvielfalt. Standorte im Sekundärwald zeigten die größte Variabilität in der Zusammensetzung ihrer Ameisenfauna, während in Ölpalmplantagen die Ameisengemeinschaften besonders einförmig waren. Unsere Studie bestätigt damit den enormen Biodiversitätsverlust in Ölpalmplantagen am Beispiel von Ameisen (Rückgang der Artenzahl um ca. 50 %). Junge Sekundärwälder wiesen hingegen eine deutlich weniger verarmte Fauna auf, was ihren naturschutzfachlichen Wert (etwa im Kontext der Waldregeneration) unterstreicht.

Keywords: *Elaeis guineensis*, Formicidae, land use, secondary forest, tropical biodiversity loss.

Introduction

Ants are among the most dominant terrestrial animals on Earth, with a high diversity and prevalence especially in tropical regions (Hölldobler & Wilson 1990, Lach et al. 2010). Besides humans and their livestock, ants are the main heterotrophic land organisms in terms of biomass. Ants play essential roles in soil perturbation and in energy and nutrient flow through ecosystems (Lobry de Bruyn & Conacher 1990, Hölldobler & Wilson 1990, Nkem et al. 2000). They are the leading predators of other insects and small invertebrates (Blüthgen & Feldhaar 2010), and they are key to many mutualistic interactions with plants, fungi or other animals (Rico-Gray & Oliveira 2007, Voglmayr et al. 2011, del Toro et al. 2012). In the Neotropical realm, leaf-cutting ants are the most destructive insect herbivores (Wirth et al. 2003). Hence, changes in ant communities may trigger massive effects on ecosystem services and functions.

Habitat loss through the ever growing demand for agricultural production areas is one of the major drivers of global biodiversity loss (Newbold et al. 2015). In humid tropical regions, the increasing cultivation of oil palms (*Elaeis guineensis*) poses a severe threat to biodiversity (Fitzherbert et al. 2008, Turner et al. 2008, Wilcove & Koh 2010, Prescott et al. 2015, Pardo et al. 2018a, 2018b). In Costa Rica, cultivation of oil palm has massively grown during the last 20 years, particularly in the Southern Pacific region, where 64 % of the current plantations and more than 50 % of the area identified as being suitable for establishing new plantations are located (Beggs & Moore 2013). The cultivation of oil palms did not necessitate forest clear-cutting (Vijay et al. 2016), as especially large tracts of areas on plain ground that had formerly been stocked with other cultivated plants or used as pastures were converted into oil palm plantations (Carlson et al. 2012, Höbinger et al. 2012). As a consequence, most old-growth lowland forest nowadays persisting in Costa Rica is situated on more or less steep slopes (Weissenhofer et al. 2008).

Multiple studies have shown that animal assemblages in oil palm plantations are dominated by only a few species, though some of these may reach very high abundance (Danielsen et al. 2008). In particular, the fate of ants in oil palm plantations has received considerable attention by scientists, though mostly in SE Asia (Brühl & Eltz 2010, Fayle et al. 2010, Pfeiffer et al. 2008). Lowest ant species richness was found in oil palm plantations relative to other forested habitats (Brühl & Eltz 2010). As oil palm plantations are structurally more homogeneous than forests, they may also have a lower beta diversity due to an increased importance of widespread generalists and a reduced number of rare species depending on specific food sources or nesting sites (Fayle et al. 2010, Wang & Foster 2015).

One reason for the dominance of just a few ant species in oil palm plantations is the massive reduction of possible nesting sites and foraging grounds in the litter layer, due to the near complete absence of leaf litter (Brühl & Eltz 2010, Fayle et al. 2010). Furthermore, the establishment and survival of shade adapted ant species is constrained by hot and dry conditions that may transiently occur in plantations during the dry season (Brühl & Eltz 2010). Also the abundance of arboreal ant species is much higher in forest than in oil palm plantations (Pfeiffer et al. 2008, Fayle et al. 2010). Thus, species as well as functional diversity of ants massively degrades when converting tropical forest into oil palm plantations. For example, Fayle et al. (2010) observed in Malaysia that total ant species richness decreased from 309 to 110 (64 % loss), and leaf-litter ant species richness even from 216 to 56 (74 % loss), between primary forest and oil palm plantations.

Spontaneously regenerating secondary forest (e.g. after agricultural or forestry use of a site has been abandoned) can be understood as a successional transition back to a more natural rainforest ecosystem. Multiple studies have shown that the fauna of tropical secondary forest has a higher species diversity than plantations, but a lower diversity than old-growth forest (for ants: Schonberg et al. 2004, Bihn et al. 2010, Brühl & Eltz 2010, Klimes et al. 2012; for other wildlife: Gibson et al. 2011). Case studies in the Golfo Dulce region in SW Costa Rica revealed that bird species diversity at forest margin sites was even higher than inside old-growth forest (Mollik 2013) and no significant differences of amphibian and reptile species diversity were observed between old-growth forest interior and forest margin sites (Gallmetzer & Schulze 2015). Similarly, moth species diversity was drastically reduced in oil palm plantations, but no substantial differences were found between old-growth forest interior, forest margin sites, and secondary forest (Alonso et al. 2017). Other taxa, such as amphibians, appear to recover even more rapidly, reaching not only similar species richness, but also a similar species composition in Costa Rican young secondary forests (10–16 years) in comparison to old-growth forests (Hilje & Aide 2012). Secondary forests are attaining ever higher importance for biodiversity conservation as well as from a functional perspective, given that remnants of near-natural forest areas are under severe pressure everywhere (Chazdon 2014).

Even though biodiversity in oil palm plantations has almost invariably turned out to be lower than in forest ecosystems, the magnitude of biodiversity loss varies across biogeographical regions and between focal taxa, and depends on the wider landscape context (e.g. Pardo et al. 2018a, 2018b). Hence, it is important to empirically assess the response of animals to conversion of forest into oil palm plantations, as well as to spontaneous forest regeneration on land that had previously been deforested, in every region where oil palm expansion is of environmental concern (Turner et al. 2008, Foster et al. 2011). Along these lines, we studied ground-layer ant assemblages in old-growth and secondary forest and compared these communities to oil palm plantations in a tropical lowland area of SW Costa Rica. Specifically, we tested the following hypotheses:

(1) Ant species richness is very substantially reduced in oil palm plantations, intermediate in secondary forest, and highest in old-growth forest. Especially leaf litter ants are underrepresented in plantations because of the near-complete absence of a litter layer.

(2) Species composition of ant assemblages differs between all three habitat types, with oil palm plantations showing the most distinct and least variable communities through the homogenizing effects of recurrent interventions during plantation management.

(3) Ant communities of oil palm assemblages are impoverished nested subsets of those found in old-growth forest.

Material and methods

Study area and study sites

Study sites were selected within marginal areas of the Piedras Blancas National Park (Puntarenas province) and in its surroundings, near the Research Station La Gamba (8°42′3″N, 83°12′6″W). The area is characterized by oil palm plantations, pasture areas and a tropical rural landscape mosaic on plain ground, while old-growth forest persists on slopes (Höbinger et al. 2012). Tracts of secondary forest are either found at the border of the national park, or occur interspersed in cultivated areas. The average annual precipitation is about 6,000 mm and the annual mean temperature is 28.5° C (Weissenhofer & Huber 2008).

We sampled ground-dwelling ants in old-growth forest, secondary regenerating forest and oil palm plantations at elevations between 70–250 m (Fig. 1). While old-growth forest and secondary forest sites were located on hilly terrain, all oil palm plantations were situated on the relatively plain valley floor. In each habitat type, five replicate plots were chosen, all 40 m × 40 m = 1600 m² in size. Neighboring study sites were separated by a minimum distance of 400 m.

Oil palm plantations are simply structured and even aged, with sparse ground cover and almost free of leaf litter (Brühl & Eltz 2010; Weissenhofer et al. 2008). They are cleared and replanted on a 25–30 year rotation (Fitzherbert et al 2008). All selected oil palm plantations in our study area were at least 2 ha in size, with palms of more than 10 m in height. The understory vegetation was free of woody shrubs and only consisted of a sparse herb layer covering less than 50 % of the ground due to frequent herbicide application. In contrast, our old-growth forest sites were strongly heterogeneous with regard to tree size and age, tall trees reaching up to 50 m in height. This forest type is characterized by a high species diversity of trees and other vascular plants and harbors a thick leaf litter layer (Weissenhofer et al. 2008). We use the term old-growth forest here to emphasize that we cannot exclude the possibility that single trees were selectively cut in the past.

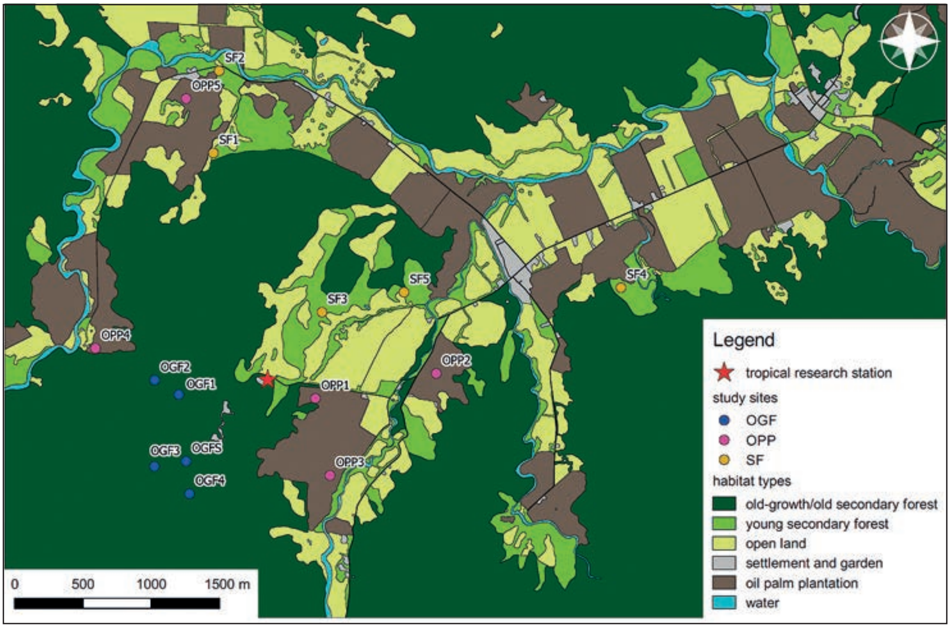


Fig. 1: Schematic map of the study area in the surroundings of the Research Station La Gamba (8°42′3″N, 83°12′6″W; red asterisk). Location of study sites in the three habitat types indicated by colored circles: old-growth forest (OGF, blue), secondary forest (SF, orange) and oil palm plantation (OPP, purple). – Abb. 1: Lage der Sammelstandorte in der Umgebung der Tropenstation La Gamba (8°42′3″N, 83°12′6″W; roter Stern). OGF: alter Regenwald (blau); SF: Sekundärwald (orange); OPP: Ölpalmplantage (violett).

However, during the last 80–100 years, these forest tracts remained largely undisturbed and their vegetation structure closely resembles natural lowland forests of the Golfo Dulce region. All old-growth forest sites were located near or on ridges (see Hofhansl 2014 for ecosystem differences between old-growth forest types in the region relative to topography). Finally, stretches of secondary forest exist on areas that had been cleared, but are now undergoing spontaneous forest regeneration for the past 10–15 years. Secondary forests show a less heterogeneous three-dimensional structure, rather dense ground cover, lower tree species diversity and also a smaller number of tree individuals per unit area than old-growth forest (Weissenhofer et al. 2008; see Alonso Rodríguez 2014 for data on stand structure from the study region). All selected secondary forest stands were larger than 1 ha.

Sampling of ants

Field work was conducted during the rainy season in June and July 2015. To representatively cover the ground-dwelling ant fauna, it is important to apply more than one method of collecting (Agosti et al. 2000, Bestelmeyer et al. 2000). Therefore, we used pitfall trapping, hand sampling and the Winkler method in combination. A schematic example for the spatial arrangement of the application of the three sampling methods per site is shown in Fig. 2.

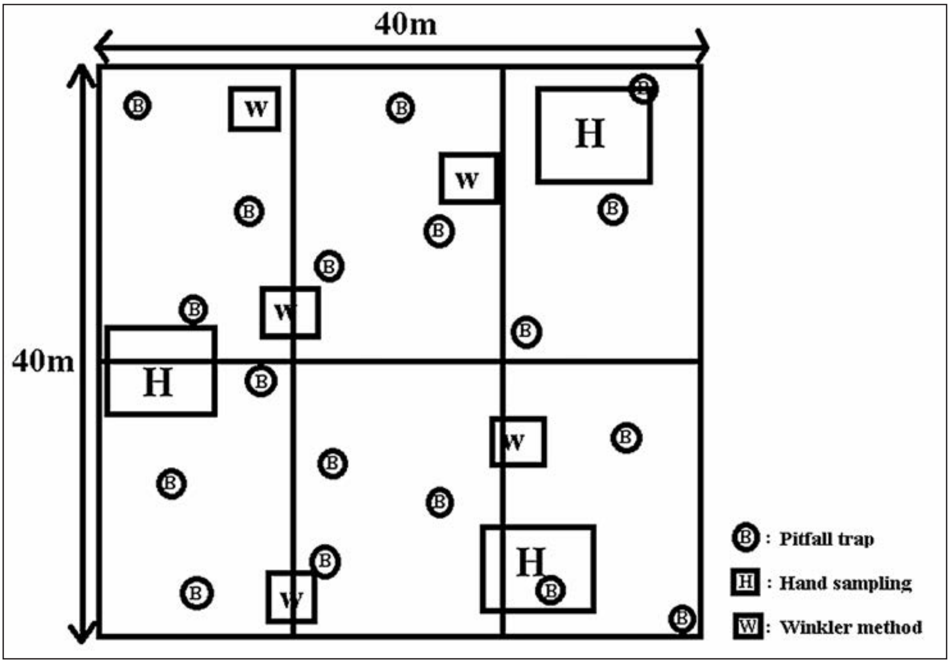


Fig. 2: Schematic representation of the locations of pitfall traps, Winkler sampling and hand sampling within each study site. In order to avoid spatial clustering of sampling micro-sites within the plot, the area was subdivided into 6 subareas for better orientation during field work. – Abb. 2: Schema der räumlichen Anordnung der verschiedenen Probennahmen (Bodenfallen, Handaufsammlung, Streuproben zur Winkler-Extraktion) auf den Probeflächen. Um eine räumliche Klumpung der Probennahmen innerhalb der Fläche zu vermeiden, wurde diese zur besseren Orientierung bei der Feldarbeit in 6 Teilflächen unterteilt.

Pitfall trapping

Pitfall traps capture surface-active ants that fall into a container placed in the ground (Agosti et al. 2000). As containers, we used 200 ml plastic drinking cups (diameter: 6.5 cm), filled with propylene glycol mixed with ethanol as preservation liquid. Pitfall traps were first left for two days in the ground, before filling them with preservation liquid. Then, traps were kept active for two more days. To prevent sample loss by inundation from rainfall a plastic soup plate (diameter: 15 cm) was placed as a roof over every trap. We distributed 18 pitfall traps randomly across every plot. After retrieval, samples were transferred into 70 % ethanol.

Hand sampling

This is a suitable method to collect ants foraging on the ground and in lower vegetation layers, thereby covering different microhabitats within an area (Agosti et al. 2000). In addition, by hand sampling one can collect larger leaf litter and slow moving ants which are not always reliably surveyed with pitfall traps. At each site, three randomly selected subplots (5 m × 6 m = 30 m^2 in size) were searched for foraging ants, each over a time span of 30 min during daylight hours. Hence, ants were sampled by hand on the entire plot for a total duration of 90 min. We caught all ants which moved about on the soil, litter or lower herb vegetation with forceps or an aspirator (Agosti et al. 2000, Tista & Fiedler 2011). We also turned stones, twigs, palm leaves or other ground material (Brühl & Eltz 2010). All sampled ants were preserved on site in 70 % ethanol.

Winkler method

Winkler extraction is an efficient method to record leaf-litter ants (Agosti et al. 2000). For this purpose, all litter from 1 m^2 of ground was removed and manually sifted with a sieve with a wire mesh of 1 cm (Tista & Fiedler 2011). The litter was then filled into the inner bag of a Winkler sack which consisted of nets with a mesh width of 6 mm. After closing the upper end of the Winkler sack, throughout the next three days ants were allowed to fall into a jar filled with 70 % ethanol at the lower end (Tista & Fiedler 2011). At each study site we randomly chose five replicate subplots for litter removal, resulting in a total area sampled of 5 m^2 per plot. Also all ants extracted from the leaf litter were preserved in 70 % ethanol.

All ant vouchers are deposited in the collections of the Natural History Museum, Vienna.

Ant identification and species' ecological traits

Worker ants were identified under a stereomicroscope to genus level and were further segregated within each genus into morpho-species, mainly using the internet data-base www.antweb.org (v7.34.4, last accessed 10/07/2018). Morpho-species of the same ant genus were differentiated from one another on the basis of multiple character differences, for example with regard to body size, color, presence or absence of spines, hairiness, cuticle sculpture, etc. In genera known for worker polymorphism, vouchers showing only slight morphological differences were treated as one species, when collected at the same place, especially if one morpho-type occurred much more often than the second. The few alate females and males obtained in pitfall or hand samples were discarded, since it is unknown whether these originated from colonies living at the sampling sites. Information on ecological traits of ants was mainly retrieved from the data-base www.antwiki.org (last accessed: 11/07/2018) and from Brandão et al. (2012).

Data analysis

For statistical analyses of ant biodiversity, we only used presence-absence coding, since ants as eusocial insects often show a highly clumped representation in ecological field samples. Accordingly, worker numbers will often be higher in samples drawn near nest sites or ant trails. After morpho-species sorting, data were entered into a spread-sheet program for subsequent management and retrieval. Primarily, we extracted two types of tabulated data: (1) the numbers of ant species and the proportions of various ant groups (subfamilies or ecologically important groups such as army ants and leaf-cutter ants) per site; and (2) a species × site × sample type matrix for analyses of species composition on habitat level.

Using the software packages PAST 3.26 (Hammer et al. 2001), box-whisker plots and one-way analyses of variance by ranks (Kruskal-Wallis ANOVA) served to analyze differences in species richness per site and for the comparison of sampling methods between habitat types. We further compared overall ant richness of the three habitat types by means of species accumulation analysis (calculated with iNext Online; Chao et al. 2016), treating each sample type from every site as a replicate unit of analysis and extrapolating (two-fold) to 30 sample units per habitat type.

Species composition was analyzed based on a Soerensen similarity matrix. We visualized sample composition by means of unconstrained ordination (non-metric MDS) and tested for possible differences between habitat types and sampling methods by a two-way permutational analysis of variance (PERMANOVA, 9999 permutations) using the software packages PRIMER 7.0.13 (Clarke & Gorley 2006) and PERMANOVA+ for PRIMER (Anderson et al. 2008), respectively. Faunal nestedness was assessed using a program developed by Strona (2015) with 100 replicates in null model analysis.

Results

Species richness

Altogether, 4752 worker ants were collected, belonging to 136 morpho-species out of 44 genera in 9 subfamilies. In total, 36 species were found in the oil palm plantations, 76 species in secondary forest, and 90 species in old growth forest. Species accumulation analysis (Fig. 3) indicated that, while the ants to be expected in oil palm plantations were already largely covered by sampling (coverage: 0.913), many more ant species awaited discovery in secondary (coverage: 0.805) and old-growth forest (coverage: 0.834). Overall, ant species richness in both forest types was significantly higher than in oil palm plantations, as indicated by the non-overlapping 95 % confidence intervals of the species accumulation curves for oil palm plantations versus the two forest types (Fig. 3).

Ant species numbers were unevenly distributed across the subfamilies (Fig. 4). Representatives of Myrmicinae were present in all three habitat types in high species numbers. Ponerinae ants were substantially more prevalent in old-growth forest than in secondary forest or plantations. Richness of Formicinae was greatly reduced in oil palm plantations compared to secondary and old-growth forest, whereas the subfamilies Heteroponerinae and Proceratiinae were only found in old-growth forest. Overall, gross taxonomic composition of ant assemblages was most complex in old-growth forest and most simplified in oil palm plantations.

Differences between habitat types in species numbers recorded per site were highly significant (Fig. 5). Oil palm plantations (mean 17.6 species/site) showed the lowest richness, old-growth forest (mean 36.4 species/site) the highest, and secondary forest (mean 26.9 species/site) an intermediate richness. On average, oil palm plantation sites reached 48.4 %

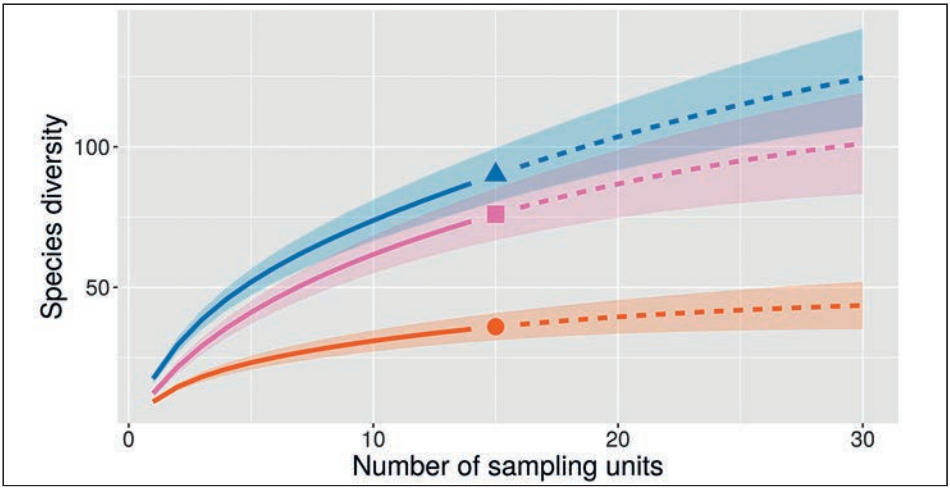


Fig. 3: Rarefaction (solid) and extrapolation curves (dashed) for ant species in oil palm plantations (brown), secondary forest (purplish) and old-growth forest (blue). Symbols: observed species richness. Shaded areas around curves are 95 % confidence limits. – Abb. 3: Interpolation (durchgezogene Kurven) und Extrapolation (gestrichelt) des Artenreichtums von Ameisen in Ölpalmplantagen (braun), Sekundärwald (violett) und Regenwald (blau). Symbole geben beobachteten Artenreichtum an, die schattierten Bereiche die 95 %-Vertrauensbereiche der Kurven.

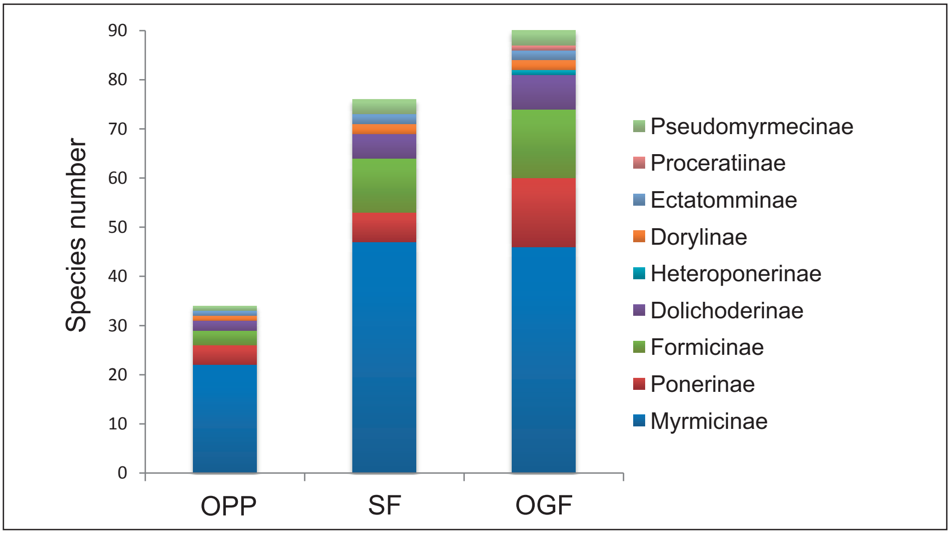


Fig. 4: Subfamily composition of ground-dwelling ant assemblages in the three studied habitat types. OPP: oil palm plantations; SF: secondary forest; OGF: old-growth forest. – Abb. 4: Zusammensetzung der Ameisen-Gemeinschaften auf Unterfamilien-Ebene. OPP: Ölpalmplantagen; SF: Sekundärwald; OGF: Regenwald.

and secondary forest 73.9 % of the mean ant species richness observed at old-growth forest sites. Interestingly, when using the combined number of worker ants recorded by the three sampling methods per site as a proxy of overall ant abundance, no consistent difference between the three habitat types was apparent ($H_{2df} = 2.94$; $p = 0.23$).

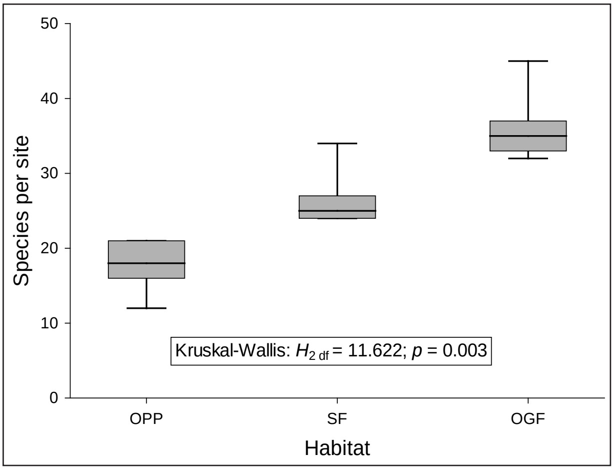


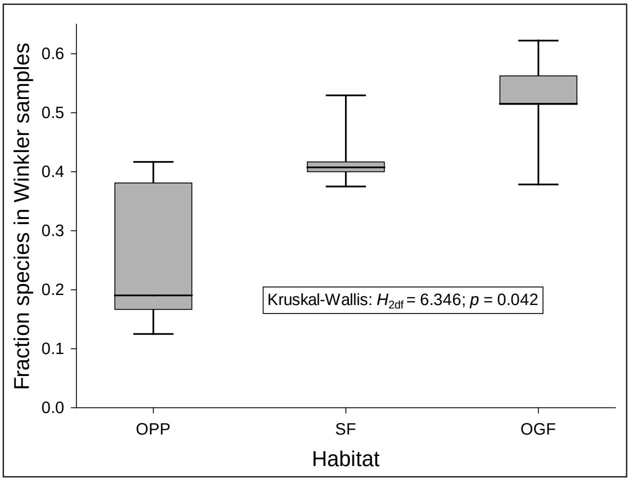
Fig. 5: Ant species richness per site compared across oil palm plantations (OPP), secondary (SF) and old-growth forest (OGF). Line: median; box: inter quartile range, whiskers: range of observed values. – Abb. 5: Artenreichtum der Ameisen pro Standort, im Vergleich von Ölpalmplantagen (OPP), Sekundärwäldern (SF) und Regenwaldstandorten (OGF). Querlinie: Median, Kasten: Interquartilbereich; Streuungsbalken: Spannweite der Werte.

Contribution of specific ant groups

In total, 64 (of 136) ant species were retrieved at least once from a leaf-litter sample by the Winkler method. These ants contributed 2–8 species per site in oil palm plantations, 9–18 species in secondary forest, and 14–28 species in old-growth forest. The proportion of ant species recorded in Winkler samples relative to the total species number per site was lowest in oil palm plantations and highest in old-growth forest (Fig. 6).

We observed three species of army ants (two species of *Labidus*, one of *Eciton*) at the 15 study sites. Army ants were observed in four plantations and at four secondary forest sites, but only in two old-growth forest sites. Hence, army ants tended to be slightly less prevalent in old-growth forest (Kruskal-Wallis ANOVA: $H_{2df} = 5.579$; $p = 0.061$).

Fig. 6: Proportion of ant species retrieved from litter samples relative to total ant species number per site, across oil palm plantations (OPP), secondary (SF) and old-growth forest (OGF). – Abb. 6: Artenanteil der Ameisen aus Laubstreu-Extraktionen, im Vergleich von Ölpalmplantagen (OPP), Sekundärwäldern (SF) und Regenwaldstandorten (OGF). Querlinie: Median, Kasten: Interquartilbereich; Streuungsbalken: Spannweite der Werte.



Three species of leaf-cutter ants (two species of *Acromyrmex*, one of *Atta*) plus a range of other fungus-growing ants (in the genera *Apterostigma*, *Cyphomyrmex*, *Myrmicocrypta*, *Sericomyrmex* and *Trachymyrmex*) showed up during our surveys. However, the relative contribution of these fungus growers to local ant species richness did not differ consistently between the three habitat types ($H_{2df} = 1.580$; $p = 0.454$).

Species composition

A representation of the species × site matrix by means of an unconstrained ordination (non-metric MDS, stress = 0.22, Fig. 7) revealed clear differences in ant species composition between all three habitat types, but with little overlap. Along the first ordination axis, ant assemblages differentiated from old-growth forest (to the left) towards oil palm plantations (to the right). The second ordination axis reflected sampling methodology, with hand samples attaining high values, pitfall samples intermediate values, and Winkler samples lowest axis scores. These differences were all significant (two-way PERMANOVA; habitat type: $F = 7.822$, sample type: $F = 3.315$; both $p < 0.001$; habitat × sample interaction: $F = 1.325$, $p = 0.044$).

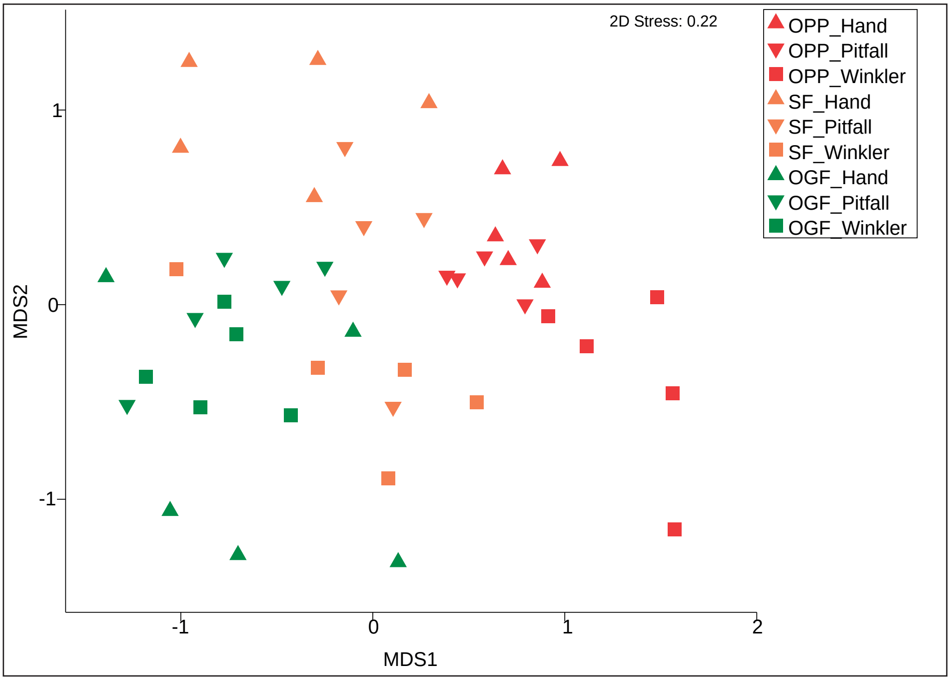


Fig. 7: Non-metric MDS ordination plot of the ant assemblages of the 15 study sites, further segregated according to the sampling method, based on species incidence data. OPP = oil palm plantations (red), SF = secondary forest (orange), OGF = old-growth forest (dark-green). Upright triangles – hand samples; inverse triangles – pitfall trap samples; squares: Winkler litter samples. – Abb. 7: Ordinationsdiagramm der Ameisen-Aufsammlungen, gegliedert nach Standorttyp und Methodik der Erfassung. OPP: Ölpalmplantagen (rot); SF: Sekundärwald (orange); OGF: Regenwald (grün). Aufrechte Dreiecke: Handaufsammlungen; umgekehrte Dreiecke: Bodenfallen; Quadrate: Winkler-Extraktionen.

Inspection of the ordination plot suggested that ant assemblages of oil palm plantations were the most homogeneous and those from secondary forest sites the most heterogeneous. An index of multivariate dispersion (MVDISP routine in PRIMER7) confirmed that notion. Here, index values below 1.00 indicate relative clumping, and those larger than 1.00 indicate high dispersion of samples. This index was 0.697 for ants in plantations, 1.005 for old-growth forest, and 1.298 for secondary forest samples.

A total of 35.3 % (48 species) of all observed ant species were only collected in old-growth forest, 21.3 % (29 species) in secondary forest, while only 4.4 % (6 species) were unique to oil palm plantations (Fig. 8). On the genus level, nine ant taxa were only found in old-growth forest (*Acanthoponera, Acropyga, Anochetus, Ectatomma, Hylomyrma, Leptogenys, Myrmecocrypta, Probolomyrmex, Tapinoma*), and six only in secondary forest (*Adelomyrmex, Cephalotes, Eciton, Linepithema, Nesomyrmex, Rogeria*), but no single ant genus only showed up exclusively in oil palm plantations. Some ant species were collected in 12 or more of the 15 study sites (for example certain representatives of *Odontomachus, Pachycondyla*, and *Nylanderia*), while others were collected in four or more study sites of one habitat type but nearly never in one of the other two habitat types (for example in plantations: *Acromyrmex* and some *Pheidole* species; in old-growth forest: *Brachymyrmex* and *Trachymyrmex*). 34 unique species (i.e. species only collected at one of the 15 study sites) were found in old-growth forest, 22 in secondary forest and only three in oil palm plantations.

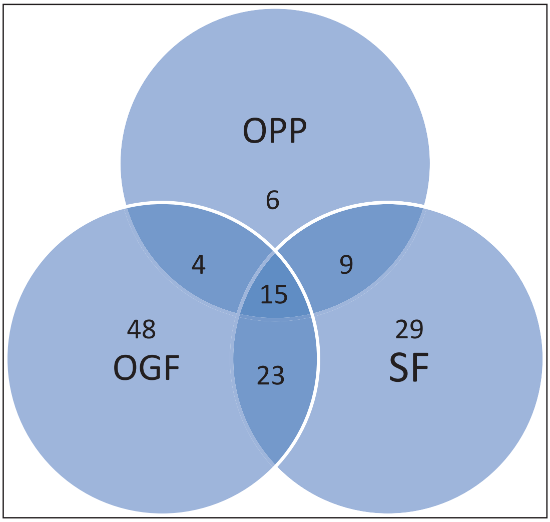


Fig. 8: Venn diagram showing numbers of exclusive and shared ant species across the three habitat types. – Abb. 8: Venn-Diagramm der Ameisen-Artenzahlen in den drei Habitattypen.

The results above indicate that species richness of local ground-dwelling ant assemblages decreases severely from old-growth across secondary forest to oil palm plantations. Yet it remains to be addressed whether the concomitant shift in ant species composition primarily reflects a pauperization of the communities or whether true species turnover also plays a role. We therefore analyzed the ant assemblages of the 15 sites for the strength of nestedness in community patterns. If communities were fully nested, all assemblages of lower richness should represent subsets of the richer communities. Our analyses indeed revealed that the local ant assemblages along the gradient from old-growth forest to oil palm plantations were highly and significantly nested ($NODF_{sites}$ = 40.671, matrix temperature $T = 33.376$; for the null model of proportional row and column totals: $z = -6.634$ and $z = 14.319$, respectively; $p<0.001$). The maximally ordered species × site matrix is shown in Figure 9.

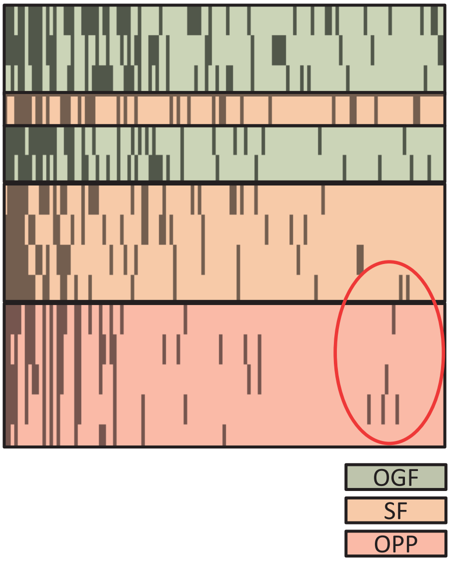


Fig. 9: Visual representation of the maximally ordered species × site matrix of ground-dwelling ants. Rows: sites; columns: ant species. Species-poor assemblages are subsets of richer ones, indicating a gradient of faunal impoverishment from old-growth forest (OGF) towards oil palm plantations (OPP). Only few ant species (marked with red ellipse) deviate from that pattern and can thus be interpreted as beneficiaries of land-use. – Abb. 9: Visualisierung der maximal geordneten Arten-Standorts-Matrix. Zeilen: Standorte; Spalten: Arten. Artenarme Gemeinschaften sind Teilmengen der artenreicheren, was einen Gradienten der Verarmung vom Regenwald (OGF) hin zu den Ölpalmplantagen (OPP) anzeigt. Nur wenige Ameisenarten (markiert mit roter Ellipse) können als Nutznießer der Plantagen angesehen werden.

Discussion

Species richness

Oil palm plantations harbored by far the lowest richness of ground-dwelling ants (36 species), i.e. about one third of the total species number observed in old-growth forest (90 species) and only a quarter of the total ant species richness observed near La Gamba in this study (136 species). This corresponds well with other studies in which species richness of ants was found to be up to five times higher in primary forest than in plantations (Brühl & Eltz 2010, Fayle et al. 2010, Pfeiffer et al. 2008). One important reason for these massive differences is the almost complete absence of leaf litter in plantations which reduces availability of nest sites and foraging opportunities (Carvelho & Vasconcelos 1999). This affects in particular those 'cryptic' ant species which predominantly forage in soil and litter and rarely show up above ground. Cryptic leaf litter ants are a hallmark of tropical rainforest ant assemblages (King et al. 1998). The distribution of a substantial litter stratum explains the higher species number of Ponerinae in old-growth forest as well as the total absence of the ant subfamilies Proceratiinae and Heteroponerinae in secondary forest and plantations. The far lower contribution of ants sampled by the Winkler method in oil palm plantations underpins the impoverishment of the litter fauna in this agro-ecosystem.

Moreover, the temporarily hot and dry microclimatic conditions in plantations, compared to primary and secondary forest (Turner & Forest 2006), may prevent colony establishment of shade-adapted forest ants in plantations. Food availability might also be critical for ant species with specialized diets, whose resources might be lacking in plantations (Andersen 2000). Surprisingly, however, we did not observe a lower representation for army ants and fungus growers in oil palm plantations relative to the forest. Nevertheless, our data confirm that the ecological conditions in oil palm plantations act as a severe filter which prevents a large fraction of ants from the regional species pool to colonize such sites.

Secondary forests harbored far more ant species than oil palm plantations, but fewer species than old-growth forest. This reflects, on the one hand, the lower complexity and thus lower number of niches in regenerating forest, compared to old-growth forest. For example, Dunn (2004) showed that ants re-colonize regenerating areas after disturbance, which leads to an increasing number of ant species during secondary forest succession. Accordingly, the recovery of ant species richness in tropical forests could be complete after 20–40 years of succession, provided that re-colonization is possible from the surrounding landscape (Hethcoat et al. 2019). On the other hand, the relatively high ant species numbers observed at our young secondary forest study sites underpins the potential of areas left to spontaneous regeneration for biodiversity conservation. Already after 10–15 years, these habitats had accumulated a substantial fraction of the ant fauna seen in old-growth forest.

The increase of ground-dwelling ant species richness from oil palm plantations across secondary towards old-growth is also explicable with the different vegetation structure of these habitat types (Philpott et al. 2008). Ant biodiversity is often very closely related to heterogeneity and structural complexity of habitats (e.g. Bestelmeyer & Wiens 2001). Plantations have the simplest, secondary forests a more strongly differentiated, and primary forests a particularly complex vegetation structure (Chazdon 2014). Alonso Rodríguez (2014) confirmed for the region around La Gamba that oil palm plantations have lowest plant species richness, vegetation density and complexity, whereas these attributes attain much higher values in secondary and especially in old-growth forest. In ants, anthropo-

Tab. 1: Species richness of ants, butterflies (Wiemers & Fiedler 2008), understory birds (Mollik 2013), bats (Freudmann et al. 2015), amphibians and reptiles (Gallmetzer & Schulze 2015) and two moth subfamilies (Alonso Rodríguez et al. 2017, figures amended by personal communications of D. Rabl and G. Brehm) in oil palm plantations around La Gamba, compared with old-growth and secondary (or disturbed) forest. – Tab. 1: Artenreichtum von Ameisen, Tagfaltern (Wiemers & Fiedler 2008), Unterwuchsvögeln (Mollik 2013), Fledermäusen (Freudmann et al. 2015), Amphibien und Reptilien (Gallmetzer & Schulze 2015) und zwei Nachtfalter Unterfamilien (Alonso Rodríguez et al. 2017, Zahlen durch persönl. Mitteilungen von D. Rabl and G. Brehm ergänzt) in Ölpalmplantagen um La Gamba, verglichen mit Regenwald und Sekundärwald (bzw. gestörtem Wald).

Group	Total	Number of recorded species (% of species total)		
		Oil-palm plantation	Secondary forest (incl. forest margins)	Old-growth forest
Ants	136	34 (25.0)	76 (55.9)	90 (66.2)
Birds	77	23 (29.9)	54 (70.1)	47 (61.0)
Bats	36	20 (55.6)	22 (63.9)	28 (75.0)
Amphibians	28	11 (39.3)	20 (71.4)	22 (78.6)
Reptiles	19	7 (36.8)	12 (63.1)	13 (68.4)
Butterflies	118	47 (39.8)	59 (50.0)	70 (59.3)
Arctiinae moths	132	34 (25.8)	78 (59.1)	111 (72.1)
Geometridae moths	154	31 (20.1)	119 (77.3)	107 (69.5)

genic simplification of vegetation structures leads to an even heavier loss of species richness than in other animal groups (Philpott et al. 2008).

A comparison of studies on various animal groups like ants, butterflies (Wiemers & Fiedler 2008), understory birds (Mollik 2013), bats (Freudmann et al. 2015), amphibians and reptiles (Gallmetzer & Schulze 2015) and two moth (sub-)families (Alonso Rodríguez et al. 2017), which were all carried out around La Gamba, invariably showed a substantial loss of species richness in oil palm plantations relative to old growth forests (Tab. 1).

Ground-dwelling ants and moths suffered the highest loss of local species richness in oil palm plantations in the Golfo Dulce region. Only about one fourth to one fifth of total species richness known to occur in the landscape from targeted surveys of those two insect taxa was also recorded in these agro-ecosystems. For birds, amphibians, reptiles and butterflies 30–40 % of the total species list also occurred in plantations, and in bats even over 55 % of the regional species at least occasionally showed up in plantations (Tab. 1). These substantial losses of biodiversity are all the more remarkable as all oil palm plantations surveyed around La Gamba were situated close to old-growth forest in the Piedras Blancas National Park. With distances to near-natural forest of less than 1 km, spill-over of animals from source areas situated in the reserve likely contributed a substantial fraction of species recorded in plantations, especially in mobile organisms such as bats, birds or butterflies. For ants, however, the species recorded in oil palm plantations were indeed an integral part of the agro-ecosystems since we only considered wingless worker ants. Hence, the presence of workers clearly indicates the existence of established ant colonies nearby.

The above compilation also sheds further light on the conservation value of secondary forests or disturbed forest habitats in the Golfo Dulce region. Species numbers known from old-growth forest were not invariably the highest. Rather, for certain groups of organisms, forest sites under not too severe human influence supported even higher local biodiversity. Therefore, secondary forest as well as disturbed forest allowed to regenerate may have a substantial conservation value, for example as buffer zones at the margins of large reserves (Chazdon et al. 2009, Chazdon 2014). Generally speaking, with the progress of succession, regenerating tropical forest communities will eventually converge to near-natural situations similar to "pristine" forest, even though this might not allow every single species to recover (Bihn et al. 2010, e.g. for plants in Mesoamerica: Dent 2013).

Species composition

Ant species composition of the three habitat types differed highly significantly from each other. Just 15 of all 136 collected morpho-species were common to all habitat types and can therefore be regarded as opportunistic habitat generalists (one species each in the genera *Crematogaster, Dolichoderus, Labidus, Monomorium, Nylanderia, Odontomachus, Pachycondyla, Pheidole, Pseudomyrmex, Sericomyrmex, Strumigenys, Wasmannia* and three species of *Solenopsis*). In these genera there are some well-known generalist and 'weedy' invasive ants (for example *Monomorium, Nylanderia, Pheidole, Solenopsis* and *Wasmannia*; Bertelsmeier et al. 2015). Otherwise, oil palm plantations shared only 4 additional species with old-growth forest and 9 species with secondary forest. In contrast, faunal overlap between secondary and old-growth forest was much larger, with 23 ant species in common in addition to the generalists. This highly nested community pattern is best explained by an impoverishment of ant assemblages, from species-rich old-growth forest characterized by a high number of available niches, across secondary regenerating forest (where niche diversity becomes successively restored), to oil palm plantations. Very few weedy ant species could be recognized as beneficiaries of land-use in oil palm plantations. Moreover, the ant assemblages of the five investigated plantations were remarkably similar to one another. Hence, faunal impoverishment of ground-dwelling ants in these agro-ecosystems was accompanied by strong homogenization.

Among ant guilds, leaf-litter ants experienced by far the strongest loss in oil palm plantations (see above). In contrast, another spectacular guild of ground-dwelling ants, viz. army ants (genera *Labidus* and *Eciton*: O'Donnell et al. 2007), showed no clear difference in their incidence across habitat types. If anything, army ants were slightly less apparent in old-growth forest, but a more extensive survey effort might well also have resulted in more frequent encounters with raid swarms there. Elsewhere in the Neotropics, army ants are well known to forage in plantations and other agro-ecosystems as long as these are situated close to near-natural forest (Monteiro et al. 2008, Roberts et al. 2000).

In summary, our study corroborated the severe negative effects that oil palm cultivation has on biodiversity (Foster et al. 2011), using ground-dwelling ants in Costa Rican lowlands as an example. Ants are important predators of other arthropods including agricultural pests. Therefore, substantial shifts in ant species richness or community composition could interfere with the ecosystem services that ants usually provide (Philpott & Armbrecht 2006). Yet, a recent study (Denmead et al. 2017) found no effect of the manipulation of ant densities on important ecosystem processes in oil palm plantations in Sumatra, even though ants turned out once more to be the most important invertebrate predators there. Hence, while the detrimental influence of oil palm cultivation on biodiversity becomes ever better documented, the potential effects that this biodiversity loss may have on the functioning of ecosystems deserves further scrutiny.

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Surface structures of *Cecropia* plants as landmarks for orientation of ant foundress queens?

Josephine S. Busch & Veronika E. Mayer

Myrmecophytes are plants which provide nesting space and food for ants. *Cecropia* plants (Urticaceae) from South and Central America are such myrmecophytes. They have a mutualistic relationship with *Azteca* ants (Formicidae, Dolichodorinae), which defend their host plant against herbivores. For this service, *Cecropia* plants offer food to their resident ants (Mullerian bodies) at the base of the petioles and nesting space in the hollow stem (= domatia). To enter the hollow stem, young *Azteca* foundress queens bite a hole into a zone where the domatium wall is thinner (the so-called prostoma), enter the cavity and close the hole. It is not yet clear how *Azteca* ants locate the prostoma zone. The study presented here indicates that surface structures on prostoma and stem could be the clue. We found a significant difference between stem and prostoma zone in three ant-associated *Cecropia* species (*C. insignis, C. obtusifolia, C. peltata*), regarding the number of trichomes and papillae. Additionally, we found an influence of internode age on the number and distribution of trichomes and papillae. The differences are interpreted as landmarks to signal to the young queens the position of the prostomata, and the age and the size of the internode. All three are important to find an internode suitable for successful colony founding.

Busch J.S. & Mayer V.E., 2019: Sind die Oberflächenstrukturen auf den Stämmen von *Cecropia* Pflanzen eine Orientierungshilfe für junge, koloniegründende Ameisenköniginnen?
Ameisenpflanzen stellen Nahrung und Nistraum für symbiotische Ameisen zur Verfügung. Die in Mittel- und Südamerika sehr häufigen *Cecropia*-Arten (Urticaceae) sind solche Ameisenpflanzen. Sie gehen eine Symbiose mit *Azteca* (Formicidae, Dolichodorinae) Ameisen ein, die ihre Wirtspflanze gegen Fressfeinde verteidigen. *Cecropia* bietet seinen „Untermietern" dafür Futterkörperchen an der Basis der Blattstiele und Wohnraum im hohlen Stamm an (= Domatium). Um ins Innere des hohlen Stammes zu gelangen, untersuchen die jungen Königinnen die Oberfläche junger *Cecropia* Stämme und beißen sich schließlich durch das sogenannte „Prostoma", eine Stelle, an der die Wand des Stamms dünner ist als das umliegende Gewebe. Bis jetzt war nicht klar, wie *Azteca* das Prostoma lokalisieren. Unsere Studie bei drei mit Ameisen vergesellschafteten *Cecropia* Arten (*C. insignis, C. obtusifolia, C. peltata*) zeigt, dass Oberflächenstrukturen der Schlüssel sein könnten. Wir fanden erstens signifikante Unterschiede bei der Anzahl und Verteilung von Trichomen und Papillen je nachdem, ob es sich um die Oberfläche des Prostomas oder der umliegenden Stängeloberfläche handelt. Zweitens wurden Unterschiede in der Anzahl der Oberflächenstrukturen bei Internodien unterschiedlichen Alters festgestellt. Unsere Ergebnisse legen den Schluss nahe, dass die Oberflächenstrukturen den jungen Koloniegründerinnen die Position als auch das Alter der Internodien signalisiert. Sie sind daher eine wichtige Voraussetzung für die erfolgreiche Koloniegründung.

Keywords: *Cecropia*, *Azteca*, surface structures, trichomes, papillae, internode age, symbiosis, ant-plant interaction, orientation cues.

Introduction

Myrmecophytes are plants which provide nesting space and food for ants. From the tropics all over the world, about 650 vascular plants living in symbiosis with ants are known (Chomicki & Renner 2015). There are two kinds of ant-plant interactions, facultative and obligate associations. In facultative associations, ants visit the plant to look for food. In obligate ant-plant interactions, the host plants provide specialised food as well as nesting space (domatia) to their symbionts (Jürgens et al. 2006). One of the oldest reported mutualistic interactions between ants and plants is the association of *Cecropia* sp. Loefl. plants (Urticaceae) with *Azteca* Forel ants (Dolichoderinae, Formicidae) (Müller 1880, Belt 1874). The ants nest in the internodes of the hollow trunk and branches. The ants harvest phytoglycogen-rich multicellular bodies (Müllerian bodies)

Fig. 1: (A) *Cecropia obtusifolia* tree. The arrows indicate the position of the prostomata; (B) Foundress queen of *Azteca alfari* biting an entrance hole into the prostoma tissue of a young *C. peltata* stem; (C) SEM detail of a *C. insignis* prostoma. Bar: 200 µm. – Abb. 1: A) *Cecropia obtusifolia* Stamm. Die Pfeile zeigen die Position der Prostomata; (B) Eine Azteca alfari Koloniegründerin beisst ein Eingangsloch in das Prostomagewebe eines jungen Stängels von *C. peltata*; (C) REM Detail eines *C. insignis* Prostomas. Maßstab: 200 µm.

produced on pads of trichome-covered tissue (trichilia) at the petiole base interface (Rickson 1971, Bischof et al. 2013) to feed their larvae.

To colonize the hollow stem of myrmecophytic *Cecropia* plants, foundress queens and later the workers must bite entrance holes into the domatia wall to enter the domatia (Fig. 1B). There are zones in many myrmecophytes where the domatium wall tissue is thinner and weaker, a so-called prostoma (Ihering 1907, Bailey 1922, Brouat et al. 2001, Sanchez 2016). The prostomata are elliptical or round areas and longitudinal structures running all over the internode (Fig. 1A, C). They consist of unvascularized tissue, lack latex vessels and have a delayed cambium formation (Bailey 1922, Davidson & Fisher 1991, Brouat et al. 2001; Federle et al. 2001). Brouat et al. (2001) showed that prostomata can act as a morphological and behavioural filter to exclude generalist ants and favor colonization with the symbiont ants. This is because, first, ants must be able to chew entrance holes, and second, the size and shape of the prostoma must match the queen's body size and shape. It is not yet known how ants find the position of the prostomata once they have landed on their host plant.

A first indication of how ants can perceive the prostoma zone was found in *Macaranga*, where ant workers use incident daylight to recognize the prostomata from inside (Federle et al. 2001). Light as an orientation cue does not work outside and is thus unsuitable for colony-founding queens. Therefore, it is suggested that prostomata have orientation cues on their outer surface to facilitate an ant queen's colony foundation.

In the present study we analysed, in three *Cecropia* species (*C. insignis, C. obtusifolia, C. peltata*) which are obligately associated with *Azteca* plant-ants, whether the surface structure of the prostoma and regular stem provides mechanical orientation cues for *Azteca* foundress queens. We show that the presence of bigger trichomes and smaller papillae exist, and that the number and density clearly differs between prostoma sites and the surrounding stem surface and between younger and older internodes.

Material and Methods

The fieldwork was conducted at the La Gamba Field Station, a research facility of the University of Vienna (Austria). The station is situated in the Golfo Dulce region in Costa Rica near the Pacific coast (N 8°42.61′, W 83°12.97′), next to the Parque Nacional Piedras Blancas and 70 m above sea level. Plants and ants were collected on plantations, along roads and in the secondary rainforest around the field station.

Three young saplings each of *Cecropia insignis*, *C. obtusifolia* and *C. peltata*, between 50–150 cm, were collected in the field, and one additional *C. peltata* was taken from the Botanical Garden in Vienna (Austria) and was unoccupied. Previous studies revealed that the foundress queens are predominantly found between the second youngest and the eighth internode (I2–I8, counted from the apex downward) (Fig. 2), therefore we investigated only the 10 youngest internodes. The collected stems were cut into c. 15 cm long stem pieces and fixated in FAA (Formaldehyde, acetic acid, 70 % ethanol – 5:5:95).

Collection and export was granted by the Sistema Nacional de Áreas de Conservación (No. INV-ACOSA-037-15) to Josephine Busch (former Schmiedel).

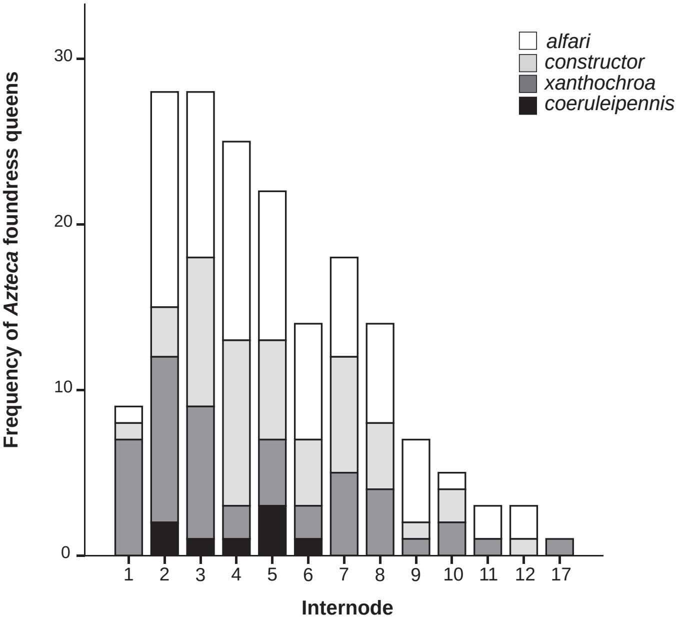

Fig. 2: Distribution of *Azteca*-foundress queens. Bars give the frequency of the azteca foundress queens found (*A. alfari, A. constructor, A. xanthochora, A. coeruleipennis*) in the internodes (n=56 *Cecropia* saplings from Monteverde and La Gamba, Costa Rica). – Abb. 2:Verteilung von *Azteca*-Jungköniginnen. Die Balken zeigen die Häufigkeit von Jungköniginnen aufgeschlüsselt nach *Azteca*-Art (*A. alfari, A. constructor, A. xanthochora, A. coeruleipennis*) in Internodien von 56 *Cecropia* Jungpflanzen (aus Monteverde und La Gamba, Costa Rica).

Electron-microscopical investigation

We investigated the stems of three *Cecropia insignis*, four *C. peltata* and three *C. obtusifolia* plants. The respective stems were fixated in the field FAA (Formaldehyde, acetic acid and Ethanol 70 %) and transported to Vienna. From each internode, the prostoma area as well as a representative area of the surrounding stem surface was cut out, dehydrated in an increasing ethanol series (70 %, 85 %, 96 %) with acetone as final step and critical point dried with a tousimis Autosamdri 815 critical point dryer and acetone as exchange medium. Sputter coating with gold was applied for 5 min at 10 kV in an Argon-atmosphere (Scd 050, Balzers Ag). After 5 min, the gold layer on the specimens is approximately 60 nm thick. Samples were then investigated with a Jeol Jsm-6390 electron microscope. It was not possible to investigate all internodes of one stem, because partly the internodes were already colonised by foundress queens and the prostoma modified, and sometimes they were destroyed by woodpeckers looking for ant larvae to feed on.

SEM pictures were taken from the center of the prostoma area and the surrounding stem surface. Then, a square of 0.5 mm × 0.5 mm was drawn into the SEM picture with ImageJ (Rueden et al. 2017) and the length of all surface structures was measured. Due to the considerable length differences of surface structures (Fig. 3A), we looked at the length distribution and distinguished two classes: the ones from maximal length to the average length were defined as "trichomes", and the ones from average to minimal length were defined as "papillae". With the same software, all trichomes and papillae that were inside the square with at least 50 % of their base were then counted, and their length as well as their base width was measured.

The youngest internode, which is normally enclosed by the caducous spathe, was not regarded. The first internode free of the caducous spathe was classified as internode I1.

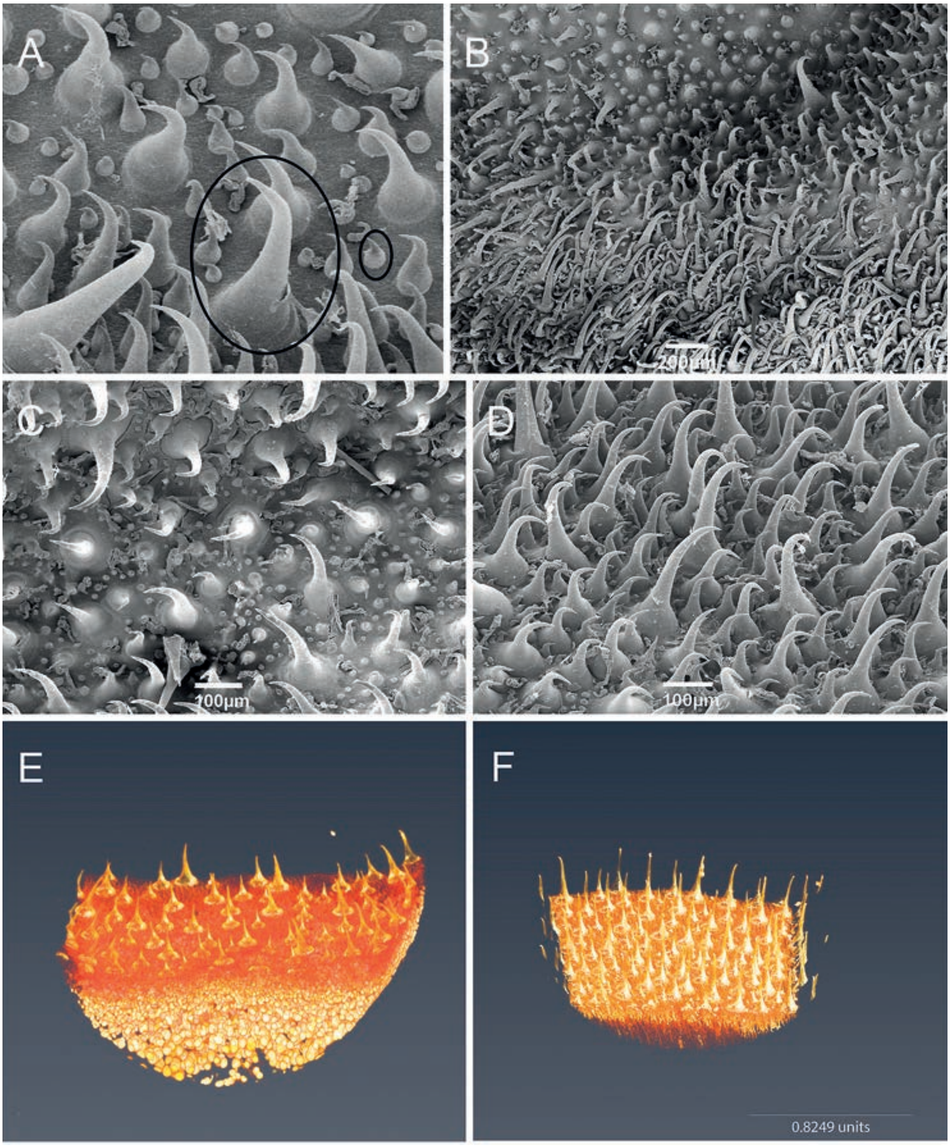

Fig. 3: (A) Trichomes (big circle) and papillae (small circle) on a *Cecropia insignis* stem; (B) transition zone between stem and prostoma of *C. peltata*; (C) prostoma surface of the second youngest internode of *C. obtusifolia*; (D) the corresponding stem surface; (E) CT-scan of the prostoma surface; (F) CT-scan of the corresponding stem surface (both from *C. peltata*). – Abb. 3: (A) das große schwarze Oval markiert ein Trichom, das kleine eine Papille; (B) Übersicht über das Prostoma; (B) Übergangszone zwischen Stängel- und der Prostomaoberfläche bei *C. peltata*; (C) Prostoma-Oberfläche des zweitjüngsten Internodiums von *C. obtusifolia*; (D) die zugehörige Stängeloberfläche; (E) CT-Scan der Prostoma-Oberfläche; (F) CT-Scan der zugehörigen Stängeloberfläche (beides von *C. peltata*).

Higher numbers mean older internodes, and they were numbered from the apex downwards to the stem base. For better handling, we made four groups comprising the first free internode as group 1, internode 2–4 as group 2, internode 5–7 as group 3, and internode 8–10 as group 4.

In total 84 prostomata and 86 stem areas (both from internode 1 to internode 17) were investigated.

Statistics

The statistical analyses were performed with IBM SPSS Statistics 23 (IBM 2015). To test whether the density of papillae and trichomes differed in general, whether the surface structure of the prostomata differed from the surface structure of the surrounding stem surface, and whether the age of the internode resulted in differences of the surface structures, a non-parametric Wilcoxon signed rank test for paired samples was used.

CT Analysis

For X-ray micro-CT scans, the FAA-fixated stem pieces were cut into smaller, 5 cm long parts and the tissue stained with a solution of 1 % (w/v) PTA (Phosphotungstic acid) in 70 % FAA (Gamisch et al. 2013; Staedler et al. 2013). The FAA/PTA solution was changed every second week.

After four months, the stained stem pieces were put into a test tube, fixed with synthetic cotton wool which was placed around the stem, and the tubes closed with parafilm. The stems were imaged with a MicroXCT-200 X-ray tomography system from Zeiss Microscopy (Jena, Germany). This system uses a 90 kV microfocus X-ray source (L9421-02 from Hamamatsu, Hamamatsu City, Japan), a cooled 2k CD camera, and switchable scintillator objective lens units. The three-dimensional reconstruction of the scans was made with the XM Reconstructor 8.1.6599 software (Zeiss Microscopy) and the scans subsequently rendered with the software Amira 5.5.0.

Results

The counts of surface structures in *Cecropia insignis*, *C. peltata*, and *C. obtusifolia* resulted in a considerable difference. The total number of all surface structures was significantly different between prostoma and stem in *C. peltata* and *C. obtusifolia*, but not in *C. insignis.* In *C. peltata* and *C. obtusifolia* the stem surface had a considerably higher number of surface structures than the prostoma site (Wilcoxon signed rank test, $P<0.05$; see also Tab. 1 and 2, Fig. 3). The CT scans additionally reinforced the difference between prostoma and stem surface (Fig. 3E, F). In *C. insignis* the average number of surface structures was more or less equal between stem and prostoma surface (Wilcoxon signed rank test, $P=0.13$).

If the total number is separated into the average number of papillae and trichomes, the prostoma site of *C. peltata* (n=25) and *C. obtusifolia* (n=29) had a significantly higher number of trichomes than of papillae, especially in the stem area (Wilcoxon signed rank test, $P=0.000$). Both had many more long trichomes than short papillae, the proportion is, however, similar with 82.9 % (*C. peltata*, n=27) and 85.9 % (*C. obtusifolia*, n=27) trichomes and

17.1 % (*C. peltata*) and 14.2 % (*C. obtusifolia*) papillae on the stem, and on the prostoma 66.5 % and 64.5 % trichomes and 33.3 % and 35.5 % papillae, respectively.

In contrast, *C. insignis* had a much higher number of papillae (70.9 % of the surface structures) and less trichomes (29.1 %) in the prostoma site. On the stem surface, 52.0 % were papillae and 47.96 % were trichomes and the average amount was more or less equal (Table 1).

Tab. 1: Average number of all surface structures per 0.25 mm², and trichomes and papillae, respectively. N gives the number of investigated internodes from 10 different plants. – Tab. 1: Durchschnittliche Anzahl aller Oberflächenstrukturen pro 0,25mm^2 und der jeweilige Anteil von Trichomen und Papillen. N entspricht der Anzahl der untersuchten Internodien von 10 verschiedenen Pflanzen.

	Prostoma					**Stem**		
Species	n	All	Trichomes	Papillae	n	All	Trichomes	Papillae
C. peltata	24	30.12	20.04	10.08	27	50.0	40.26	9.74
C. obtusifolia	29	49.35	31.83	17.52	27	61.30	52.67	8.63
C. insignis	30	38.96	11.33	27.63	32	36.76	17.63	19.13

Tab. 2: Comparison of trichome and papillae density, of stem and prostoma surface with respect to total number of surface structures, and the influence of the internode age on the surface structures. The significance was calculated with a non-parametric asymptotic Wilcoxon signed rang test for related data with the assumption (H_0) that surface structures are the same, independent of area and internode age. In most cases H_0 had to be rejected. – Tab. 2: Vergleich der Dichte von Trichomen und Papillen, der Gesamtanzahl der Oberflächenstrukturen im Prostomabereich und dem umliegenden Stängel, sowie der Einfluss des Internodienalters auf die Oberflächenstrukturen. Die Signifikanzwerte wurden mit einem nicht-parametrischen Wilcoxon-Vorzeichen-Rangtest für gepaarte Stichproben berechnet. Es wurde von der Nullhypothese ausgegangen, dass die Oberflächenstrukturen am Stamm gleich sind unabhängig von Prostoma und Internodienalter.

Species	**Trichome and papillae density**	**Surface structures on prostoma and stem**	**Internode age and surface structures**
C. peltata	Z=-3.21, **p=0.001**	Z=-3.824, **p=0.000**	Z=-3.824, **p=0.001**
C. obtusifolia	Z=-4.359, **p=0.000**	Z=-6.510, **p=0.000**	Z=-6.372, **p=0.000**
C. insignis	Z=-1.513, p=0.130	Z=-8.464, **p=0.000**	Z=-8412, **p=0.000**

Comparing internode age and surface structures, we found the highest trichome number on prostoma and stem of the youngest internode (Fig. 4). The papillae number is only significantly higher in the youngest internode in the prostoma site (Friedman ANOVA p=0.000). In older internodes (i2–4, i5–7 and older), the number of trichomes and papillae is the same on stem surface and prostoma area (Friedman ANOVA p=0.000) (Fig. 4).

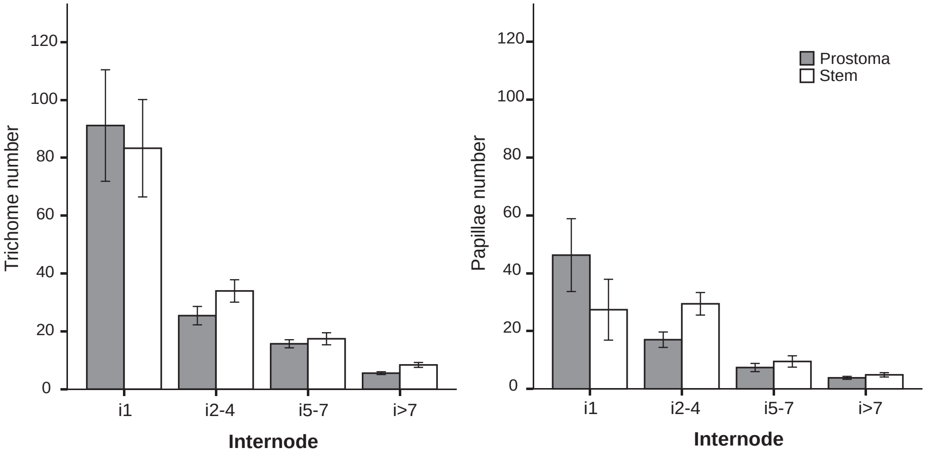

Fig. 4: Comparison of the average number of trichomes and papillae depending on internode age and surface area (prostoma or stem). The three *Cecropia* species are pooled. Bars represent the standard error. – Abb. 4: Vergleich der durchschnittlichen Anzahl von Trichomen und Papillen in Relation zu Internodienalter und Oberflächenbereich (Prostoma oder Stängel). Die drei *Cecropia* Arten wurden zusammengefasst. Die Fehlerbalken repräsentieren den Standardfehler.

Discussion

After analyzing the surface structure of the prostoma site and the surrounding stem surface of three *Cecropia* species associated with *Azteca* ants, we found that the total number of trichomes and papillae differed significantly between prostoma site and stem surface in two *Cecropia* species. The number of trichomes and papillae was significantly lower on the prostoma surface and higher on the stem (see Tab. 1; Fig. 3B-F). The prostoma surface can, therefore, be clearly distinguished from the stem surface based on the number and density of trichomes and may be a kind of guiding system for the ant queen. Ant foundress queens once landed on a *Cecropia* stem were observed to antennate the stem thoroughly (J. Busch, personal observation), which indicates that ant queens are investigating the surface. The queens were observed to explore the plant from the apical end. It did not matter where the queen first landed, she first climbed to the top of the plant and worked her way down from there, constantly antennating (J. Busch, personal communication). Additionally, the length of the surface structures was smaller on the prostoma site than on the surrounding stem surface. The smaller size and lower trichome density probably facilitates chewing a hole, therefore the structural difference of the prostoma site might be important for the decision of the foundress ants where to bite in.

Survival rate of foundress queens in domatia seems to increase with increasing stem diameter (Nishi & Romero 2008). Also, *Azteca* foundress queens need an internode of a certain size and a big enough spongy parenchyma layer on the inner domatia wall to start their fungiculture on piles of scraped parenchyma, which seems to be important for a successful colony foundation (Mayer et al. 2018). Interestingly, the youngest internodes of a *Cecropia* stem were hardly colonized, though the internode wall is softer and it is certainly easier to chew a hole in a younger than in an older internode wall. The youngest internodes always had many more surface structures per square µm on the prostoma and stem surface than older ones.

Too many surface structures are a structural barrier which prevent the foundress queen from gnawing an entrance hole and investing energy in an internode which is not suitable for successful colony founding. A slight tendency can be seen that the overall number of surface structures decreases with internode age. Thus, the surface structures may not only indicate to the foundress queen the prostoma zone itself, but also the age of the internode and the degree of lignification of the internode wall.

Apart from *Cecropia*, surface structures also seem to be important in myrmecophytic *Macaranga* species for the final decision of a foundress queen to stay on a certain host plant (Jürgens et al. 2006). Federle et al. (2001) could show that *Crematogaster* sp. living in *Macaranga* stems use incident daylight to select the position of their entrance hole and use visual landmarks for decision making.

In summary, the density of surface structures seems to act as landmarks for (1) the position of the prostoma, and (2) for the age and size of an internode. Finding the right site for biting the entrance hole is important for the colony founding queen. First, because the stem tissue is much thinner at the prostoma site than in the surrounding stem, second the internode should be young enough to allow to gnaw an entrance hole. Both make it easier for the ant queens to gnaw the entrance hole and shortens the time considerably during which a foundress queen is unprotected and exposed to predators. Third, the internodes should be old enough to have a parenchyma layer on the inner domatia wall surface big enough to start the fungiculture. As young queens make parenchyma piles before laying eggs, they seem to be crucial for colony foundation (Mayer et al. 2018). In *Macaranga*, it was shown that chemical cues have an important impact on long distance attraction of *Crematogaster* foundress queens (Jürgens et al. 2006). Whether this is the case in the *Cecropia*/*Azteca* association is not yet known and requires further investigation.

Future work has to be done with broader sampling and, in addition to the surface structures, the anatomy of the stem wall should also be taken into account. Another important factor may be the diameter of the prostoma: as *Azteca* queens vary in size, diameter differences with increasing internode age could be important for the distribution of foundress queens in *Cecropia* saplings.

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Current state and future directions of research on stingless bees in La Gamba

Johannes Spaethe, Martin Streinzer & Frank Sommerlandt

Stingless bees are important pollinators of crops and wild plants in tropical regions. All species possess a highly eusocial lifestyle including division of labor, a complex communication system and diverse foraging strategies, ranging from solitary foraging to mass recruitment and chemically marking of rewarding feeding sites. In the area surrounding Gamba, which is a small community in the Golfo Dulce region, 26 species of stingless bees have been identified so far, comprising ca. 45 % of all Costa Rican species. Here, we summarize the research on these fascinating bees conducted at the 'La Gamba' field station during the past 12 years and discuss promising directions for future research.

Spaethe J., Streinzer M. & Sommerlandt F., 2019: Aktueller Stand der Forschung und zukünftige Forschungsthemen bei stachellosen Bienen in La Gamba. Stachellose Bienen sind wichtige Bestäuber von Kultur- und Wildpflanzen in tropischen Regionen der Erde. Alle Arten zeigen einen hoch eusozialen Lebensstil, mit reproduktiver Arbeitsteilung und komplexer Kommunikation. Die verschiedenen Nahrungssammelstrategien reichen von solitärem Fouragieren bis zur Massenrekrutierung mit chemischer Markierung der Futterstellen. In der Umgebung von Gamba, einer kleinen Gemeinde in der Golfo Dulce Region von Costa Rica, wurden bisher 26 Arten von stachellosen Bienen nachgewiesen, was einem Anteil von ca. 45 % aller aus Costa Rica bekannten Arten entspricht. In diesem Artikel fassen wir die bisherige Forschung zusammen, die in den letzten 12 Jahren in der Forschungsstation 'La Gamba' an dieser faszinierenden Bienengruppe durchgeführt wurde und zeigen Richtungen für zukünftige Projekte auf.

Keywords: Meliponini, Apidae, chemical communication, eusocial insects, pollination, social information, vision.

Introduction

With over 500 described species, stingless bees (Meliponini, Apidae) are the largest group of eusocial bees and show a pantropical distribution with the highest diversity in Central and South America (Michener 2013, Hrncir et al. 2016). They are important pollinators for native plants and also for many agriculturally important species. Heard (1999) summarized that stingless bees visit flowers of about 90 crop species and significantly improve yield, for example, in macadamia, eggplant and coffee (Heard 1987, Veddeler et al. 2008, Nunes-Silva et al. 2013). Furthermore, beekeeping with stingless bees has a long tradition, particularly in Meso- and South America, and the produced honey is used as a nutritional resource, for medication and for ancient ceremonial practices (Weaver & Weaver 1981, Crane 1992, Vit et al. 2013).

All stingless bees are highly eusocial with a reproductive queen and functionally sterile workers, age-related division of labor among workers, and a complex communication system predominantly based on chemical cues and signals (Wille 1983, Leonhardt 2017). The number of colony members can range from a few hundred to more than one hundred thousand, thus exhibiting the largest colonies of eusocial bees (Michener 1974, Roubik 1983). To provision their brood, stingless bees forage for nectar and pollen from a large variety of plant species (Engel & Dingemans-Bakels 1980, Biesmeijer & Slaa

2006, Eltz et al. 2001). They also utilize non-nutritive resources, such as resin, for nest building or defense against predators and pathogens (Roubik 2006, Leonhardt 2017). Recruitment strategies vary considerably, from individual foraging to mass recruitment where hundreds of foragers can appear at a profitable food source within minutes (Fig. 1D; Nieh 2004). Species differ in size and level of aggression, and aggressive species sometimes push away heterospecific foragers at resources due to aggressive interactions or simply by outnumbering them (Johnson 1983, Howard 1985, Biesmeijer & Slaa 2004, Lichtenberg et al. 2010, Keppner & Jarau 2016).

Nest constructions are extraordinarily diverse among species of stingless bees and are mainly built from propolis, which is a mixture of wax and resin collected from plants. Other materials used are mud, vertebrate feces, plant fibers, and chewed leaf material (Wille & Michener 1973, Roubik 2006). Depending on species, nests can be found in termite and ant nests, tree cavities or in the ground, and the entrance is usually built as a tunnel made of propolis and other materials to protect against floods or predators such as ants (Fig. 1; Roubik 1983, Couvillon et al. 2008).

Stingless bees are affected by the 'global pollinator crisis' (Potts et al. 2010, Lichtenberg et al. 2017), but due to the complex interaction of biotic and abiotic factors, we still have

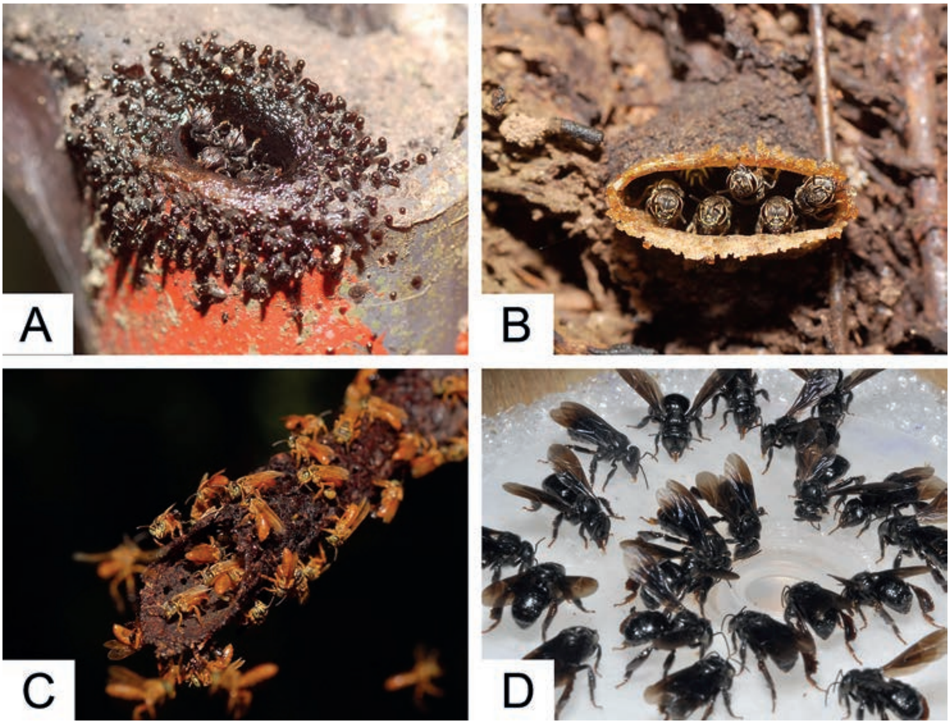

Fig. 1: Stingless bee species in La Gamba. Examples of nest entrance construction **A** *Trigonisca pipioli*, **B** *Paratrigona opaca*, and **C** *Tetragona ziegleri*. **D** Mass recruitment of *Trigona corvina* at an artificial feeder containing 1M sucrose solution. – Abb. 1: Stachellose Bienen aus La Gamba. Konstruktion der Nesteingänge von **A** *Trigonisca pipioli*, **B** *Paratrigona opaca,* und **C** *Tetragona ziegleri*. **D** Massenrekrutierung von *Trigona corvina* an einer künstlichen Futterquelle mit 1M Zuckerlösung.

an incomplete understanding of how stingless bees respond to land-use transformation, agricultural intensification, pesticide use and climate change. Brosi (2009), for example, showed that species richness and abundance of stingless bees in Costa Rica were strongly correlated with forest cover and significantly decreased with increasing deforestation. Besides deforestation due to logging or land-use transformation, the intensive use of agrochemicals, in particular pesticides, may also impact stingless bee diversity and abundance (Freitas et al. 2009). For example, the addition of imidacloprid, which is a neonicotinoid insecticide, to the diet of *Melipona quadrifasciata* worker larvae reduced their survival rate and negatively affected the development of the mushroom bodies, i.e. the neuronal integration centers of the bee's brain, which are important in learning and memory retention (Tomé et al. 2012). A variety of pesticides are used in intensified agriculture, almost all of which have a significant hazardous effect on stingless bees (Valdovinos-Núñez et al. 2009).

While the ecology and general behavior of stingless bees have been investigated in a large number of studies, research on (sensory) physiological and neurobiological aspects is relatively scarce compared to honey bees and bumble bees. In the Golfo Dulce region, 26 species of stingless bee have been recorded so far (Jarau & Barth 2008), comprising almost half of all species described for Costa Rica (N=58; Aguilar et al. 2013). During the past years, several projects on stingless bees have been initiated at the 'La Gamba' field station, addressing various aspects of visual and chemical ecology of stingless bees. Here we present a brief overview of the conducted studies, and discuss possible directions for future research.

Multimodal information use at a food sources

Sympatric species of stingless bees often compete for the same limited food sources. Therefore, different strategies have evolved to exploit a feeding site. Such strategies include aggressive behavior or adaptation of the peak foraging activity to less favorable environmental conditions (such as dim light or rainy weather conditions; Hubbell & Johnson 1978, Keppner & Jarau 2016). Moreover, an efficient recruitment system allows mass-recruiting species to monopolize a profitable food source. Providing olfactory information along the trail and at a food source allows for a rapid recruitment of nestmates and defense of the site against conspecific or heterospecific competitors. The chemical information used for communication and recruitment in bees is obtained from different sources, such as deliberately deposited anal droplets or gland secretions (reviewed in Jarau 2009). Moreover, foragers leave chemical footprints at food sources. These scent marks are produced by glandular epithelia of the claw retractor tendon and are likely left incidentally (Jarau et al. 2004). Both intentionally and unintentionally deposited scents can be exploited by nestmates and members of other bee species (reviewed in Barth et al. 2008). The durability of the information transmitted by scent marks depends on its function. Repellent odor marks are usually rather short-lived and allow foragers, for example, to avoid visitation of flowers which were recently depleted. In contrast, attracting scents are often long-lasting and are used by foraging bees to advertise particularly rewarding food sources (Stout & Goulson 2001, Jarau 2009). Another source of chemical information is provided by the profile of cuticular hydrocarbons (CHC). With a rather short action range, CHCs provide species-, nest-, and task-specific information (Jungnickel et al. 2004, Leonhardt et al. 2009, Ferreira-Caliman et al. 2010) and allows for recognition of nestmates. Nest-specificity has also been demonstrated for the chemical composition as well as the behavioral attractiveness of food site marking pheromones secreted from a forager's labial glands (JARAU et al. 2010, 2011, JOHN et al. 2012). Besides the variety of olfactory cues and signals, foragers of stingless bees can also exploit visual information. The physical presence of other bees provides a visual stimulus which can affect decision making at the food site (BIESMEIJER & SLAA 2004). Particularly in group- and mass-recruiting bees, multimodal information might be used under natural conditions. The cues can be either attractive or repellent to a forager and can lead to local enhancement or inhibition. Major questions in research on information use at a food source are: which cues are used for decision-making and do cues of different modalities have redundant, hierarchical or additive functions?

Trigona corvina is a species of mass-recruiting stingless bee, which plays an important role in plant pollination in the neotropics. Its recruitment system includes field-based mechanisms, such as pheromone trails and deposition of scent marks (ROUBIK 1981, AGUILAR et al. 2005, JARAU et al. 2010). At a feeding site, newly arriving forgers prefer to land at food sources that are associated with conspecific cues and are attracted by both chemical and visual cues (Fig. 2; SOMMERLANDT et al. 2014). Freshly killed conspecific specimens, which provided their typical CHC profile, had the strongest attractive effect at a food source. Interestingly, the CHC profile, even though the range of its perceptibility is rather

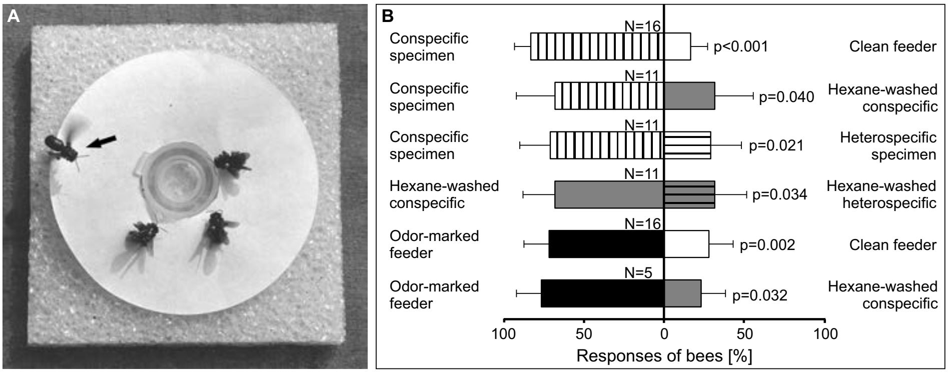

Fig. 2: Behavioral assay testing attractiveness of olfactory and visual cues at an artificial feeder. **A:** Experimental setup; a *Trigona corvina* forager (marked with an arrow) approaches a test feeder equipped with three freshly killed specimens of the same species. **B:** Responses of bees to cues presented in dual choice tests. The attractiveness of the following cues was tested in different combinations: three dead specimens of *T. corvina*, freshly killed by freezing; three dead heterospecific specimens of *T. ziegleri*; footprint-markings of 10–15 *T. corvina* foragers, which previously walked over the filter paper; three specimens of *T. corvina* that were hexane-washed to remove their CHC profile of the cuticle; three heterospecific specimens of hexane-washed *T. ziegleri*; and as control clean feeders without any cues (method and data adapted from SOMMERLANDT et al. 2014). – Abb. 2: Verhaltensversuch zum Test der Attraktivität olfaktorischer und visueller Reize an einer künstlichen Futterstelle. **A:** Versuchsaufbau; eine Arbeiterin von *Trigona corvina* (durch einem Pfeil markiert) nähert sich einer Futterstelle mit drei frisch getöteten Exemplare der eigenen Art. **B:** Wahlentscheidungen von Bienen auf unterschiedliche Reize. Die Attraktivität der folgenden Reize wurde in verschiedenen Kombinationen getestet: drei durch Einfrieren getötete Individuen der eigenen Art; drei getötete Individuen der Art *Tetragona ziegleri*; Fußspuren von 10–15 *T. corvina* Arbeiterinnen, die zuvor über Filterpapier gelaufen sind; drei Individuen von *T. corvina*, deren CHC-Profile durch Hexanwaschung entfernt wurden; drei in Hexan gewaschene Individuen der Art *T. ziegleri*; als Kontrolle dienten saubere Futtergefäße ohne zusätzliche Reize (Methode und Daten adaptiert aus SOMMERLANDT et al. 2014).

short, seemed to be an important cue. Foragers strongly preferred conspecifics possessing their typical CHCs over hexane-washed specimens (Sommerlandt et al. 2014). This indicates that stingless bees use the CHC profile to recognize nestmates not only at the nest (Leonhardt 2017), but also in the field. Besides the chemical information obtained from the body surface, deposited odor marks also have a strong attracting effect (Boogert et al. 2006). Recruited *T. corvina* foragers land repeatedly on a novel food source and are likely to scent mark the site (Aguilar & den Held 2003).

Depositing attractive scent marks at the feeding site is a common but not the exclusive strategy in mass-recruiting bees. Flaig et al. (2016), for example, showed mass recruitment in *Partamona orizabaensis*, but without deposition of any scent marks at the feeding site. In *T. corvina*, the strength of the scent is correlated with the number of visiting foragers. The attractive effect of the scent marks remains even when deposited by conspecifics of a different colony (Boogert et al. 2006). Even though scent marks are more important than visual cues at a feeding site (a fact which holds true for other stingless bees, such as *Tetragonisca angustula* and *Scaptotrigona mexicana*; Villa & Weiss 1990; Sánchez et al. 2011), the visual stimulus of present conspecifics alone is sufficient to attract approaching bees. In the absence of chemical information, foragers of *T. corvina* can still discriminate conspecific individuals from heterospecifics of similar size but different color (*T. ziegleri*, Fig. 2; Sommerlandt et al. 2014). This indicates that color vision, which is used for flower recognition in stingless bees (Villa & Weiss 1990, Spaethe et al. 2014), may also play a role in species discrimination at feeding sites. However, when chemical and visual cues are simultaneously presented, the multimodal information has no additive effect (Sánchez et al. 2011). The bias towards olfaction, which is observed when chemical and visual information is available in competing situations, could be caused by differences in the sensitivity for olfactory and visual stimuli, or an innate preference of the bees for scent marks over visual stimuli. Except for CHCs, which are non-volatile, odor marks at food sources can transmit information over several meters (Nieh 1998; Schmidt et al. 2003). In contrast, due to the relatively low spatial resolution of the bee's apposition eyes, objects which are the same size as conspecifics (5–6 mm body length in *T. corvina*) are recognized only in the range of several centimeters (Zeil & Wittmann 1993). The pronounced local enhancement observed in *T. corvina* enables a rapid increase in the number of foraging nestmates at a profitable food source in order to monopolize it. The presence of a nestmate at a food site seems to be a general attractive cue in social bees (Biesmeijer & Slaa 2004). However, in *Trigona amalthea* foragers, this preference depends on their previous experience. Naïve bees initially prefer to land on a flower next to nestmates, but with increasing experience they tend to completely avoid resources occupied by nestmates (Biesmeijer & Slaa 2004). Thus, strategies to exploit profitable food sources differ not only among species but also among foragers of a single colony that have different past experiences.

Visual ecology

Vision plays an important role in the ecology of stingless bees, e.g. for the detection and recognition of food plants, inter- and intraspecific communication, navigation and orientation between nest site and food sources and for mate detection (Srinivasan 2010, Sommerlandt et al. 2014). Bees use their compound eyes for (spatial) vision. The eyes are of the apposition type and consist of several thousand units, termed ommatidia. Each of these repetitive units can sample a small region of space through a tiny facet lens. The quality of the images mostly depends on the number and angular separation of ommatidia and their light sampling capacity (Land 1997). Individual ommatidia are usually equipped with two or three different photoreceptor types (Kelber et al. 2003). Perception of color, as a separate visual modality, may allow bees to detect flowers faster in cluttered visual environments and to reliably recognize rewarding food sources during foraging trips leading to an increase in foraging efficiency (Spaethe et al. 2001, Morawetz & Spaethe 2012). The basic requirement for perceiving color independently of object brightness is the existence of at least two photoreceptor types with different spectral sensitivities that sample the same region in space, and a color opponency mechanism to compare their outputs (Kelber et al. 2003). Electrophysiological recordings show a highly conserved set of three different receptor types among most bees, with maximum sensitivity in the ultraviolet, blue and green range of the light spectrum (Peitsch et al. 1992). Most of our knowledge about color vision and visual ecology in bees stems from observations and experiments in honey bees and bumble bees (e.g. Giurfa et al. 1996, Dyer et al. 2008), while our knowledge about stingless bees and other bee species is marginal.

Using a similar experimental approach as Nobel-laureate Karl von Frisch did in his pioneering study more than 100 years ago (von Frisch 1914), we tested for functional color vision in stingless bees (Spaethe et al. 2014). Workers of *Trigona* cf. *fuscipennis* were trained to associate a sucrose reward with a colored stimulus. After training, bees were tested in an unrewarded test, where they had to recognize the previously learned color among a set of 12 different shades of grey. The proportion of bees that landed on the chromatic (colored) target was significantly higher than the landings on any achromatic (grey) card. The bees did not mistake any of the grey shades for the previously rewarded stimulus in experiments with four different colors (yellow, blue, purple and pink), which proves that true color vision is present in *Trigona* cf. *fuscipennis* (Spaethe et al. 2014). In a follow-up experiment we tested the accuracy of their color discrimination abilities by using a simultaneous color discrimination paradigm, which was previously used to determine color discrimination in honey bees and bumble bees (Dyer & Neumeyer 2005, Dyer et al. 2008). Bees were first trained to associate a saturated blue or yellow star on an achromatic grey background with a reward and then tested with colored stars of decreasing saturation to determine the threshold of color perception (Spaethe et al. 2014). The workers were able to perceive the colored star down to ca. 0.07–0.08 hexagon units (a dimensionless value which describes the similarity of two different colors), which is significantly poorer than the 0.01–0.02 hexagon units found in honey bees and bumble bees (Dyer & Neumeyer 2005, Dyer et al. 2008). However, the observed performance of *T.* cf. *fuscipennis* is comparable to the performance of another stingless bee species of similar size, the Australian *Tetragonula carbonaria* (Spaethe et al. 2014).

Currently, the neural processing of color information is only superficially understood in stingless bees (Garcia et al. 2017). Distinct differences exist even among closely related species, such as honey bees and bumble bees (Dyer et al. 2008). At the current stage of research, we have not yet determined whether differences in the acuity of color vision are related to the phylogenetic background, physiological or morphological differences or other factors. It is entirely possible that the relatively poor visual abilities of the tested species are due to their small body size. Small eyes suffer from lower spatial acuity and capture less light, which likely affects signal-to-noise ratios (Dyer et al. 2016). As a consequence of the lower light gathering abilities, small species may also be limited by the light conditions at which they can forage. Apposition eyes are relatively insensitive and usually restrict flight activity to the bright daylight hours. Specific morphological and neuronal adaptations have been described in bees, which allow them to shift their temporal activity towards twilight and night-time (Warrant & Dacke 2011), but in general smaller eyes are less sensitive (Kapustjanskij et al. 2007). Vice versa, larger and thus more sensitive eyes may enable bees to forage in the relatively dark understory of tropical rain forests and to shift their activity to earlier and later times of the day, as was observed for *Partamona orizabaensis* at the La Gamba field station (Keppner & Jarau 2016). The latter is particularly important, since many flowering plants produce ample amounts of nectar and pollen in the early morning or late afternoon, which can be more efficiently exploited by species that are able to forage under low light conditions (Wcislo & Tierney 2009).

In a recent study, we investigated the scaling of compound eyes and sought to understand its functional consequences in a number of stingless bee species (Streinzer et al. 2016). Eye size, ommatidia number and size, and ocellar diameter were found to be positively correlated with body size. Based on theoretical considerations we anticipated that larger species would benefit from higher light sensitivity. To test this assumption, we quantified flight activity of several bee species in and around the La Gamba field station and measured the minimum light levels at which they commence foraging in the morning and cease flying in the evening. Smaller species were found to be more restricted and began flying about half an hour to an hour later in the morning and ended activity by the same period earlier in the evening (Fig. 3, Streinzer et al. 2016).

To summarize, all stingless bee species most likely possess color vision, which permits reliable detection and identification of rewarding flowers. However, due to the strong link between light sensitivity and spatial resolution on the one hand, and eye size on the other, smaller species are more restricted in their daily activity and need to deploy behavioral strategies to successfully compete with larger species.

Future directions

The La Gamba field station is an excellent location for research on Meliponini due to the high diversity of stingless bee species, the available infrastructure including an extensive trail system and air-conditioned laboratory, and a multitude of different habitats in close vicinity. Based on these fortuitous conditions, we recommend two major directions of future research.

First, the combination of behavioral and physiological experiments in situ enables us to address questions regarding the chemical ecology of bees. As mentioned above, stingless bees use a multitude of chemical cues and signals in their daily life. Behavioral studies usually take place in the field in close vicinity to the colonies, since most species cannot easily be transferred into the lab. Thus, if one intends to analyze potentially relevant chemical cues, odors, or CHCs involved in a specific behavior, the potential (but still unidentified) substances must be sampled and transported to a suitable laboratory for subsequent analyses, which is time consuming and impedes the direct testing of substances (Etl et al. 2016). However, with the availability of an air-conditioned laboratory in La Gamba, sensitive equipment such as a gas-phase chromatograph (GC) can be operated in close proximity to the experimental field site. The currently available GC is combined with an electrophysiological setup for electroantennogram recordings (GC-EAD), which allows one

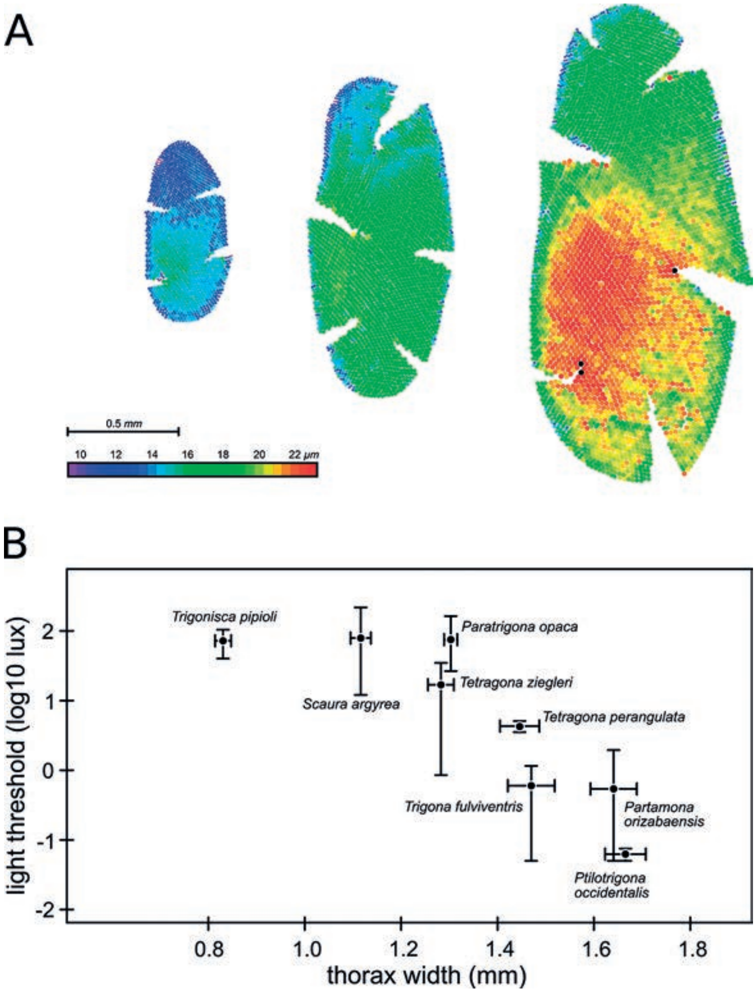

Fig. 3: Eye morphology and light intensity-dependent flight activity of stingless bees. **A:** Eye maps generated from replicas made of nail polish (for methods see Streinzer et al. 2016), showing facet diameter (color coded) of compound eyes of a small (*Trigonisca pipioli*), intermediate (*Paratrigona opaca*) and large (*Ptilotrigona occidentalis*) sized stingless bee species. **B:** Light thresholds at which bees are just able to fly, plotted against body size (measured as inter-tegulae span), in eight species of stingless bees from La Gamba, Costa Rica. Large species with larger eyes and facet lenses were able to start earlier in the morning and fly longer in the evening due to a higher light sensitivity. Colored scale bar in **A** indicates facet diameters. – Abb. 3: Augenmorphologie und Lichtintensität abhängige Flugaktivität stachelloser Bienen. **A:** Anhand von Nagellackabdrücken der Komplexaugen generierte Augenkarten, die die Verteilung der Facettendurchmesser (farbcodiert) von einer kleinen (*Trigonisca pipioli*), einer mittelgroßen (*Paratrigona opaca*) und einer großen (*Ptilotrigona occidentalis*) stachellosen Bienenart zeigen (Methode in Streinzer et al. 2016). **B:** Minimale Lichtintensität, bei der acht stachellose Bienenarten aus La Gamba fähig waren, zu fliegen, aufgetragen gegen ihre Körpergröße (gemessen als Distanz zwischen den Flügelschuppen des Thorax). Größere Arten mit größeren Augen und Facettendurchmessern und daraus resultierender höherer Lichtempfindlichkeit, konnten früher am Morgen und später am Abend fliegen. Der Farbbalken in **A** zeigt die jeweiligen Facettendurchmesser.

to measure antennal olfactory responses towards individual and identifiable compounds (Struble & Arn 1984). The behavior of bees, in particular when they use chemical cues (Sommerlandt et al. 2014), can now be efficiently investigated by sampling the potential cue from the bees' bodies or other surfaces, separating it into its individual substances, and testing a bee's antennal response towards each substance by means of the GC-EAD. Subsequently, the identified substances that elicit a response can be tested directly in the field. This approach can also be used to investigate bee-flower relationships, e.g. to identify floral odors that are attractive to stingless bees (Burger et al. 2012).

The second major direction of research is to quantify stingless bee diversity as a tool for evaluating the success of forest restoration. During the past 50 years, large areas of the primary tropical rainforest in the Golfo Dulce region have been deforested and transformed into agricultural land or urban areas, thus isolating the lowland Pacific rainforest area of the Piedras Blancas National Park from the eastern mountain range Fila Cal. The La Gamba field station has acquired several degraded plots in the area over the past years and has initiated reforestation efforts using local tree species (Weissenhofer et al. 2008). To evaluate the success of ecological restoration on wildlife and ecosystem function, it is necessary to design and implement suitable monitoring studies (Block et al. 2001, Ruiz-Jaen et al. 2005). Stingless bees are very sensitive to forest degradation (Brosi 2009) and thus may be a suitable insect taxon to evaluate restoration progress, since they rely on both sufficient flowering plants to meet their nutritional requirements, and proper trees for nesting sites (Eltz et al. 2002).

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(indicates studies conducted at least partially in and around the field station of La Gamba)*

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Correlates of display activity and perch residency of male orchid bees in cage experiments

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Males of the neotropical orchid bees collect volatile chemicals from various environmental sources, including leaves, fungi and flowers, to accumulate species-specific blends in hind tibial pouches. Subsequently, male bees expose perfumes during display at perches in the forest understory. While there is as yet no direct proof that females are attracted to male perfumes, conspecific males often approach perch holders in the natural habitat and are known to respond to tibial perfumes when those are presented at perch sites in bioassays. Here we ask whether the tibial perfume phenotype of male *Euglossa imperialis* is related to display activity or the success in holding a perch following intrusions of conspecific males in cage experiments. Neither the amount nor the complexity of tibial perfume correlated with display activity or perch residency in field-caught males observed for four days in a 9 × 9 × 3 m flight cage. Instead, we found a positive relationship between display activity and the amount of labial gland lipids in hind tibial pouches, suggesting that the most actively displaying males had more recently collected volatiles. The success of males in holding a display perch against intruding males was also unrelated to tibial perfume phenotype. Instead, males that had previously occupied a given perch were more likely to win a contest over that perch than males that had not. Overall, our results suggest that stored perfume is not a key determinant of male display intensity or perch residency. These findings are in broad agreement with results from a field study conducted at La Gamba in which males caught during display had perfume compositions similar to those of males caught at chemical baits.

Pokorny T., Millahn E., Schlütting P., Ramírez S.R. & Thomas Eltz T., 2019: Korrelation von Balzaktivität und Balzplatz-Besetzung männlicher Prachtbienen in Käfigversuchen.
Die Männchen der neotropischen Prachtbienen (Euglossini) sammeln Umweltdüfte und kombinieren diese in speziellen Taschen auf ihren Hintertibien zu einem komplexen „Parfüm“, das sie während ihres Balzverhaltens im Unterwuchs des Waldes freisetzen. Eine Lockwirkung auf Weibchen ist bislang nicht bewiesen, aber Männchen scheinen auf Parfümsignale von Artgenossen zu reagieren, wenn sie in deren Balzterritorien eindringen und mit ihnen interagieren. Wir untersuchen hier die Frage, ob der Parfüm-Phänotyp eines Männchens mit seiner Balzaktivität bzw. seinem Erfolg, Balzplätze zu behaupten, korreliert ist. Weder die Menge noch die Komplexität des Parfüms von Köder-gefangenen Männchen waren mit der mittleren Balzaktivität oder der Anzahl von Balzplätzen (vertikale Sitzwarten) korreliert, die diese in einem 9 × 9 × 3 m großen Flugkäfig im Garten der La Gamba-Forschungsstation besetzten. Stattdessen gab es einen positiven Zusammenhang zwischen der individuellen Balzaktivität und der in den Tibien enthaltenen Menge von Labialdrüsenlipiden, was darauf hindeutet, dass stark balzende Männchen kürzlich Düfte gesammelt haben. Der Erfolg beim Verteidigen von Balzplätzen zeigte ebenfalls keinen Zusammenhang mit dem gespeicherten Parfüm. Stattdessen wurden Balzplätze hauptsächlich dann erfolgreich behauptet, wenn das balzende Männchen bereits in der Vergangenheit an der jeweiligen Sitzwarte aktiv war. Insgesamt stimmen unsere Ergebnisse mit denen anderer Käfig- und Freilandstudien überein und verstärken den Eindruck, dass Menge und Komplexität der gespeicherten Parfüme keine starken Einflussfaktoren des Balzverhaltens von Prachtbienenmännchen sind.

Keywords: Euglossini, courtship, territory, intrasexual conflict, residency effect.

Introduction

The males of neotropical orchid bees (Euglossini) are characterised by their use of volatiles collected from their environment for intraspecific signalling. These volatiles, derived from flowers, resin, sap or decaying substrates, are not ingested. Instead, they are stored in specialized sponge-like cavities on the hind legs, where complex and broadly species-specific blends of "perfume" accumulate (Vogel 1966, Zimmermann et al. 2009). Individual males found in nature vary markedly in the amount and the number of compounds collected, with few individuals possessing many compounds and some having stored only small quantities of few compounds. While the reasons behind perfume marking remain uncertain, it has been shown that males expose the chemicals in a sexual context, i.e. during a characteristic male display behaviour at the places where mating occurs. These display sites are usually vertical structures (trees) in the forest understory on which the males perch and assume a typical posture (Kimsey 1980, Stern 1991, Eltz et al. 2003, Pokorny et al. 2017 and see Fig. 1).

In the genus *Euglossa*, males display by performing repetitive short hovering flights and somewhat more extensive patrolling flights intermittent with perching behaviour. It is during the hovering flights that males expose perfume from their hind leg pockets by stereotypic leg movements. Display has been observed to continue for lengthy periods of time, and both the general location of the perch (exposed to the wind on hills and ridges) as well as the angular orientation of the male on the perch (almost always exactly downwind) suggest that perfume dispersion is the central function of male display (Pokorny et al. 2017).

However, the potential recipient(s) of the perfume signal is (are) uncertain. While females have been (rarely) observed to copulate with displaying males on the perch (Kimsey 1980, Eltz et al. 2003, Pokorny et al. 2017, Zimmermann et al. 2006), direct bioassay evidence for attraction of females to male perfumes has not been forthcoming. On the other hand, conspecific males arrive quite regularly and engage in interactions with the resident male (Dodson 1966, Kimsey 1980, Stern 1991, Eltz et al. 2003, Zimmermann et al. 2006). This behaviour appears to be mediated by perfume stimuli: Zimmermann et al. (2006) exposed hind leg extracts of two closely related sympatric species of *Eulaema* at their respective active perch trees in Panama and French Guiana, and found that males arrived quickly at the 'correct' (conspecific) extracts, behaving as if encountering conspecific displaying males. In general, the behaviour of interacting euglossine males at display sites appears competitive rather than mutualistic, with males engaging in ritualized zig-zag or sustained circling flights near the perch. The interactions can last from a few seconds up to dozens of minutes (Kimsey 1980, Stern 1991, Roubik & Hanson 2004, T. Pokorny, pers. obs.), and in almost all cases end with one of the males leaving the site and the other resuming display (see Stern 1991).

For the experiments presented here we assumed that the success of male display, be it for intersexual or intrasexual communication or both, depends on the male perfume phenotype. Therefore, we predicted that the general likelihood of a male to occupy perches and perform display behaviour should increase with perfume amount and complexity. Furthermore, we assumed that perfume communication is used among males to settle conflicts and predicted that the likelihood of defending or taking over a perch increases with perfume amount and complexity. To test these predictions we conducted two behavioural experiments in a large flight cage.

Materials and methods

Two experiments were carried out in the gardens of the La Gamba Tropical Station, Puntarenas, Costa Rica. The Display Activity experiment took place in March/April 2014 and the Interaction experiment in March/April 2018. For both experiments, males of *Euglossa imperialis* were captured in the nearby forest at chemical baits of 1,8-cineole, and placed individually in Eppendorf tubes with breathing holes for transport. Back at the field station they were released into small insectaries containing branches with leaves and artificial flowers providing sugar water (40 % sugar). All artificial flowers were refilled with sugar water every morning, and bees soon learned to drink from them.

Display Activity experiment

After having habituated for two days in the small insectaries, 16 males were marked individually with one coloured bee tag (star symbols; Graze, Endersbach, Germany) on the scutum, plus a combination of three coloured paint dots (enamel paint, Revell) on the second metasomal tergite (Fig. 1b). They were then introduced into a large experimental cage (9 × 9 x 3 m, see Fig. 1a) containing flowering plants (*Calathea lutea*, *Stachytarpheta* sp.), artificial flowers mounted on corner poles, and 21 numbered stems of forest trees with diameters (3.1 to 5.4 cm; all branches removed) preferred for display by *E. imperialis* spread evenly throughout the cage. The first display was observed on the 4th of April 2014, five days after the males had been introduced into the experimental cage. During the next four days (5th to 8th of April 2014), individual display activity and perch occupancy was quantified by doing rounds of observations of all perches between 0600 and 0930 hours, the time of peak display activity on sunny days. During each round the perches were observed in a pre-defined sequence with each perch being observed only once for a few seconds. In case a bee was seen to be displaying at a given perch it was identified and the round was continued. Thus, an individual bee was never counted twice on a given perch per round. An observation round took between 5 and 7 minutes, and 15 to 20 rounds were completed per day, totalling 66 over the four days.

We calculated an index of daily display activity by dividing the number of observations per individual by the number of rounds of observation on that day. Daily activity was averaged over four days to estimate the overall display activity per individual. Perch occupancy was calculated as the mean of the number of different perches used by an individual per day. After completion of observations on the 8th of April 2014, all 16 males were caught, killed by freezing, and both their hind legs were extracted by submersing them permanently in 0.5 ml of n-hexane for GC/MS analysis (in Bochum). The bees were pinned and dried for subsequent size measurements (head width) using callipers. For each bee, two measurements were taken, and the average value for each individual was used in the downstream statistical analysis.

Interaction experiment

In addition to the small insectary and the large experimental cage, this setup included a medium-sized mesh cage (2 × 2 x 2 m) that was equipped in the same manner as the small insectary. Display opportunities in the large experimental cage were provided by the same plants as during the Display Activity experiment, but this time only three additional tree stem perches were installed.

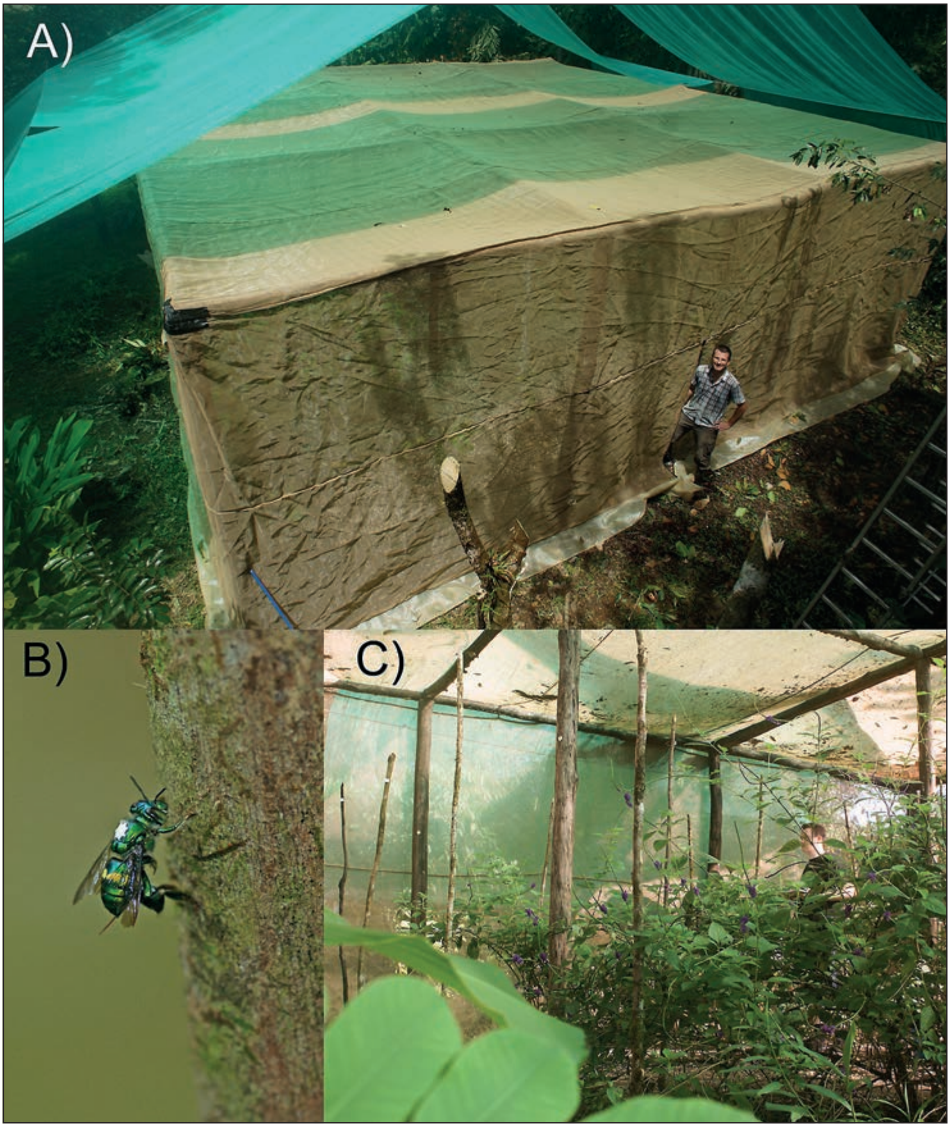

Fig. 1: A) Experimental cage, viewed from the top. B) Marked *Euglossa imperialis* male in the typical posture during display at a perch. C) Researcher observing display behaviour in the cage. – Abb. 1: A) Der Versuchskäfig, Ansicht von oben. B) Markiertes *Euglossa imperialis*-Männchen in der typischen Display-Haltung an der Ansitzwarte. C) Forscher beim Beobachten des Displayverhaltens im Käfig.

One day after capture and acclimatization to the small insectary, bees were marked on scutum, scutellum and abdominal tergites using four different colours of enamel paint (Revell). Paint was applied in small dots using the tip of a wooden toothpick, and after a short drying time each bee was released back into the small insectary. The next day, about two thirds of the marked bees were released into the large experimental cage. These bees were defined as ‘experienced’, i.e. they had had time to familiarize themselves with the experimental environment, while the remaining bees remained in the small insectary. After 24 h, the bees in the experimental cage were recaptured and transferred to the medium-sized mesh cage, which was positioned directly adjacent to the experimental cage. Trials on perch residency, i.e. defence or take-over of a perch tree after a male-male interaction, commenced the following day, with observations conducted between 0600 and 1500 hours, depending on weather conditions. After one week, new bees were captured, and the procedure was repeated.

For each trial, a group of four males (either all four experienced or two experienced males and two males that had remained in the small flight cage) was released into the large experimental cage and observed for three hours for display activity and male-male interactions at perch trees. Display activity was documented, including the identity of the displaying male and the perch. Once an interaction took place, its duration was noted, as well as its outcome, i.e. the male that returned to the contested perch for display was considered the winner of the interaction. Both interacting males were then captured, singly transferred to clean Eppendorf vials and placed in a freezer (-20° C) for later sample preparation. The remaining two males were observed for up to three more hours or until they too interacted, after which they were captured and frozen. Subsequently, a new group of males was released into the experimental cage and observed in the same manner. When the bees became inactive or it was past 1500 hours before the allotted observation time had expired, observations were stopped and continued the next morning after onset of bee activity. Extractions of hind legs for chemical analysis and measurements of head widths were performed as described above.

Chemical analyses

Hind leg extracts were analysed using coupled gas chromatography (GC: HP 5890 II) and mass spectrometry (MS: HP 5972) in splitless mode and with a sample volume of 1 µl. The GC was fitted with a non-polar DB-5MS column (30 m x 0.25 mm x 0.25 µm). The oven program ran from 60° C to 300° C at 10° C per min, with the final temperature held for an additional 15 min. Based on previous experience with *E. imperialis* tibial samples (Zimmermann et al. 2009; Weber et al. 2016), we sorted peaks into two groups of compounds: exogenous volatiles (perfume compounds, before 20.5 min) and endogenous labial gland lipids (straight chain saturated and unsaturated acetates, diacetates and alcohols, after 20.5 min). For each sample, we assessed the total amount of perfume compounds (summed peak areas < 20.5 min), perfume complexity (number of different perfume compounds), the total amount of labial gland lipids (summed peak areas > 20.5 min), and labial gland lipid complexity (number of different lipid compounds).

Statistical analyses

Data of the Display Activity experiment were analysed using Spearman rank correlation in Statistica v13 (Statsoft, Tulsa, Oklahoma, USA). Data of the Interaction experiment was analysed in R 3.3.3 (R Development Core Team 2017), testing for the impact of the different factors that might influence the outcome or duration of male-male interactions. These were: perfume amount, perfume complexity, body size (via the proxy head width), experience in the experimental cage and previous occupation of the contested perch.

Results

Display Activity experiment

Thirteen of the 16 males displayed during the four-day observation period, with 9 to 12 males displaying per day. The mean individual likelihood to be observed displaying during an observation round varied from 0 to 39.2 % (mean 13.5 %) over all four days (Fig. 2), peaking on the first observation day (16.3 %), with a slight decline towards the last day of the experiment (12.1 %). The amount and complexity of exogenous perfumes in hind leg extracts taken at the end of the experiment varied over approximately half an order of magnitude among the 16 individuals. However, neither the amount nor the complexity of tibial perfume was correlated with display activity or perch occupancy (Tab. 1, Fig. 2). The only significant correlates were a positive relationship of mean display activity with the amount of labial gland lipids, and a negative relationship between mean display activity and the ratio in the amount of perfume/lipids (on day 4 and overall). There was also a positive but non-significant relationship ($P < 0.1$) between head width and display activity.

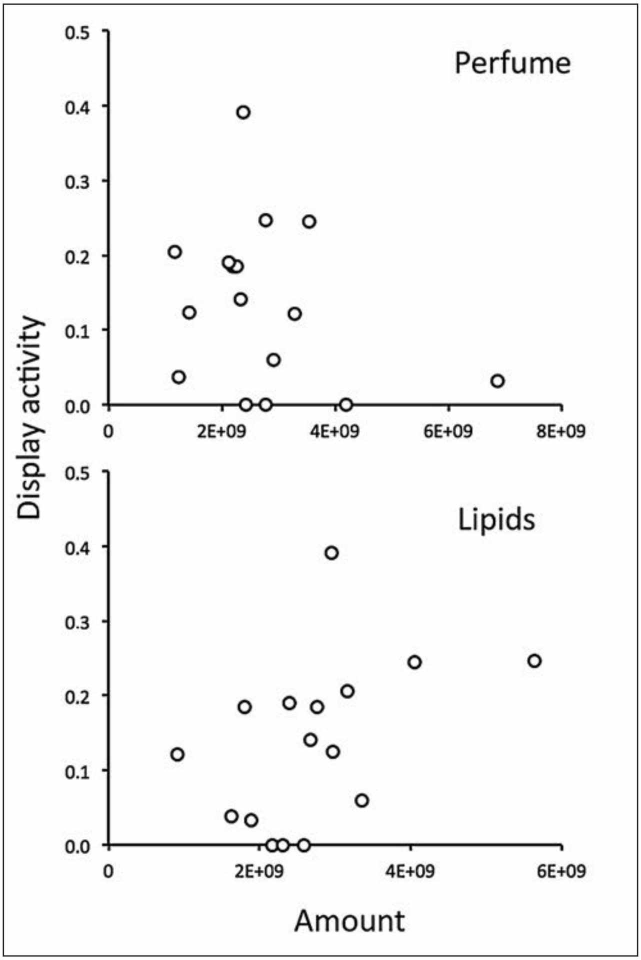

Fig. 2: Relationship between the amount of exogenous perfume (top) and the amount of endogenous labial gland lipids (bottom) in hind leg extracts and the display activity of male *Euglossa imperialis* (N = 16) summed over four days of the Display Activity experiment. See text and Tab. 1 for statistics. – Abb. 2: Korrelation der Menge exogenen Parfüms (oben) beziehungsweise der Menge endogener Labialdrüsenlipide (unten) von Hinterbeinextrakten und der Displayaktivität, aufsummiert über vier Tage, von männlichen *Euglossa imperialis* (N = 16) im Experiment zur Displayaktivität. Siehe Text und Tabelle 1 für die Ergebnisse der statistischen Analyse.

Tab. 1: Results of Spearman rank correlation tests between chemical/morphological (variable 1) and behavioural (variable 2) traits of male *Euglossa imperialis* participating in the Display Activity experiment. – Tab. 1: Ergebnisse der Spearman Rangkorrelationstests zwischen chemischer/morphologischer Eigenschaften (Variable 1) und dem Verhalten (Variable 2) von *Euglossa imperialis* Männchen im Experiment zur Displayaktivität.

Variable 1	Variable 2	N	Rs	t(N-2)	p
Amount of perfume	Mean display activity	16	-0,330	-1,310	0,211
	Mean no. of perches	16	-0,342	-1,361	0,195
No. of perfume compounds	Mean display activity	16	-0,050	-0,188	0,853
	Mean no. of perches	16	-0,081	-0,303	0,766
Amount of labial gland lipids	Mean display activity	16	0,555	2,494	0,026
	Mean no. of perches	16	0,401	1,637	0,124
No. of labial gland compounds	Mean display activity	16	0,178	0,678	0,509
	Mean no. of perches	16	0,058	0,219	0,830
Ratio amount of perfume/lipids	Mean display activity	16	-0,552	-2,475	0,027
	Mean no. of perches	16	-0,478	-2,035	0,061
Head width	Mean display activity	16	0,479	2,039	0,061
	Mean no. of perches	16	0,283	1,105	0,288

Interaction experiment

A total of 23 interactions were documented during 15 of the 17 trials. Variation in perfume amount and complexity spanned more than an order of magnitude in this experiment. Neither perfume amount nor perfume complexity correlated with male body size (Spearman rank correlations, n.s.). Males winning interactions did not differ from the losing males in the amount and complexity of perfumes or labial gland lipids (Wilcoxon paired tests, n.s.) or body size (paired t-test, n.s.). Males with experience in the experimental cage did not win interactions more often than naïve males (Chi^2 test, n.s.); however, males that

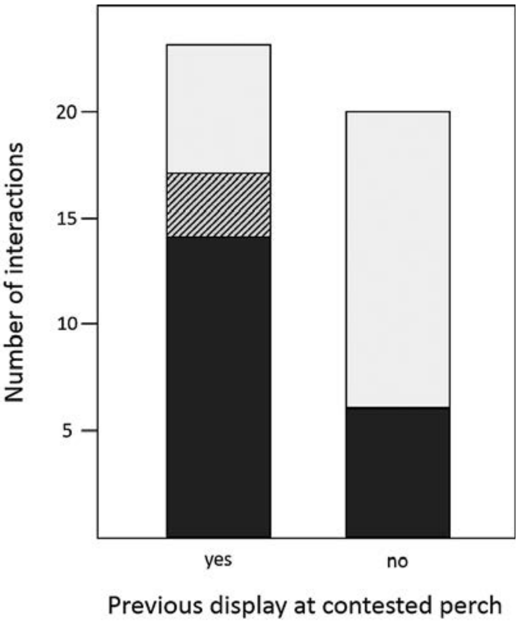

Fig. 3: Number of male-male interactions won (black) and lost (grey) in relation to whether a male had previously shown display behaviour at the contested perch or not. In three cases (hatched area), both interacting males had previously shown display at the contested perch. In all three cases, the male that had held the perch first, but not directly before the interaction, won against the newly displaying male. See text for statistics. – Abb. 3: Anzahl der Interaktionen zwischen Männchen, die gewonnen (schwarz) und verloren (grau) wurden, in Bezug darauf, ob ein Männchen bereits an der umkämpften Ansitzwarte Displayverhalten gezeigt hatte oder nicht. In drei Fällen (schraffierte Fläche) hatten beide interagierenden Männchen bereits an der entsprechenden Ansitzwarte Display gezeigt. In allen drei Fällen gewann das Männchen, welches als Erstes, aber nicht direkt vor der Interaktion, an dieser Ansitzwarte Displayverhalten gezeigt hatte. Statistik siehe Text.

had previously shown display activity at the contested perch won significantly more often than intruding males (Chi² test, chi² = 4.33, $P < 0.05$, see Fig. 3). In three cases, both of the interacting males had previously shown display at the contested perch. In all of these cases, the male that had displayed at the perch first won the interaction against the currently displaying male.

The duration of the interaction was not correlated to absolute differences in perfume amount or perfume complexity between the contestants. However, interactions were concluded faster when the males exhibited pronounced body size differences (Spearman rank correlation, r = -0.43, $P < 0.05$, Fig. 4), irrespective of whether the smaller or the larger male won the interaction.

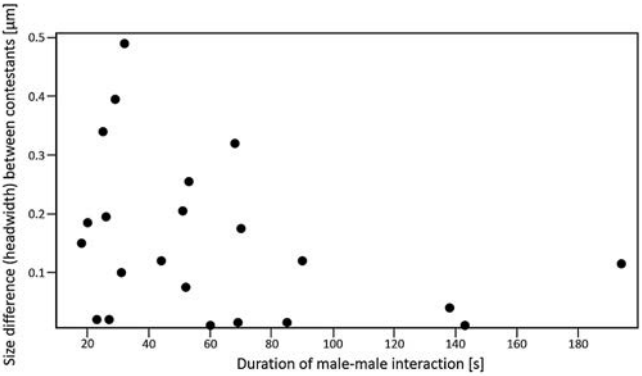

Fig. 4: Relationship between the size differences (head width) between pairs of males interacting at a perch and the duration of the interaction (N = 23). Spearman rank correlation, r = -0.43, $P < 0.05$. – Abb. 4: Korrelation der Differenz der Körpergrößen (Kopfbreite) zwischen den jeweils interagierenden Männchen und der Dauer der Interaktion (N = 23). Spearman Rangkorrelation, r = -0.43, $P < 0.05$.

Discussion

Our experiments suggest that the perfumes stored by individual male orchid bees are a poor predictor of their display activity, the number of perches they occupied for display, or the likelihood of a male winning an interaction contest over display sites.

There are a number of possible explanations for this lack of effects, some experimental, others biological. First, it is possible that the circumstances in our experiments were too confined or too artificial in abiotic conditions (temperature, wind), preventing males from displaying in accordance with the status of their perfume signal. Considering that males were caught in the wild, it is possible that the short timeframe of observation, perhaps in combination with individual differences in how fast bees habituate to the cage situation, was insufficient to demonstrate perfume effects. While this is difficult to rule out completely, there is evidence from other studies suggesting that stored perfumes are indeed not strongly linked to display activity or the onset of display. For example, the longer-term (14 days) display activity of caged male *Euglossa hemichlora* varied among individuals but was also unrelated to perfumes extracted at the end of the observation period (Eltz et al. 2003). In the field, the amount of perfume stored by male *E. imperialis* captured while displaying along forest trails at La Gamba, Costa Rica, did not differ from that of control males captured at chemical baits (Pokorny et al. 2017). This suggests that there is no perfume-dependent shift in male orchid bee behaviour, with younger (or perfume-poor) individuals engaging in volatile collection and older (or well-stocked) males switching to display. Instead, display appears to be a rather stereotyped behaviour that is controlled by endogenous mechanisms independent of volatile uptake, a notion that was recently supported by the observation of display occurring in very young, cage-reared *Euglossa dilemma*, which had not had the opportunity to collect volatiles at all (J. Henske, pers obs.).

It should be emphasized that the lack of a stored perfume-effect on the onset and undertaking of display does not preclude perfume effects on downstream sexual success, i.e. mate attraction and mating. Second, it is possible that stored perfumes do affect display, but we have not measured the relevant perfume trait. The amount and complexity of individual perfumes are highly variable and easy to quantify, but the relative contribution of certain (unknown) compounds to the blend or the overall chemical composition (template match) could be more important in determining behaviour. Third, display activity might be stimulated by recent collection of compounds rather than the overall amount and composition of accumulated volatiles. This would be in agreement with our finding of a positive correlation between display activity and the amount of labial gland lipids (both in absolute terms and in proportion to exogenous perfume).

Labial gland lipids serve as carriers during perfume collection (Whitten et al. 1989), and it has been shown that they are specifically sequestered from the hind leg pouches to be subsequently reused (Eltz et al. 2007). A high concentration of labial gland lipids in the hind leg pouches thus indicates a recent collection event, which might even have occurred in the experimental cage (e.g., from damaged and possibly fungus-infected leaves; T. Eltz, pers. obs.). Positive feedback from recent perfume collection might lead to higher intrinsic motivation to show display activity, or reduce the chance of interrupting or terminating display behaviour in comparison to males that have not experienced a recent collection opportunity.

Recent volatile collection has previously been implicated to stimulate display in *E. imperialis* (Schemske & Lande 1984). When these authors allowed caged males to collect synthetic 1,8-cineole from filter pads, there were more displaying males and a higher number of occupied perches than in a second cage without 1,8-cineole. Finally, the lack of a stored perfume-effect on display activity could be due to the possibility that the possession of perfume does not play a role in the acquisition of mates in male orchid bees. While perfume exposure during male display is strongly suggested by specific stereotypic leg movements, associated specific morphological structures and fluorescent tracer experiments (Eltz et al. 2005), there is still no direct evidence that females use chemical information contained in the perfumes exposed by males (see discussion in Pokorny et al. 2017). Carefully controlled mating experiments are required to fill this knowledge gap.

While female attraction to male perfumes remains uncertain, it seems likely that males respond to exposed perfumes when they approach and interact with conspecific perch-holders (see Zimmermann et al. 2006). Intrasexual conflicts can occur when competing for mates or for mating territories through which mates can be acquired (e.g. Wickman 1985). In orchid bees, all hitherto observed matings took place at or near the perch tree and with the current perch holder (Kimsey 1980, Eltz et al. 2003, Pokorny et al. 2017), and the only correlate of euglossine male mating success to date is the amount of time spent displaying (in a cage experiment, see Eltz et al. 2003).

While display itself is likely a stereotypic behaviour, success in occupying and holding a perch territory (and thus, presumably, mating success) might be dependent on display experience. This notion is in part corroborated by our finding that neither perfume phenotype nor body size were related to the outcome of male-male interactions at perch trees, while previous occupancy of the contested display site led to males winning a contest significantly more often than males that intruded upon a territory.

In insects, many territorial contest situations are won by the owner of the territory ('residency effect', see Takeuchi & Honda 2009 and Kemp & Wiklund 2001) and this had also been observed for interactions in *Eulaema bombiformis* (Stern 1991). The outcome of territorial disputes can be influenced by individual motivation based on previous experience (Takeuchi 2006, Bergman et al. 2010) or invested resources. Studies on the wood butterfly *Pararge aegeria* suggested that residency effects might in part be due to inherently aggressive males dominating the available territories (Kemp & Wiklund 2004). Interestingly, we found that contests between interacting male *E. imperialis* were concluded faster when the difference in head width (i.e. body size) between opponents was more pronounced. In fact, the average contest duration for cases in which the opponents differed by less than the average size difference was twice as long (13 cases, average duration of the interaction: 80 s) as that of the cases in which size differences were larger than average (10 cases, average duration of the interaction: 39 s). These results could be explained by self-assessment of body size in relation to that of the opponent in combination with the opponent's apparent aggressiveness/motivation.

While it is unclear which additional factors could lead to a fast conclusion of a contest with either a clearly larger male or a clearly smaller male, it seems plausible that contestants interact for a longer period of time the more similar they are in body size before the contest is resolved. Escalation of contests, i.e. a longer duration, was also observed in *Melanitis leda* when the combatants were more similar in age, although in this case the parameter 'age' influenced the final outcome as well (Kemp 2003).

Further controlled experiments including other potentially influential parameters for contest outcome apart from prior residence might shed light on the factors leading to successful perch residency in orchid bees.

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Pollinator specificity and seasonal patterns in the euglossine bee-orchid mutualism at La Gamba Biological Station

Santiago R. Ramirez

The plant family Orchidaceae exhibits some of the most spectacular and intricate adaptations for insect pollination. Across the Neotropical region male euglossine bees provide pollination services to approx. 700 orchid species that have evolved scent production in exchange for sexual reproduction. Male orchid bees collect scents from flowers and other sources to concoct perfume mixtures that they use as pheromone analogs during courtship display. Although the pollination biology of some of these associations has been studied in detail for some orchid taxa, community-wide analyses of this mutualism are lacking. Here I present an analysis of the plant-pollinator affiliation patterns and phenology among scent-producing orchids and male euglossine bees based on 960 bee-orchid interactions obtained over the course of five years of sampling at La Gamba Biological Station (south-western Costa Rica). I identify a highly nested plant-pollinator network that is composed of 24 bee species and 17 orchid genera. Some orchid genera exhibit pronounced flowering seasonality, with most of the diversity of interactions taking place during the dry season (March-April) and few orchid taxa blooming throughout the year. The architecture of the plant-pollinator network also revealed a substantial degree of pollinator sharing among orchid genera, suggesting that distantly related lineages independently converged on the use of similar pollinator bee assemblages.

Ramirez S.R., 2019: Bestäuberspezifizität und jahreszeitliche Variation in Prachtbienen-Orchideen Mutualismen an der Tropenstation La Gamba.
Die Pflanzenfamilie Orchidaceae weist einige der spektakulärsten und komplexesten Anpassungen zur Insektenbestäubung im Pflanzenreich auf. In der gesamten Neotropis liefern männliche Prachtbienen Bestäubungsdienste für ca. 700 Orchideenarten, die im Gegenzug für die sexuelle Reproduktion Düfte entwickeln und bereitstellen. Männliche Prachtbienen sammeln Düfte von Blüten und anderen Quellen, um daraus Duftmischungen zu bilden, die während der Partnerwerbung als Pheromonanaloge eingesetzt werden. Obwohl die Bestäubungsbiologie für einzelne dieser Orchideentaxa und Assoziationen bereits eingehend untersucht wurde, fehlt es nach wie vor an einer umfassenden Analyse dieser Mutualismen. Hier präsentiere ich eine Analyse der Pflanzen–Bestäuber Zugehörigkeitsmuster und der Phänologie bei Duft-produzierenden Orchideen und männlichen Prachtbienen, basierend auf 960 Bienen–Orchideen Interaktionen, die im Laufe von fünf Jahren Datenerhebung an der Tropenstation La Gamba (im südwestlichen Costa Rica) beobachtet wurden. Es zeigt sich ein stark geschachteltes Pflanzen–Bestäuber Netzwerk bestehend aus 24 Bienenarten und 17 Orchideengattungen. Manche Orchideengattungen zeigen eine deutliche Blütesaisonalität, wobei der Großteil der Interaktionen während der Trockenzeit (März bis April) stattfindet und nur wenige Orchideentaxa während des ganzen Jahres blühen. Die Struktur des Pflanzen–Bestäuber Netzwerkes zeigte auch einen erheblichen Anteil an Bestäubern, der von mehreren Orchideengattungen geteilt wird. Dies legt die Vermutung nahe, dass weit-entfernte Orchideenlinien unabhängig auf die Interaktion mit ähnlichen Bestäuberbienen konvergierten.

Keywords: Euglossa, Gongora, orchid pollination, pollinaria, pollination networks.

Introduction

Plant-pollinator associations have profoundly influenced the evolution of both flowering plants and insect pollinators (Willmer 2011). Some plant-pollinator associations are highly specialized, with many intricate and fascinating examples occurring in the tropical regions of the world. Specialized plant-pollinator interactions provide excellent opportunities for studying the evolution of floral adaptation, pollinator-mediated selection, and the origin of new species via floral isolation (Kay & Sargent 2009, Schiestl & Schlüter 2009). However, many of these associations remain poorly investigated and even basic information about their natural history, phenology and association patterns remains scarce.

Euglossine bees are one of the most important insect pollinators in tropical America (Roubik 1989, Ramírez et al. 2002). Male and female euglossine bees (>230 spp.) collectively pollinate thousands of flowering plant species while foraging for nectar, pollen, and resins (Ramirez-Arriaga & Martinez-Hernandez 1998, Ramírez et al. 2002, Ospina-Torres et al. 2015, da Silva et al. 2016). Additionally, male euglossine bees exhibit unique adaptations for the acquisition and storage of perfume compounds from flowers and other sources, including hundreds of orchid species (Dressler 1982, Williams & Whitten 1983, Ramírez et al. 2002). Male euglossine bees collect perfume mixtures throughout their life and continually expose them during elaborate courtship displays (Pokorny et al. 2017, Eltz et al. 2019). Because euglossine bees often fly long distances while foraging for perfume compounds, they provide crucial pollination services to numerous orchid species with fragmented and/or low-density populations (Janzen 1971, Pokorny et al. 2014).

Approximately 700 species of orchids, equivalent to 10 % of the Neotropical Orchidaceae, have evolved a suite of traits that enable pollination by male euglossine bees (Ramírez et al. 2002). Euglossine-pollinated orchids emit concentrated floral scents as rewards and exhibit a variety of mechanisms that ensure the release and proper attachment of pollinaria onto the body of the male bees. It was previously believed that euglossine-pollinated orchids depended exclusively on male bees for cross-fertilization and that male bees depended exclusively on orchid flowers for perfume acquisition (and therefore access to mates). However, it is now clear that, while orchids depend exclusively on male euglossine bees for pollen transfer (and therefore sexual reproduction), male euglossine bees can acquire perfume compounds similar to those produced by orchids from other hosts, including fungi, leaves, and rotting vegetation (Ackerman 1983, Whitten et al. 1993, Pemberton & Wheeler 2006, Ramírez et al. 2011). Using comparative methods, I showed that the association between euglossine bees and their orchid hosts is highly asymmetric (Ramírez et al. 2011). Under this scenario, the opportunities for reciprocal selection (coevolution) were significantly reduced during the diversification of these two lineages (Ramírez et al. 2011).

Most euglossine-pollinated orchids belong to closely related lineages within the orchid tribe Cymbidieae, with the majority of species concentrated in the subtribes Stanhopeinae, Catasetinae and Zygopetalinae. Each of these three orchid lineages is monophyletic (Chase et al. 2015) but they are interspersed among lineages that are not pollinated by euglossine bees and instead exhibit food deceptive pollination syndromes (Whitten et al. 2007, Ramírez et al. 2011, Whitten et al. 2014). Therefore, the euglossine pollination syndrome appears to have evolved multiple times independently. Moreover, although the phylogenetic relationships within Zygopetalinae are not fully resolved (Whitten et al. 2014), it is likely that male euglossine pollination evolved more than once within this group. As a result, the total number of independent origins of euglossine pollination may be as high as 4 or 5 events.

Some euglossine-orchid associations have been studied in great detail (Dodson & Frymire 1961, Dodson 1962, Dressler 1968, Hills et al. 1972, Zimmerman et al. 1989, Milet-Pinheiro et al. 2015, Hetherington-Rauth & Ramírez 2015, Pansarin et al. 2018). However, most of the interactions are known from a few focal orchid taxa or a narrow seasonal time window. With the exception of the study conducted by Ackerman in Central Panama (Ackerman 1983), community-wide and year-round surveys of bee-orchid associations are lacking. Thus, the affiliation networks, the diversity patterns, and the phenological cycles of euglossine-orchid mutualisms remain largely understudied.

Here I report the results of a survey of euglossine-orchid associations conducted by my students, colleagues and myself over a five-year period at La Gamba Biological Station in south-western Costa Rica. Because all orchids produce pollen masses (pollinaria) that are attached to the pollinator's body while visiting flowers, it is possible to track plant-pollinator associations by capturing pollinaria-laden bees using synthetic chemical baits. In most cases orchid pollinaria can be readily identified to genus based on morphological traits. The analysis I present here is based on 960 pollinaria-laden bees collected along the trail system around La Gamba Biological Station. Here I analyze the diversity, phenology, and association patterns of euglossine-pollinated orchids and their euglossine bee pollinators.

Materials and Methods

Pollinaria-laden bees were lured and collected using six different chemical baits that attract a wide variety of euglossine bee species: methyl salicylate, eugenol, 1,8-cineol, 1,4-dimethoxybenzene, vanillin and methyl cinnamate. All compounds were obtained from Sigma-Aldrich. The latter three compounds were purchased as solid crystals and dissolved in 200-proof ethanol. Each compound was applied separately to a blotter paper pad (5 × 5 cm) attached to a tree trunk 1.5 m above ground, separated from other baits by at least 5 meters. Bees were captured with hand nets as they approached the chemical baits. Pollinaria were carefully removed with forceps and deposited and preserved in 1.5 mL vials containing silica gel. Bees were pinned, identified and stored in the bee collection at UC Davis. Baits were typically presented between 8:00AM and noon and replenished as needed. Baits were presented along the trail system around La Gamba Biological Station at the same locations.

The sampling area was composed of secondary tropical forest, surrounded by patches of primary forest. The area receives an average annual rainfall of approximately 6,000 mm. The sampling was conducted between 2013 and 2018, with most of the collecting effort taking place during the dry season (March-April), but additional collections were made once a week throughout the year between 2015 and 2016. Pollinaria were identified to genus and bees were identified to species using a reference collection. I analyzed data using basic R packages. Bipartite networks were visualized and analyzed using the R package *bipartite v*2.07.

Rainfall patterns were calculated as averages for the past 20 years, and were measured with an automated meteorological station located at La Gamba Biological Station.

Results and Discussion

In previous surveys conducted by Dr. Tamara Pokorny, Dr. Thomas Eltz and myself, we found that the euglossine bee community occurring around La Gamba Biological Station is composed of 35 species belonging to four genera (*Euglossa*, *Eufriesea*, *Eulaema* and *Exaerete*, see Fig. 1 for four exemplary bee-orchid interactions). In this study, I found a total of 24 species of euglossine bees carrying orchid pollinaria, including three of the four genera present in the area (*Euglossa*, *Eulaema* and *Exaerete*, Fig. 2). I found pollinaria from a total of 17 orchid genera (Fig. 2), most of which belong to the three major orchid lineages that are known to exhibit scent-production and pollination by male euglossine bees (*i.e.* orchid subtribes Stanhopeinae, Catasetinae and Zygopetalinae, Fig. 2). Additionally, I found pollinaria belonging to other orchid lineages, including the genus *Lycaste* (subtribe Maxillariinae), the genus *Macroclinium* (subtribe Oncidiinae) and the genus *Notylia* (subtribe Oncidiinae). The first genus has not been reported for the area but the latter two are known to occur and are common around La Gamba Biological Station (Gegenbauer et al. 2013). These three genera have been previously described to exhibit male euglossine

Fig. 1: Scent producing orchids and their euglossine bee pollinators: an orchid *Gongora sp.* with bee *Euglossa tridentata* (top left; photo by T. Eltz), orchid *Mormodes sp.* with bee *Euglossa tridentata* (top right; photo by T. Eltz), orchid *Catasetum maculatum* with bee *Eulaema cingulata* (bottom left; photo by S.R. Ramírez), orchid *Notylia barkeri* with its bee pollinator *Euglossa championi* carrying pollinaria on clypeus (bottom right; photo by S.R. Ramírez). – Abb. 1: Duft-produzierende Orchideen und ihre jeweiligen Prachtbienen-Bestäuber: Orchidee *Gongora sp.* mit Biene *Euglossa tridentata* (oben links; Foto: T. Eltz), Orchidee *Mormodes sp.* mit Biene *Euglossa tridentata* (oben rechts; Foto: T. Eltz), Orchidee *Catasetum maculatum* mit Biene *Eulaema cingulata* (unten links; Foto: S.R. Ramírez), Orchidee *Notylia barkeri* und Biene *Euglossa championi* mit Pollinaria am Clypeus (unten rechts; Foto: S.R. Ramírez).

pollination syndrome (Ramírez et al. 2002). In addition, I found pollinaria from other orchid groups that lack floral scent production and male euglossine pollination syndrome, including *Sobralia* and *Vanilla,* both of which have been proposed to rely on food deception for pollinator attraction (Neubig et al. 2015).

The resulting bee-orchid pollination network was highly nested (Fig. 2; nestedness index=11.74). This network architecture is typical of plant-pollinator mutualistic interactions (Jordano & Bascompte 2013), and conforms well to what has been described for the

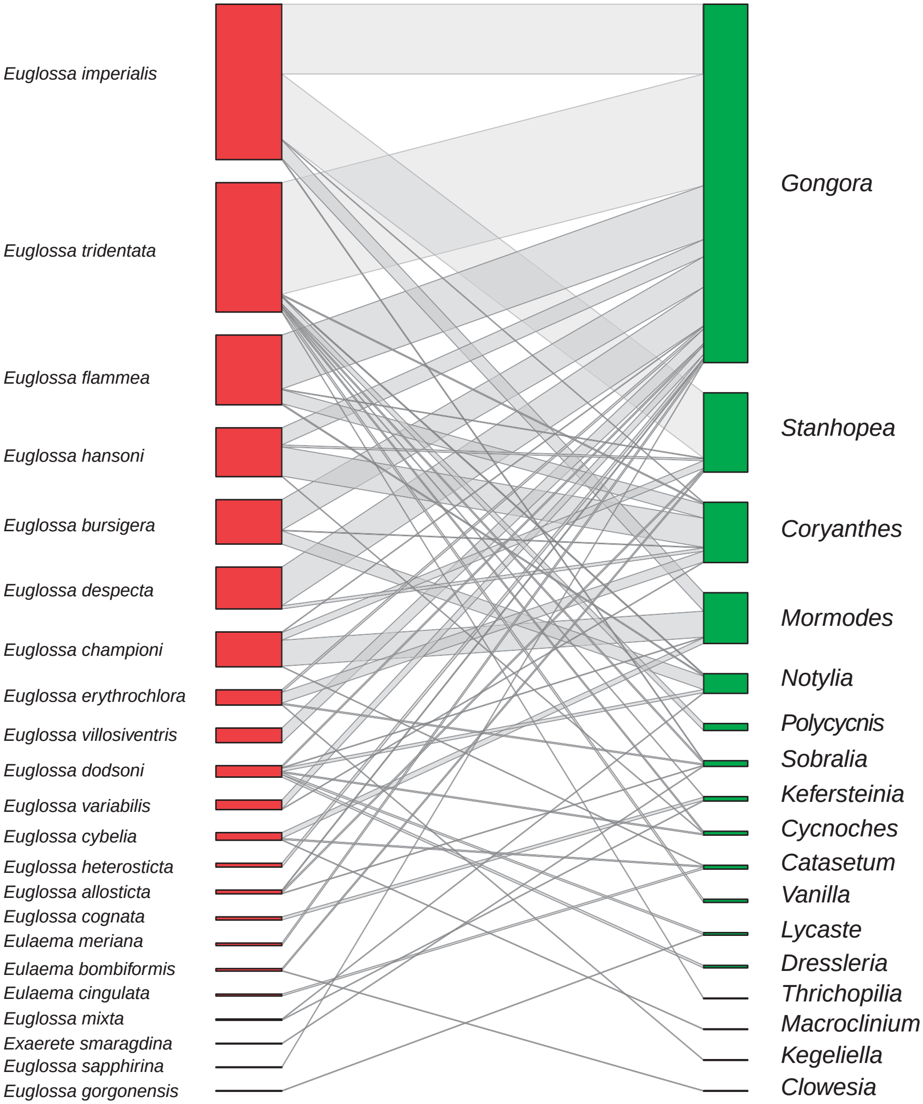

Fig. 2: Orchid-euglossine bee interaction network inferred from pollinaria records of all orchid genera and bee species. – Abb. 2: Orchideen-Prachtbienen Interaktionsnetzwerk, abgeleitet aus Pollinaria-Nachweisen aller Orchideengattungen und Bienenarten.

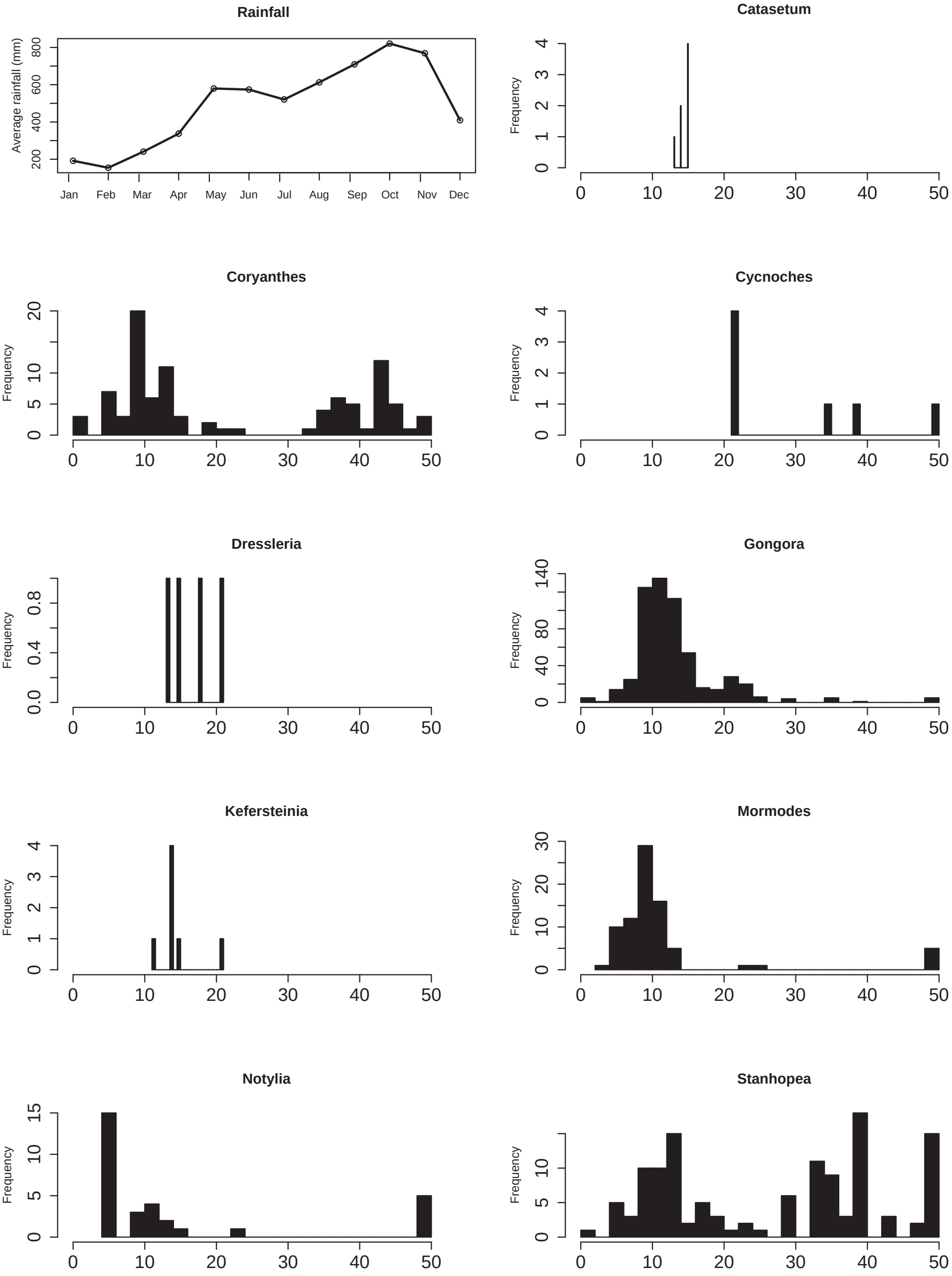

Fig. 3: Average monthly rainfall (1997–2017) and blooming phenology of the most common orchid genera based on weekly census data. – Abb. 3: Durchschnittlicher Monatsniederschlag (1997–2017) und Blütephänologie der häufigsten Orchideengattungen, basierend auf wöchentlichen Erhebungen.

architecture of other euglossine bee-orchid networks (Ramírez et al. 2011). The numerically most abundant bee species in the network were *Euglossa imperialis* (245), *E. tridentata* (204), *E. flammea* (110) and *E. hansoni* (77) (Fig. 2). Moreover, the bee species with the highest degree indices (*i.e.* the number of connections to different orchid nodes) were *E. dodsoni* (9 orchid genera), *E. imperialis* (6 orchid genera), *E. hansoni* (4 orchid genera) *E. flammea* (4 orchid genera), *E. erythrochlora* (4 orchid genera) and *E. championi* (4 orchid genera). Conversely, the numerically most abundant pollinaria were those of the orchid genera *Gongora* (571), *Stanhopea* (125), *Coryanthes* (95), *Mormodes* (80) and *Notylia* (31). The orchid genera with the highest degree indices in the network were *Gongora* (15 bee species), *Coryanthes* (8 bee species), *Stanhopea* (6 bee species), *Sobralia* (5 bee species) and

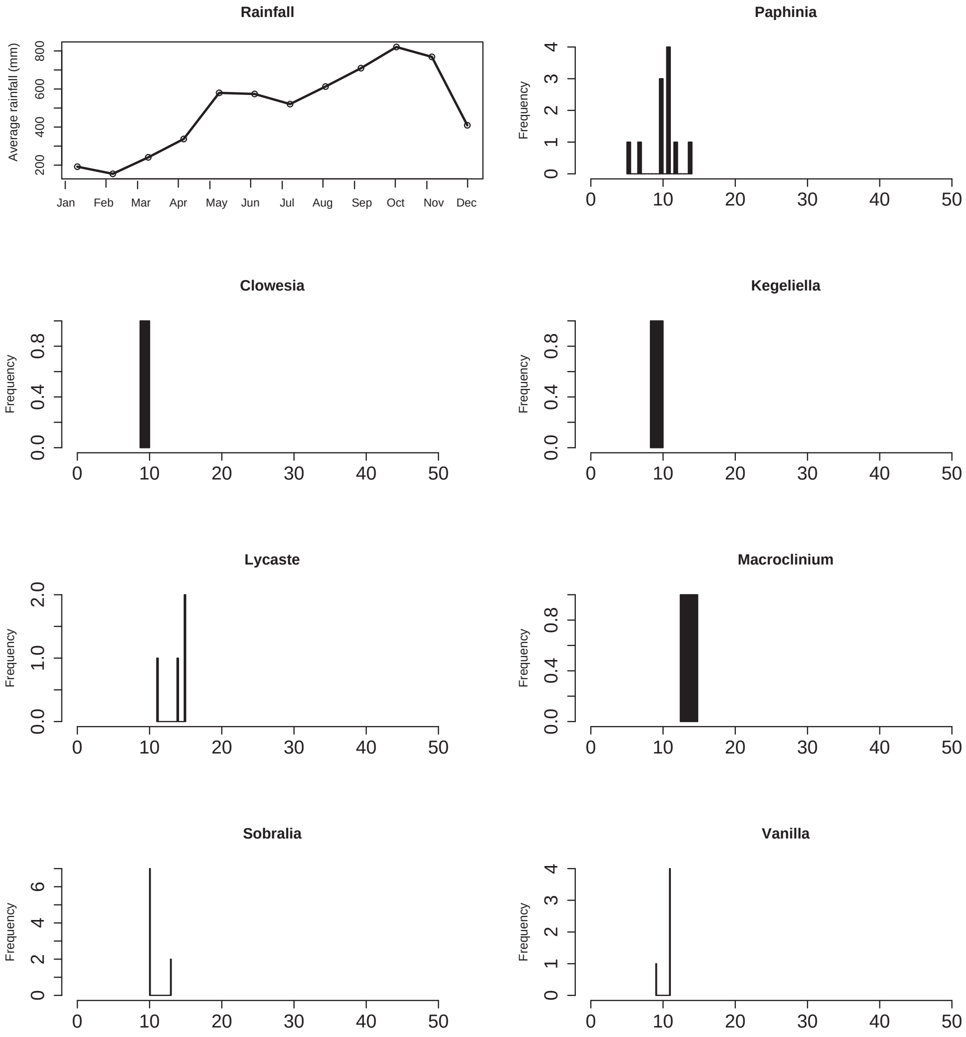

Fig. 4: Average monthly rainfall (1997–2017) and blooming phenology of rare orchid genera based on weekly census data. – Abb. 4: Durchschnittlicher Monatsniederschlag (1997–2017) und Blütephänologie seltener Orchideengattungen, basierend auf wöchentlichen Erhebungen.

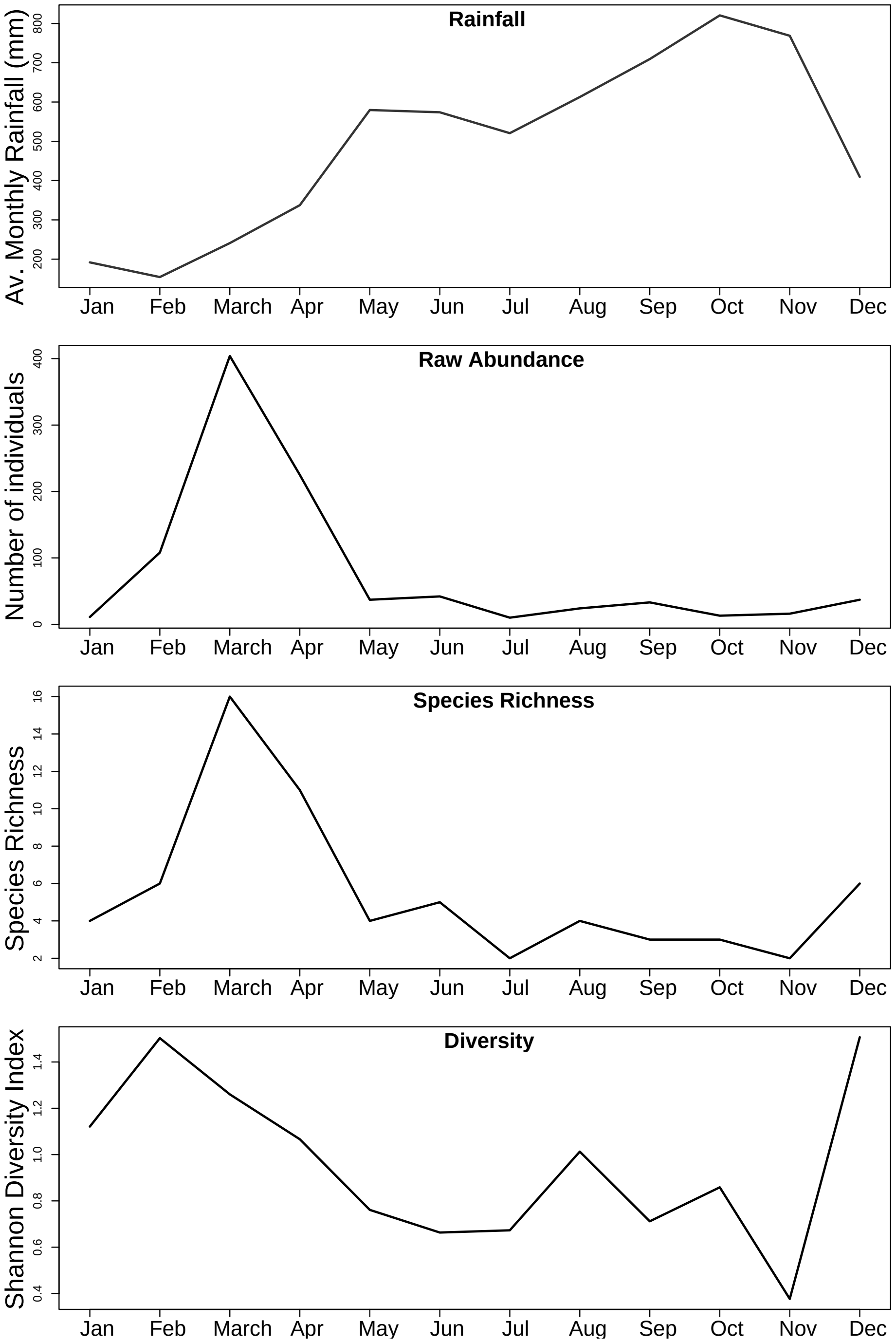

Fig. 5: Average monthly rainfall (1997–2017) and phenology in raw abundance, species richness, and diversity. – Abb. 5: Durchschnittlicher Monatsniederschlag (1997–2017) und Phänologie der Abundanz, Artenvielfalt und des Diversitätsindex.

Notylia (5 bee species). The pollination network also revealed a substantial amount of pollinator sharing among orchid genera. Since these orchid lineages represent independent origins of the euglossine pollination syndrome, the observed pollinator sharing likely corresponds to convergent evolution of traits that facilitate pollinator attraction. Future studies on the chemical composition of the floral scent of these orchid taxa should reveal whether the same or different scent molecules mediate the attraction of similar bee assemblages.

The analysis of the phenology of bee-orchid associations revealed that the majority of interactions occur during the dry season in the early months of the year (March-April, Figs. 3, 4). Specifically, I found that abundance, species richness and diversity of orchid taxa peaked during the dry season (Fig. 5). In fact, species richness and diversity were negatively correlated with monthly rainfall (Pearson's correlation $p<0.05$ for both richness and Shannon diversity). This pattern is similar to the phenology patterns described previously in Central Panama (Ackerman 1983), where flowering phenology also peaks during the dry season. Although most species of *Euglossa* and *Eulaema* are active year-round, it has been proposed that orchid flowering times have evolved to peak during the dry season to coincide with the highest levels of bee activity (Ackerman 1983). It appears that the seasonality patterns that I observed here are more pronounced than in Central Panama, possibly due to a more severe rainfall differential.

Interestingly, the genera *Stanhopea* and *Coryanthes* exhibit a bimodal blooming pattern (Fig. 3). Moreover, careful inspection of the pollinator associations of *Coryanthes* revealed that the pollinaria collected during the dry season were recovered from several bee species including *E. bursigera*, *E. despecta*, *E. erythrochlora*, *E. flammea*, *E. hansoni*, *E. tridentata* and *E. variabilis*, whereas the pollinaria recovered during the wet season were found only on *E. hansoni* bees. This observation may indicate the presence of two (or more) species with distinct blooming periods in the genus *Coryanthes*. However detailed pollinator observations and genetic work are required to confirm this assertion. This pattern was detected despite the greater sampling effort during the dry season, therefore indicating that sampling bias is unlikely to result in equivocal patterns of phenology. These patterns of flowering asynchrony with respect to the period of highest bee activity (dry season) are similar to the pattern described for two species of *Catasetum* in Panama (Zimmerman et al. 1989). Multiple factors may conspire to shape the timing of flowering phenology, including plant growth, leaf production, herbivore pressure, seed predation, and flower predation. The balance of these forces may ultimately shift flowering phenology towards time periods of lower (suboptimal) bee activity.

The phenology analysis also revealed that the majority of orchid genera present in the bee-orchid network bloom during a relatively narrow seasonal period, with most orchid genera being observed in the dry season (Figs. 3, 4). The genus *Gongora* was by far the most abundant pollinarium type in this study and also showed a pronounced blooming peak during the dry season (March-May, Fig. 3). Although at first glance this pattern may suggest the presence of a single species of *Gongora* in the area, ongoing work by colleagues and myself has indicted the presence of several cryptic species that can only be differentiated via floral scent chemistry, each of which attracts a unique set of bee pollinators (Hetherington-Rauth & Ramírez 2016). However, morphological examination of the pollinaria collected in this survey did not reveal any diagnostic traits that allow the separation of cryptic species. Other genera with narrow blooming times may also contain several cryptic species, and additional work is needed.

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Flower visitors of *Calathea lutea* (Marantaceae): The role of the hummingbird *Threnetes ruckeri*

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Most representatives of the pantropical monocot family Marantaceae have highly specialized flowers either adapted to bee or bird pollinators. However, bee-pollinated species are often reported to also be visited by birds, which may then act as co-pollinators or extract nectar without pollinating the flowers. A variety of flower visitors including orchid bees, butterflies and hummingbirds have been observed on *Calathea lutea*, a common Marantaceae species from Central and South America. While orchid bees are believed to be the main pollinators of *C. lutea*, butterflies have been observed to steal nectar without pollinating the flowers. The interactions with hummingbirds have not been studied in detail so far. In this study, we observed the flower-visiting behavior of the hummingbird *Threnetes ruckeri* (Trochilidae: Phaethornithinae) on *C. lutea* in southwestern Costa Rica. Our study focused on nectar removal by this bird species and on its potential role as pollinator. Nectar volume of unvisited flowers was measured at different times of day and compared to flowers that were visited by hummingbirds. We provide evidence that *T. ruckeri* does not pollinate these flowers, but acts as a nectar robber. The hummingbird pierces the sides of the corollae of fresh flowers with its beak and subsequently removes all nectar without touching the reproductive parts of the flowers.

Etl F., Brandauer S.S., Brandauer P., Prader S., Neier V., Dötterl S. & Schönenberger J., 2019: Blütenbesucher von *Calathea lutea* (Marantaceae): die Rolle des Kolibris *Threnetes ruckeri*.

Vertreter der pantropisch verbreiteten einkeimblättrigen Familie Marantaceae haben spezialisierte Blüten, die entweder an bestimmte Bienen- oder Vogelarten angepasst sind. Bienenbestäubte Vertreter der Marantaceae werden jedoch oft von Vögeln besucht. Bisher ist unklar, ob Vögel als Co-Bestäuber agieren oder nur den Nektar der Blüten trinken. An *Calathea lutea*, einer häufigen Marantaceae aus Süd- und Mittelamerika, wurden Prachtbienen, Schmetterlinge und Kolibris als Besucher beschrieben. Prachtbienen gelten als Bestäuber und Schmetterlinge wurden als Nektardiebe von *C. lutea* klassifiziert. Ob Kolibiris zur Bestäubung der bienenbestäubten *C. lutea* beitragen, ist nach wie vor unklar. Wir beobachteten Blütenbesuche und Nektarentnahme des Kolibris *Threnetes ruckeri* (Trochilidae: Phaethornithinae) an *C. lutea* im südwestlichen Costa Rica. Die Nektarmenge von unbesuchten Blüten wurde zu verschiedenen Tageszeiten gemessen und mit der Nektarmenge von Kolibri besuchten Blüten verglichen. *T. ruckeri* kann als Bestäuber von *C. lutea* ausgeschlossen werden, da die Vögel mit ihrem Schnabel die Kronröhre der Blüten seitlich anstechen und den Nektar entnehmen, ohne mit den reproduktiven Blütenteilen in Kontakt zu kommen.

Keywords: Band-tailed Barbthroat, nectar robbery, nectar removal, nectar amount, pollination.

Introduction

The pantropical family Marantaceae comprises approximately 525 species that are abundant understory herbs in many tropical ecosystems (Andersson 1998, Costa 2006, Hammel et al. 2003, Poulsen & Balslev 1991). Interactions with pollinators are highly specialized within this family due to a unique pollination mechanism that involves secondary pollen presentation and an irreversible explosive style movement (Kennedy 1978). The style is held under tension until a pollinator touches the trigger appendage of the style to release it. Once released, the style curls up explosively, moving the stigma forward to scrape off pollen from the pollinator, while the plant's own pollen is applied together with a sticky substance from the backside of the style to the same spot on the pollinator (for details see Classen-Bockhoff 1991, Jerominek & Classen-Bockhoff 2015, Kunze 1984, Endress 1996, Vogel 1984).

In most bee-pollinated Marantaceae species the pollen is pushed under the bee's tongue into the proboscidial fossa from where it cannot be removed by the animal (Andersson 1981, Ley & Classen-Bockhoff 2009). Large, long-proboscid orchid bees (Apidae: Euglossini) are the main pollinators in the Neotropics, while various short-proboscid bees are the principle pollinators in the Old World tropics (Dressler 1968, Kennedy 1978). Marantaceae are important nectar plants for bees due to the high amount of nectar and the long flowering period of each inflorescence, which can last for several weeks or even months. Euglossine bees are known to visit the same plants each day on a regular basis, following certain routes using a trap-lining strategy (Ackerman et al. 1982).

Notable exceptions of the bee-pollination syndrome are the ornithophilous taxa, e.g. three African species that are specialized in pollination by sunbirds (Ley & Classen-Bockhoff 2009) and the South American species *Calathea crocata* and *C. timothei*, both adapted to pollination by hummingbirds (Kennedy 1978, Nolasco et al. 2013). *C. schunkei* from the lowlands of Peru is also believed to be ornithophilous based on its red showy bracts and unusual floral morphology (Kennedy 2000). Adaptation in flower orientation and shape, stigma morphology as well as in the color of bracts were apparently necessary to ensure successful pollination by such long-beaked birds (Kennedy 1978, 2000, Ley & Classen-Bockhoff 2009).

The floral trigger mechanism of Marantaceae allows for distinction between possibly pollinated and clearly unpollinated flowers, and can also provide clues for the classification of visitors as potential pollinators or potential nectar thieves. For example, Davis (1987) noted that butterflies drink nectar but do not trigger the flowers. This has also been shown in extensive studies on butterflies visiting *Calathea lutea* and *C. crotalifera* in La Gamba, Costa Rica. Several species of Hesperiidae and one species of Riodinidae, *Eurybia lycisca*, were identified as nectar thieves because they extracted nectar without triggering the flower with their extremely long mouthparts (Bauder et al. 2013, 2011, 2015). The hummingbirds *Glaucus aeneus* and *Threnetes ruckeri* also visit the flowers of *C. crotalifera* without releasing the explosive style (Bauder et al. 2011). It has not yet been clarified whether they act as nectar thieves that steal nectar without damaging the flower, like the aforementioned butterflies, or as nectar robbers that actively make a hole in the flower to gain access to nectar. According to Stiles & Skutch (1989), *T. ruckeri* slits or pierces flowers of *Calathea* spp., and Kennedy (1978, 2000) suspected that hummingbirds visit bee-pollinated Marantaceae mainly to rob nectar by piercing flowers and to take up water from the phytotelmata which are formed by the bracts subtending the flowers. However, in another study conducted in La Gamba, Costa Rica (Classen-Bockhoff & Heller 2008) on *C. lutea* and *C. crotalifera* (*C. platystachia* in that study), hummingbirds were also seen triggering flowers during nectar drinking and were therefore assumed to occasionally co-pollinate these plant species in addition to the orchid bee pollinators. A similar situation was found in *Saranthe klotzschi-*

ana from Brasil and in the Costa Rican *Thalia geniculata* (Davis 1987, Locatelli et al. 2004).

While the antagonistic role of flower-visiting butterflies on *C. crotalifera* and *C. lutea* is clearly understood and well documented, the role of hummingbirds has not been investigated in detail (Bauder et al. 2011, 2015, Classen-Bockhoff & Heller 2008, Kennedy 2000). In this study, we focused on the interaction of the hummingbird *T. ruckeri* with *C. lutea* flowers in southwestern Costa Rica. We measured the nectar availability and sugar concentration in flowers at different times of a day and documented the hummingbirds' nectar removal in order to clarify their potential role as mutualistic pollinators or antagonistic nectar thieves/robbers.

Materials and Methods

Study area, study sites and time

The study was conducted at the Tropical Field Station La Gamba and in the nearby Piedras Blancas National Park in southwestern Costa Rica. Fieldwork was conducted during the dry season (January - March) of 2011 and 2019.

Three sites with *C. lutea* were studied. Site 1 is located at the border of the P.N. Piedras Blancas. This site has an area of approximately 1000 m² and is densely covered with *Dieffenbachia aurantiaca* and > 100 individuals of *C. lutea*. Site 2 consists of approximately 50 individuals of *C. lutea*, which are situated along a small stream in the garden of the research station. This site is smaller than site 1 and of open habitat type. Site 3 consists of approximately 50 individuals growing along the 100 m long main trail through the research station and about the same distance along the road to the town of La Gamba. The distance between these sites is in the range of 50–200 m (1–2: 150 m, 1–3: 200 m, 2–3: 50 m).

Study plants, inflorescences and flowers

Plant, inflorescence and flower morphology as well as descriptions of anthesis of *C. lutea* are given in detail elsewhere (Bauder et al. 2015, Classen-Bockhoff & Heller 2008, Düster et al. 2018, Hammel et al. 2003) and are summarized here.

C. lutea grows to a height of 2–4 m in populations of variable size, often along streams or in open areas that are fully exposed to the sun (Hammel et al. 2003). The large inflorescences are located 1–3 m above the ground and are composed of several approx. 20 cm long partial inflorescences ('florescences'), each bearing a total of approx. 100 flowers. The flowers emerge from phytotelmata (water filled compartments) that are formed by stiff reddish bracts. Each bract holds five to seven pairs of flowers that develop successively, so that each florescence holds an average of three open flowers per day. A florescence may flower for several months. Flower buds are only visible on the evening before anthesis, which usually starts around 5:00 in the morning. The flowers last for a few hours and start to wilt at around 10:00 of the same morning, before falling to the ground at around 18:00 of the same day (Classen-Bockhoff & Heller 2008). The perianth is rather inconspicuous and has a similar brown-reddish colour as the bracts. Once the perianth opens, the modified yellow staminodes and the style that form the trigger mechanism are presented.

The length of the floral tube, formed by the staminodes, is about 31 mm (Bauder et al. 2015). Each flower contains approx. 12 µl of nectar with a sugar concentration of 40 % (Düster et al. 2018, Ruppel & Morlock 2015).

Hummingbird flower visitation and nectar extraction

Flowering individuals within two sites (Site 1 and 2) were observed by sitting in front of a few chosen inflorescences for several hours per day (see below). We recorded all hummingbirds visiting inflorescences, noted the daytime of each visitation and documented their feeding habits by photographs and video recordings using digital cameras (Canon Eos 60D, Canon Ixus 970 IS, Panasonic Lumix DMC-FZ200EG9, Sony HDR-PJ10). The observations took place in February 2011 (5 days) and February 2019 (3 days).

We paid special attention to the way the birds inserted their beaks into flowers and the condition of a flower before and after visitation, i.e., whether the trigger mechanism was released or not. Two *C. lutea* sites were observed in 2011. Site 1 was observed for three days, and site 2 for two days. Observations took place three times per day, during a 60-minute period in the early morning (6:00–7:00) and one 90-minute period each during late morning (8:00–9:30) and late afternoon (15:00–16:30). Identification of hummingbirds was undertaken by bird identification books (Garrigues & Dean 2013, Stiles & Skutch 1989).

In 2019, we paid special attention to nectar extraction by hummingbirds at site 1. To test if flowers visited by hummingbirds contained smaller volumes of nectar than unvisited flowers, and whether hummingbirds pierced the perianth to access the nectar, we studied the floral tube and nectar level in flowers that had been visited by hummingbirds as well as unvisited control flowers. For this purpose, we removed all flowers from 21 florescences of two different individuals from site 1 in the evenings on three successive days and bagged these florescences with fine mesh nylon gauze. During the next days, we unbagged all the florescences at 06:00, 08:00, 10:00, 14:00 and 16:00 for 45 minutes at any one time allowing for animal visits. All flowers visited by a hummingbird were removed immediately after visits for morphological investigation and nectar analyses. Additionally, the same number of unvisited flowers from the same individual was removed to perform the same morphological and nectar analyses. Both types of flowers were analyzed for marks of nectar robbery (i.e., slits in the floral tube) using a stereo microscope (Leica-EZ4). The nectar volume was determined based on the nectar level in the floral tube by observing it against a light source. This method was tested prior to the experiment on eleven randomly selected flowers from which we measured the level of nectar in the floral tube and extracted it with capillaries to determine the volume. Given that nectar level was a good predictor of volume, as shown by a correlation analysis (Pearson $r = 0.84$, $p < 0.01$, $n = 11$ flowers), we used the linear fit function for all further analyses to determine the nectar amount.

Nectar volumes of hummingbird-visited flowers and unvisited control flowers were compared by a t-test for dependent samples (Statistica 12; StatSoft Inc. 2013) after tests for normality (Kolmogorov-Smirnov) and homogeneity of variances (Hartley).

Frequency of hummingbird nectar extraction and influence on nectar volume

To determine the frequency of nectar extraction by hummingbirds, 60 unbagged flowers were randomly collected in 2019 in the afternoon of February 25th from sites 1 (30 flowers) and 3 (30 flowers). All were checked for penetration marks and nectar volume. In addition to the vertical marks left behind by hummingbirds, we also detected marks with a different shape (see results) and thus classified the flowers into the categories 'hummingbird damage', 'other damage' and 'no damage'.

Nectar volumes of the different classes were analyzed by a Kruskal-Wallis ANOVA followed by a Tukey-HSD post hoc test for non-parametric data (Statistica 12; StatSoft Inc. 2013).

Number of open flowers, nectar volume and nectar concentration over the course of a day

Four plant individuals from site 1 and six individuals from site 3 were randomly selected to determine the number of open flowers, nectar volume, and nectar concentration over the course of a day. Several florescences (5–20, depending on availability) of each individual were bagged with fine mesh nylon gauze to exclude floral visitors. At 16:30 of three successive days, all open flowers within the bags were removed. During the following mornings, the number of open flowers available in the bagged florescences was determined at 06:00, 08:00, 10:00, 14:00 and 16:00 to learn about the number of flowers available at a certain point in time. In addition, one bagged flower per individual (n = 10 individuals) and time was randomly selected to measure the nectar properties (if available, 5 flowers per individual and day; 15 flowers during the three days of the experiments per individual; due to a sometimes small number of flowers available per individual, only 126 and not 150 flowers were sampled in total). Nectar concentration was measured by removing the ovary of each flower and pressing a droplet of nectar on an Eclipse handheld refractometer (0–50 Brix; Bellingham + Stanley Ltd.) and the nectar volume was determined using the method described above in section 'Hummingbird flower visitation and nectar extraction'.

PERMANOVA analyses (time as fixed factor; 10000 permutations) based on pairwise Euclidean distances among the nectar properties / flower counts, were calculated in Primer 7.0.13 with the add-on package Permanova+1 (Anderson et al. 2008, Clarke & Gorley 2015) to test for differences in the nectar volume of flowers, nectar concentration and the number of available flowers at different times of day. Given that plant individuals were repeatedly sampled, we included individual as a random factor in the analyses.

Complete names of plant and animal species treated or mentioned in the text

Plants: *Dieffenbachia aurantiaca* Engl., *Calathea crocata* E. Morren & Joriss., *C. crotalifera* S.Watson, *C. lutea* (Aubl.) Schult., *C. platystachya* Standl. & L.O.Williams, *C. schunkei* H.A.Kenn., *C. timothei* H.A.Kenn., *Heliconia stilesii* J.W.Kress, *Saranthe klotzschiana* Eichl., *Thalia geniculata* L.

Animals: *Amazilia tzacatl* (De la Llave, 1833), *Eulaema cingulata* (Fabricius, 1804), *Eufriesea surinamensis* (Linnaeus, 1758), *Eurybia lycisca* Westwood, 1851, *Glaucus aeneus* Law-

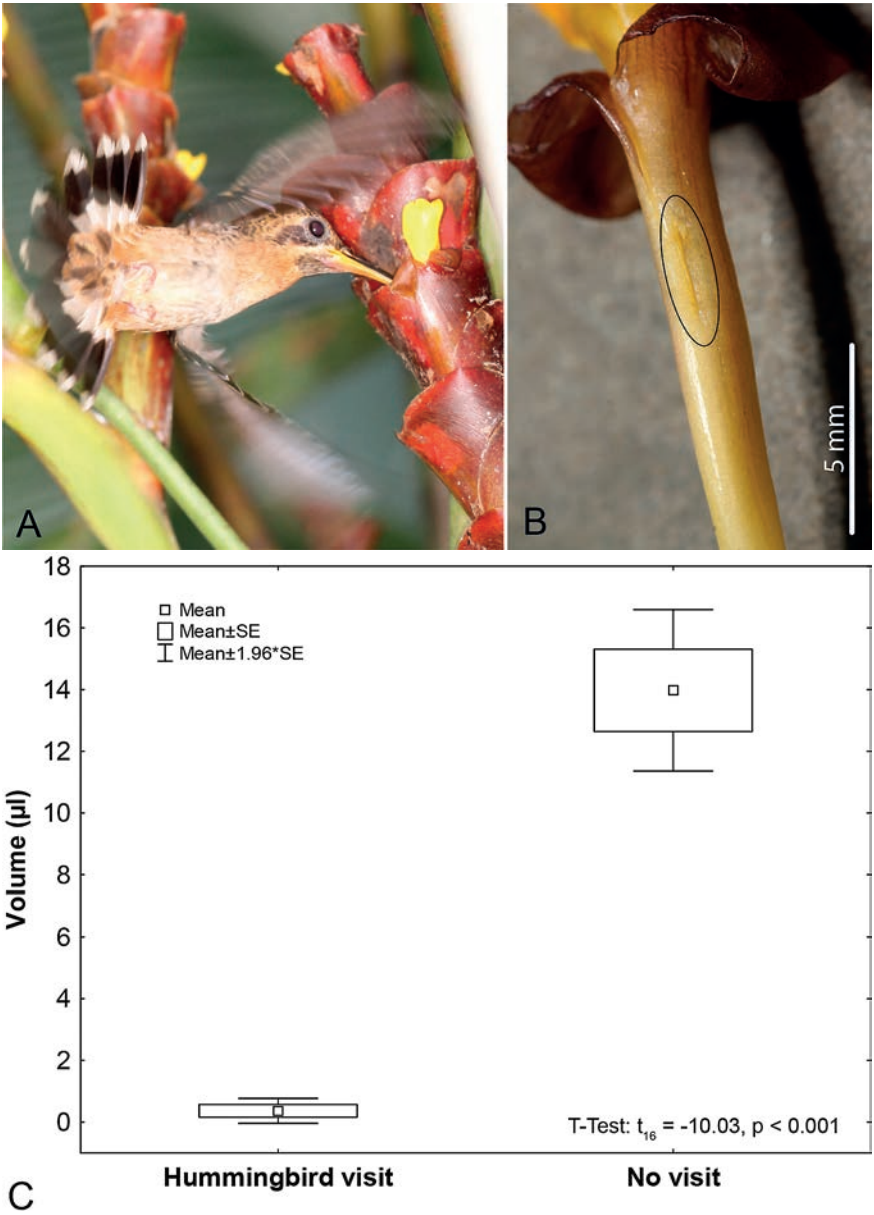

Fig. 1: A: The hummingbird *Threnetes ruckeri* piercing a flower of *Calathea lutea* from the side (Photo: Etl). B: A characteristic vertical slit in the floral tube of the flower left by the beak of *T. ruckeri* (Photo: Etl). C: Nectar volume of flowers of *C. lutea* visited by *T. ruckeri* (n = 26 flowers) and unvisited flowers (n = 26 flowers). Flowers visited by *T. ruckeri* contained less nectar than unvisited control flowers. – Abb. 1: A: Der Kolibri *Threnetes ruckeri* sticht seitlich in eine Blüte von *Calathea lutea* (Photo: Etl). B: Die charakteristische vertikale Einstichstelle in der Kronröhre einer Blüte von *C. lutea*, verursacht durch den Schnabel von *T. ruckeri* (Photo: Etl). C: Nektarmenge von *C. lutea* Blüten die von *T. ruckeri* besucht wurden (n = 26 Blüten) und unbesuchte Blüten (n = 26 Blüten). Blüten die von *T. ruckeri* besucht wurden enthielten weniger Nektar als unbesuchte Kontrollblüten.

rence, 1868, *Phaetornis longirostris* (Delattre, 1843) [formerly *P. superciliosus* (Linnaeus, 1766)], *Threnetes ruckeri* (Bourcier, 1847).

Results

Hummingbird flower visitation and nectar extraction

Threnetes ruckeri, a common hummingbird of the region, was the only hummingbird species documented during our observations. Observations over a period of five days in 2011 showed 25 visitations of *T. ruckeri* on inflorescences of *C. lutea*. The birds strictly visited flowers from the side (Fig. 1A, online video: https://youtu.be/cCqA2tYEUhQ) and pierced the flowers in the middle of the corolla (Fig. 1B). They pressed their beak deep into the floral tube without triggering the pollination mechanism. Visitations occurred mainly in the afternoon but also during the morning (Fig. 2). Typically, the birds consecutively visited several flowers (both triggered and untriggered ones) from different florescences. Sometimes they also stuck their beak into a phytotelmata without any flowers and poked around in it.

During the three days of observations in 2019, eleven visitations by *T. ruckeri* were documented in which the birds visited a total of 26 flowers. We noted a vertical penetration mark on the side of the floral tube at about 1.5 cm below the top of the flower in all visited flowers (Fig. 1B), whereas control flowers had no penetration marks. The flowers visited by hummingbirds contained less than 1 µl of nectar, whereas unvisited flowers contained around 14 µl (Fig. 1C).

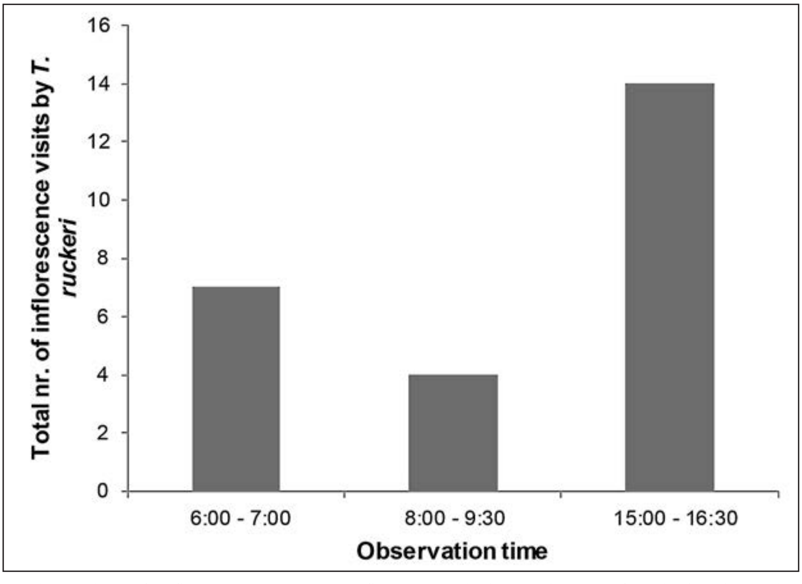

Fig. 2: Total number of inflorescence visits (to flowers and phytotelmata of *Calathea lutea*) by *Threnetes ruckeri* over five days of observations during specific time intervals. Depending on time of day, between four and 14 visitations were observed per time interval. – Abb. 2: Anzahl von Infloreszenzbesuchen (zu Blüten und Phytotelmata von *Calathea lutea*) durch *Threnetes ruckeri* innerhalb bestimmter Zeitintervalle während fünf Beobachtungstagen. Je nach Tageszeit wurden zwischen vier und 14 Besuche beobachtet.

Frequency of hummingbird nectar extraction and influence on nectar volume

At site 1, flowers with 'no damage' constituted the largest portion (53.3 %) of randomly collected flowers, and with 13 µl they also contained the highest mean nectar volume, followed by 'hummingbird damage' with 33.3 % and a mean nectar volume of 4 µl. Flowers with 'other damage', i.e. round cuts along the floral tube caused by other, unknown visitors, were the least frequent (13.3 %), with the mean nectar volume being similar to 'hummingbird damage' flowers. The flowers collected from site 3 showed no signs of hummingbird damage. Half of the flowers had 'no damage', the other half had 'other damage'; intact flowers contained a higher mean nectar volume (9 µl) compared to the 'other damage' flowers (4 µl). Overall (pooled among sites), flowers with no damage had a median of eight times more nectar than flowers with damage by hummingbirds or other unidentified visitors (Fig. 3).

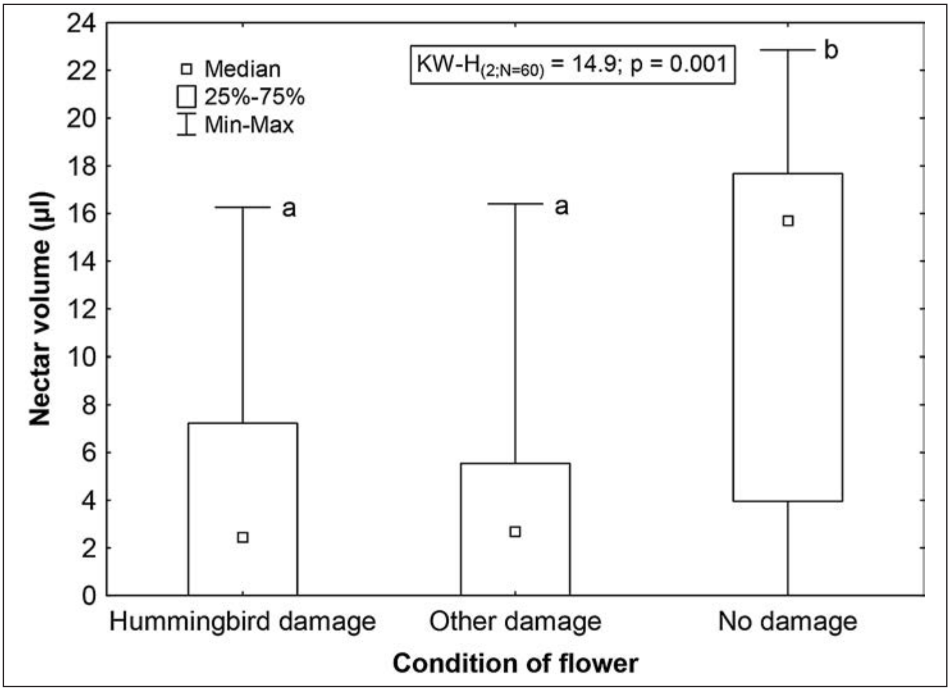

Fig. 3: Nectar volumes of differently damaged and undamaged flowers (n = 60) of *Calathea lutea*. Undamaged flowers contained higher nectar volumes than damaged flowers independent of the kind of damage. Different letters (a, b) indicate significant differences. – Abb. 3: Nektarmenge von unterschiedlich beschädigten und unbeschädigten Blüten (n = 60) von *Calathea lutea*. Unbeschädigte Blüten enthielten mehr Nektar als beschädigte Blüten, unabhängig von der Art der Beschädigung. Verschiedene Buchstaben (a, b) geben signifikante Unterschiede an.

Number of open flowers, nectar volume and nectar concentration over a day

The number of anthetic flowers per time varied over the day. At 6:00 a.m. a smaller number (76 % of the maximum number of flowers open per time) of flowers was available than later during the day (80 % - 84 %). The number of open flowers was constant from 8:00 a.m. onwards (Fig. 4).

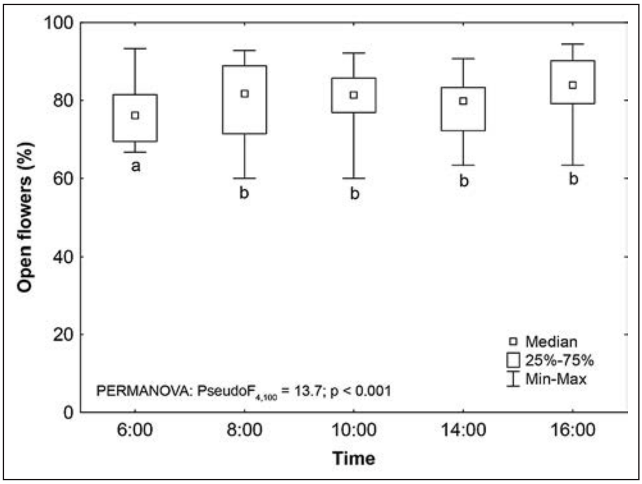

Fig. 4: Percentage of open flowers of *Calathea lutea* at different times of a day standardized using the maximum flower number per time category per day and individual. Mean values of the replicate measurements (over three days) per individual and time category were used for plotting purposes; statistical analyses were based on the original count data. At 6:00 a smaller number of flowers was available than later during the day. Time categories with different letters (a, b) differ among each other according to post-hoc analyses. – Abb. 4: Prozentzahl offener Blüten von *Calathea lutea* zu unterschiedlichen Tageszeiten, standardisiert unter Verwendung der maximalen Anzahl an Blüten je Zeiteinheit pro Tag und Individuum. Mittelwerte der Replikate (Messungen über drei Tage) pro Individuum und Zeiteinheit wurden geplottet, statistische Analysen basieren auf den ursprünglichen Zählungen. Um 6:00 waren weniger Blüten vorhanden als zu den späteren Messungen. Zeitkategorien mit verschiedenen Buchstaben (a, b) unterscheiden sich laut post-hoc Analyse voneinander.

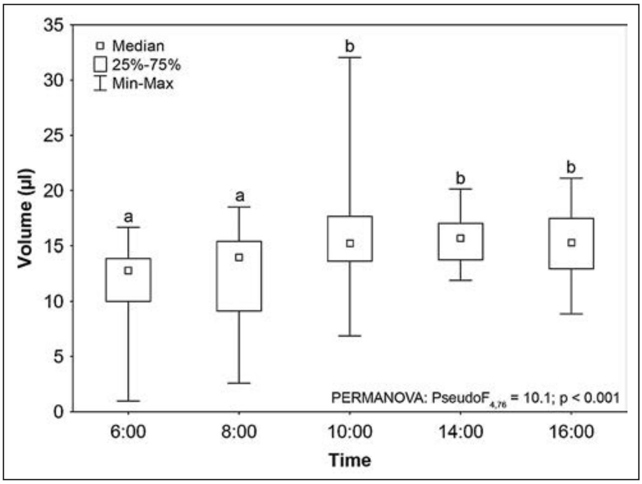

Fig. 5: Nectar volumes of bagged flowers (n = 126 flowers) of *Calathea lutea* measured at different times of day. The nectar volume was smaller in the early morning hours than later during the day. Different letters (a, b) indicate significant differences in volume. – Abb. 5: Nektarmengen von eingepackten Blüten (n = 126 Blüten) von *Calathea lutea* zu verschiedenen Tageszeiten. Die Nektarmenge war am frühen Morgen geringer als zu den späteren Messungen. Signifikante Unterschiede sind durch unterschiedliche Buchstaben (a, b) gekennzeichnet.

We found differences in the nectar volume of flowers over the day (Fig. 5), whereas nectar concentration did not differ over the day (median nectar concentration = 35 % and 40 %, n = 126 flowers; Fig. 6). The median nectar volume of flowers measured at 6:00 and 8:00 was 13 µl and thus significantly lower than the nectar volumes measured later in the day (15 µl; see Fig. 5; n = 126 flowers).

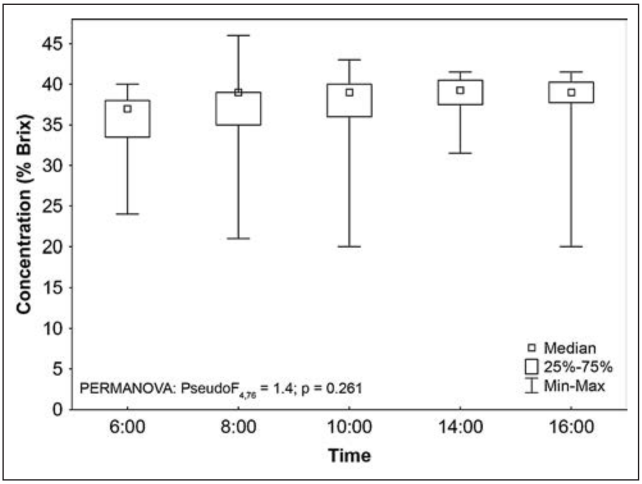

Fig. 6: Nectar concentrations of bagged flowers (n = 126 flowers) of *Calathea lutea* measured at different times of day. Concentration did not change significantly in the course of the day. – Abb. 6: Nektarkonzentrationen von eingepackten Blüten (n = 126 Blüten) von *Calathea lutea*, gemessen zu verschiedenen Tageszeiten. Es gab keine signifikanten Änderungen der Nektarkonzentration im Tagesverlauf.

Discussion

Our data show that individuals of the hummingbird species *Threnetes ruckeri* regularly and repeatedly visit inflorescences of *C. lutea* to pierce flowers and remove most of the nectar available in the flowers without touching reproductive parts of the flower, thus making them nectar robbers. Nectar robbery by hummingbirds is a widespread phenomenon across several families of Angiosperms in the Neotropics, especially on long-tubed flowers (Boehm 2018, Lara & Ornelas 2001). Darwin (1876) mentioned short-billed hummingbirds as nectar robbers that pierce the base of deep corollas to access nectar. Hummingbirds can rob from flowers that are pollinated by other hummingbirds (Lara & Ornelas 2001) or from insect-pollinated flowers, as shown in this study.

Though we provide evidence that *T. ruckeri* acted as a nectar robber, this does not mean that all hummingbirds that have been reported to visit *C. lutea* behave in this way. The two hummingbird species *Phaetornis longirostris* (former *P. superciliosus*) and *Amazilia tzacatl* were reported to insert their beak into the floral tube of several flowers of *C. lutea* to drink nectar while sometimes triggering the flowers (Classen-Bockhoff & Heller 2008). Their beak might be better suited for insertion into the corolla compared to the beak of *T. ruckeri*. However, it is unclear if pollen is deposited onto the beaks of these species and finally onto the stigmas of *C. lutea*. It is also uncertain whether *T. ruckeri* acts as a legitimate pollinator of other plant species in the area, or if nectar robbery is its preferred way of nectar extraction in all visited species. Gill (1987) reported on nectar thievery by *T. ruckeri* at flowers of *Heliconia stilesii* on the nearby Osa Peninsula in Costa Rica.

As we observed hummingbirds visiting phytotelmata with flowers and without flowers (see also Classen-Bockhoff & Heller 2008, Kennedy 1978), we assume that they occasionally also drink water from the phytotelmata, and likely also feed on various unidentified arthropod larvae inside the liquid (Etl et al. unpub. data). As many old flower parts become macerated within the phytotelmata and likely still contain leftover nectar from pierced flowers, we suppose that the liquid inside the phytotelmata also contains sugar. However, sugar was not evidenced by preliminary measurements using the refractometer described above. Further investigations of the chemical content of the liquid might reveal other nutrients that are possibly taken up by the hummingbirds.

Nectar removal by nectar thieves and robbers might affect reproduction of *C. lutea* species, as the amount left for orchid bee pollinators is reduced (Bauder et al. 2015). How this influences the behavior of bees, and if they visit more or less flowers on an inflorescence than usual on their foraging trips has not been clarified so far and could have negative or positive effects on plant fitness (Burkle et al. 2007, Irwin et al. 2010, Maloof & Inouye 2000). Bees might avoid patches where *T. ruckeri* and long-proboscid butterflies are abundant and exclude such patches from their daily routes.

The bee species *Eulaema cingulata* and *Eufriesea surinamensis* recognize and actively open the flowers of *C. lutea* in late bud stage (Etl unpub. data), while they are still covered by brownish petals. Given that the trigger mechanism already works at this floral stage and pollen is already available, these visitations can also lead to pollination. This behavior might be a response to high rates of nectar robbery by hummingbirds and nectar thievery by butterflies. Such early stage flowers might have a higher probability of still holding nectar. As the flowers are still closed and inconspicuous during the bud stage, butterflies most likely cannot insert their proboscises. It remains unclear if hummingbirds visit such flowers. Their method of flower-piercing (sideways) would also allow nectar to be extracted from closed flowers. On the other hand, hummingbird visitation was highest in the afternoon, which might be a strategy to decrease nectar competition with bees in the morning, the time of highest bee activity on flowers of *C. lutea* (Etl unpub. data). As shown in this study, open flowers of *C. lutea* are also available in the afternoon and nectar volume is slightly higher at that time, which could be another advantage for *T. ruckeri*.

Conclusion

Based on its abundance in the region and the amount of described interactions, *C. lutea* is of great importance for various butterfly, bee and hummingbird species looking for nectar (Bauder et al. 2015, Classen-Bockhoff & Heller 2008). As hummingbirds of the species *T. ruckeri* did not make contact with the style when removing nectar by flower piercing, they are classified as nectar robbers. These nectar robbers as well as nectar thieves (butterflies) visit the flowers frequently, and extract considerable amounts of nectar (Bauder et al. 2015, this study). They likely have a strong influence on the behavior of legitimate pollinators and on plant reproduction. Their role in the evolution of plant-pollinator mutualisms may be underestimated (Irwin et al. 2010) as floral traits are under selective pressures by both mutualists and antagonists (Ehrlén et al. 2012, Irwin et al. 2001, 2004, Knauer et al. 2018, Schiestl 2015).

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We like to thank D. Kollarits for correcting the English and two reviewers as well as A. Weber for helpful comments on the manuscript. Furthermore, we thank A. Weissenhofer, W. Huber, D. Schaber and the staff of the Tropical Field Station La Gamba for logistic support.

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Testing functional hypotheses on poricidal anther dehiscence and heteranthery in buzz-pollinated flowers

Agnes S. Dellinger, Léa Pöllabauer, Milan Loreti,
Janis Czurda & Jürg Schönenberger

Poricidal anther dehiscence is characteristic of pollen-rewarding flowers specialized on buzz-pollinating bees. Pollen dosing as a strategy of avoiding excessive pollen loss as a reward to the bees is believed to have played a major role in the evolution of buzz-pollination. Such dosing strategies should, however, be flexible and allow dispensing schedules to be adjusted to pollinator visitation frequency and flower age in order to avoid risks of dispersing too small amounts of pollen. In addition, many pollen-rewarding flowers have evolved different stamen types within the same flower (heteranthery), usually involving at least one showy and one cryptic type. Heteranthery is commonly explained by 'division-of-labour' between stamen types with one type depositing pollination pollen on the bee's back (which cannot be reached by grooming) and the other depositing feeding pollen on the bee's belly. Recently, however, differential pollen dosing between stamen types as a strategy to maximize male fitness has been proposed as an alternative driver of the evolution of heteranthery.

We used three pollen rewarding species (*Senna reticulata* (Fabaceae), *Adelobotrys adscendens*, *Conostegia subcrustulata* (both Melastomataceae)) with poricidal anthers to test 1) whether pollen dosing strategies change with flower age and 2) whether the different stamen types of heterantherous species differ in dosing strategies. In *A. adscendens*, 3) we also tested whether pollen from two different stamen types is deposited in different areas of the pollinator's body (mimicked by a microscope slide) as proposed by the 'division-of-labour' hypothesis.

Pollen was dosed in all three species, but pollen release increased with flower age only in *S. reticulata*. Differential dosing by the different stamen types was strong in both heterantherous species. In *A. adscendens*, we could not detect pollen deposition in different areas of the pollinator's body, but significantly more pollen was released by the cryptic (feeding) stamen type. Our results suggest that heteranthery may indeed be understood as a complex combination of spatial and temporal 'division-of-labour'. As proposed by other authors, heteranthery may function both in differential pollen placement (spatial aspect) but also in differential dosing between stamen types via 'shift-working' (temporal aspect).

Dellinger A.S., Pöllabauer L., Loreti M., Czurda J., Schönenberger J., 2019: Untersuchungen zur Funktion von poriziden Antheren und Heterantherie bei vibrationsbestäubten Blüten.

Eine porizide Antherenöffnung charakterisiert Blüten, die auf Vibrationsbestäubung spezialisiert sind und Pollen als einzige Bestäuberbelohnung anbieten. Um nicht zu viel Pollen als Belohnung zu verlieren, haben sich in vibrationsbestäubten Blüten spezialisierte Mechanismen zur Pollendosierung entwickelt. Diese Dosierungsmechanismen sollten jedoch so flexibel sein, dass auch bei niedriger Frequenz von Blütenbesuchen und in älteren Blüten nur kleine Mengen an Pollen in den Blüten zurückbleiben. Zusätzlich weisen viele vibrationsbestäubte Blüten unterschiedliche Staubblatttypen auf (Heterantherie), zumeist einen auffälligen Typ und einen unauffälligen Typ. Heterantherie wird oft mit einer „Arbeitsteilung“ erklärt, bei der ein Staubblatttyp Pollen für die Bestäubung unerreichbar auf dem Rücken der Biene ablädt (Befruchtungsstaubblätter), und der andere Futterpollen auf dem Bauch der Biene deponiert (Beköstigungsstaubblätter). Unterschiedliche Dosierungsstrategien zwischen den zwei Staubblatttypen wurden als alternative Gründe für die Evolution von Heterantherie vorgeschlagen.

An drei vibrationsbestäubten Pflanzenarten (*Senna reticulata* (Fabaceae), *Adelobotrys adscendens*, *Conostegia subcrustulata* (beide Melastomataceae)) untersuchten wir 1) ob sich die Pollendosierungsstrategien mit dem Älterwerden der Blüte verändern und 2) ob sich die zwei Staubblatttypen heterandrischer Blüten in ihren Pollendosierungsstrategien unterscheiden. An *A. adscendens* testeten wir außerdem, 3) ob Pollen der unterschiedlichen Staubblatttypen gemäß der Hypothese der Arbeitsteilung an unterschiedlichen Körperstellen des Bestäubers (repräsentiert durch einen Objektträger) deponiert wird. Alle drei Arten dosierten ihren Pollen, aber nur Staubblätter von *S. reticulata* setzten beim Älterwerden mehr Pollen frei. In beiden heterandrischen Arten bestanden große Unterschiede in der Pollendosierung zwischen den zwei Staubblatttypen. Bei *A. adscendens* konnten wir keine Hinweise auf Pollendeposition an unterschiedlichen Stellen feststellen, jedoch setzten die unscheinbaren Beköstigungsstaubblätter deutlich mehr Pollen frei. Unsere Ergebnisse legen damit nahe, dass Heterantherie eine komplexe Kombination aus räumlicher (Pollendeposition an unterschiedlichen Körperteilen des Bestäubers) und zeitlicher (schnellere Pollenfreisetzung bei einem der beiden Staubblatttypen) Arbeitsteilung zwischen den unterschiedlichen Staubblatttypen darstellen könnte.

Keywords: buzz-pollination, poricidal anthers, artificial buzzing, pollen dosing, division-of-labour, Melastomataceae, Fabaceae.

Introduction

Pollination, i.e. the successful transfer of pollen (containing the male gametes) to stigmas (receptive female organs), is a quintessential step in plant reproduction. Various vectors for pollen transfer exist, including animals, wind and water, or pollination may also occur autonomously by self-pollen-transfer within flowers (Proctor et al. 1996). Pollination by animals is believed to be a major driver of diversification in angiosperms (flowering plants, van der Niet et al. 2014). Approximately 80 % of angiosperms rely on animal pollinators, including insects, mammals, birds and reptiles (Ollerton et al. 2011). Animals visit flowers for various reasons, most importantly to obtain food or shelter or to find mates (Proctor et al. 1996). The most common food rewards offered by flowers are pollen and nectar (Simpson & Neff 1981). Offering pollen as a reward is risky, however, as excessive pollen consumption by flower visitors reduces pollen available for pollination and ovule fertilization (De Luca & Vallejo-Marín 2013). Hence, selection for optimizing efficient pollen transfer and minimizing pollen loss has led to the evolution of a tremendous diversity of pollination strategies (Westerkamp 1997, Castellanos et al. 2006, Hargreaves et al. 2009, Minnaar et al. 2018).

Buzz-pollination by bees represents one such strategy (Buchmann 1983, Bernhardt 1996). Buzz-pollinated flowers usually only offer pollen as a reward. The pollen is concealed in elongated tubular anthers, which open at a small pore at the tip of the anther (Buchmann 1983). Normally, the only way to excise pollen from such tubular anthers is by applying high-frequency vibrations to the stamens. Only certain bee and bumblebee species can produce vibrations at the required frequencies (240–405 Hz, DeLuca et al. 2013, Cardinal et al. 2018). Despite this pollination strategy being functionally very specialized, buzz-pollination has evolved many times independently across angiosperms and is currently found in approximately 6 % of species (Endress 1994, Vallejo-Marín 2019). In some groups, like the genus *Solanum* (approx. 1500 sp.) or the family Melastomataceae (ca. 5000 sp.), buzz-pollination is even the dominant pollination strategy, likely representing extraordinary evolutionary success (Macior 1971, Renner 1989, Berger et al. 2016, Dellinger et al. 2019).

The tubular, poricidal anther structure of buzz-pollinated flowers is interpreted as the result of selection to minimize pollen loss to pollinators (Endress 1996, Amorim et al. 2017, Vallejo-Marín 2019). Pollen can be strictly dosed through the anther pore and may be dispensed to many different pollinators throughout the flower's life-span (anthesis, Harder & Barclay 1994). In theory, dispensing small doses of pollen to several pollinators may increase chances of successful outcross-pollen transfer (Harder & Wilson 1994, LeBuhn & Holsinger 1998, Castellanos et al. 2006, Muchhala et al. 2010). The balance between optimal and overly strict dosing can be delicate, however. If pollinator visitation frequencies are very low, strict dosing is disadvantageous (Castellanos et al. 2006). Also, at the end of anthesis, no pollen should remain in the anthers. The few studies that have addressed these questions indicate that flowers can adjust their dosing strategies to pollinator visitation frequencies and change dispensing schemes over the course of anthesis (Larson & Barrett 1999, Sargent 2003, Castellanos et al. 2006). To maximize reproductive success, flowers would hence dose pollen more strictly at the beginning of anthesis, and dispense it more freely at the end (Larson & Barrett 1999). In buzz-pollinated flowers, the only outlet of pollen is the stamen pore. Thus, we hypothesize that adjustments of dosing may happen primarily by gradual enlargement of the stamen pore during anthesis.

Flowers may not only dose pollen within single stamens, but also between stamens within a flower, i.e. when presenting structurally different types of stamens. This condition is referred to as 'heteranthery' (Endress 1994, Vallejo-Marín 2010). Buzz-pollination, pollen rewards and heteranthery are significantly correlated across angiosperms (Vallejo-Marín 2010). The evolution of heteranthery has been explained by a 'division-of-labour' hypothesis, stating that different stamen types within a flower can carry different functions (Müller 1881, Luo et al. 2008, Vallejo-Marín et al. 2009, Mesquita-Neto et al. 2017). Specifically, it has been suggested that one stamen type may be involved in producing fodder pollen ('feeding' stamens) as pollinator reward while the other type produces pollination pollen ('pollination' stamens, Müller 1881). The different stamen types are often aggregated in different areas of the flower and differ in size so that they can deposit pollen on different parts of the pollinator's body (Luo et al. 2008, Amorim et al. 2017). Ideally, pollen from 'feeding' stamens should be deposited on body parts the bee can easily groom while pollen from 'pollination' stamens should be deposited on 'safe sites' like the bee's back (Luo et al. 2008). Recently, Kay & Jogesh (2017) proposed an alternative functional explanation for the evolution of heteranthery in that it may also provide a means of pollen dosing. Following this reasoning, one would presume that pollinators first exploit the showy stamens (e.g. more vividly coloured) and only later forage on the less attractive ones. In contrast to the 'division-of-labour' hypothesis, however, pollen from both stamen types would function in pollination and in feeding pollinators (Kay & Jogesh 2017).

We selected three buzz-pollinated plant species (*Senna reticulata* (Willd.) H.S.Irwin & Barneby (Fabaceae), *Adelobotrys adscendens* (Sw.) Triana, and *Conostegia subcrustulata* (Beurl.) Triana (both Melastomataceae)) to assess the adaptive significance of poricidal anthers in functioning as dynamic pollen dosing mechanisms. First, we artificially buzzed flowers of all three species to test whether pollen dosing changes over the course of anthesis, i.e. if older flowers release more pollen than younger flowers. Second, in the two heterantherous species (*S. reticulata*, *A. adscendens*), we tested whether the different stamen types differ in their pollen dosing strategies. Finally, in the heterantherous species *A. adscendens*, we assessed whether the different stamen types indeed deposit pollen in different areas of the pollinator's body as assumed by the 'division-of-labour' hypothesis.

Material and Methods

Study site and study species

Fieldwork for all three study species was conducted in a lowland tropical rainforest site in Costa Rica, i.e. in the area of the Tropical Research Station La Gamba, between February 13th and 22nd 2018. *S. reticulata* are shrubs or treelets with many multi-flowered inflorescences (Parolin 2001). Flowers are relatively large (diameter ca. 2 cm), pentamerous with free petals which form cup-shaped corollas (Amorim et al. 2017). *S. reticulata* is heterantherous and has four different stamen types (Fig. 1A): two long, lateral fertile stamens ('pollination stamens'), one central median stamen (often infertile), four short, central fertile stamens ('feeding stamens') and three sterile reduced staminodes (Marazzi et al. 2007). Recent pollination experiments have given support to the 'division-of-labour' hypothesis in *Senna* and complex co-functioning between petals and stamens in a ricochet pollination mechanism (Amorim et al. 2017). *A. adscendens* grows as a liana up to ca. 20 m into the canopy and also presents several densely flowered inflorescences. Flowers are ca. 1 cm in diameter, pentamerous and also have cup-shaped corollas (Fig. 1B, Dellinger et al. 2019). *A. adscendens* flowers are heterantherous and have two stamen types, five long, narrow stamens with corrugated thecal walls (Fig. 1D) and five short, wider stamens with smooth thecae (Fig. 1E, Dellinger et al. 2019). The function of these stamens has never been studied, but following 'division-of-labour' theory, we hypothesize that the long stamens may be 'pollination' stamens and the short ones 'feeding' stamens. *C. subcrustulata* grows as shrubs or trees with multi-flowered inflorescences but has smaller, hexamerous flowers (ca. 0.7 cm in diameter) with twelve isomorphic stamens (Fig. 1C, Kriebel 2016).

From our own observations prior to the start of experiments, we know that flowers of all species are anthetic for one day only. Flowers open in the early hours of morning (ca. 5 am) and wither in the late afternoon (*S. subcrustulata*, ca. 5 pm) or close in the early evening (*A. adscendens*, *C. subcrustulata*, ca. 7 pm).

Experiment 1 – Testing the effect of flower age and heteranthery on pollen dosing

In order to test if pollen dosing strategy changes over the course of anthesis, we performed artificial buzzing experiments on the three study species. For this purpose, several inflorescences were selected in four individuals of *S. reticulata* and *C. subcrustulata* each, and in two individuals of *A. adscendens*. In the selected inflorescences, all open flowers were removed and discarded and the entire inflorescences were bagged with mesh-nets (mesh size < 1mm) to prevent pollinator visitation. The following day, newly opened flowers were picked at three different times to capture different anthetic stages: shortly after sunrise (6:00–7:30, young), at midday (12:00, medium) and in the late afternoon before daily rainfall (16:30–17:00, old). In each species, a total of 25 flowers were collected per anthetic stage. 20 of these flowers were used for artificial buzzing experiments, and five flowers were used for pore size measurements (see section 'Pore size measurements'). Flowers were transferred to the air-conditioned laboratory of the Tropical Research Station La Gamba immediately after collection to perform artificial buzzing experiments. Following the method of Amorim et al. (2017) developed for studying pollination in *Senna*, we modified an electric toothbrush (Philips Sonicare Flexcare Platinum HX9111/20) by exchanging the brush with a sewing needle to apply artificial vibrations to flowers.

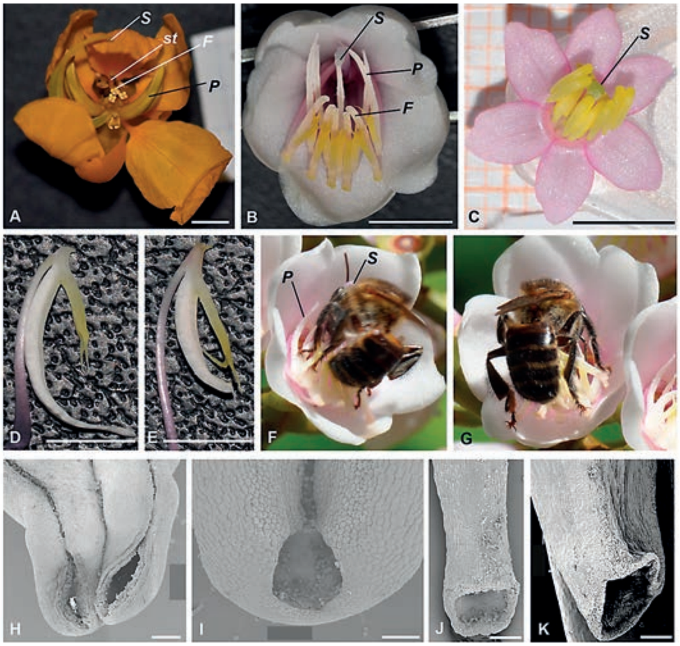

Fig. 1: Flowers of the three study species, details of stamen dimorphism and pollination of *Adelobotrys adscendens* by *Melipona costaricensis*, and SEM images of stamen pores. A) Enantiostylous (S) and partially asymmetric flower of *Senna reticulata* with two large pollination stamens (P, lateral) and four centrally arranged feeding stamens (F) and staminodes (st), the curved petals possibly function in the ricochet-mechanism of pollen transfer (as documented for other *Senna* species, see Amorim et al. 2017). B) Flower of *A. adscendens* with dimorphic and monosymmetrically arranged stamens. C) Flower of *Conostegia subcrustulata* with isomorphic stamens arranged in a weakly monosymmetric pattern; note green, capitate stigma. D) Long stamen (hypothesized pollination stamen) of *A. adscendens*. E) Short stamen (hypothesized feeding stamen) of *A. adscendens*. F), G) *A. adscendens* buzzed by its main pollinator, *M. costaricensis*; note how the bee crouches above the entire androecium, the short stamens pointing at its belly and the long stamens at its head. H) Stamen pore of pollination stamen of *S. reticulata*. I) Stamen pore of *C. subcrustulata*. J) Stamen pore of long (pollination) stamen of *A. adscendens*. K) Stamen pore of short (feeding) stamen of *A. adscendens*. P – pollination stamen, F – feeding stamen, S – stigma, st – staminodes. Scale bars: A–E: 0.5 mm, H: 200 µm, I–K: 100 µm. – Abb. 1: Blüten der drei Untersuchungsarten, Details der unterschiedlichen Staubblatttypen und Bestäubung bei *Adelobotrys adscendens* durch *Melipona costaricensis* sowie SEM Bilder von Staubblattporen. A) Enantiostylie (S) und teilweise monosymmetrische Blüte von *Senna reticulata* mit zwei großen Bestäubungsstaubblättern (P, seitlich), und vier zentralen Futterstaubblättern (F) und Staminodien (st), die gekrümmten Petalen fungieren möglicherweise als Reflektoren beim Pollentransfer (wie bei anderen Arten der Gattung *Senna* dokumentiert, Amorim et al. 2017). B) Blüten von *A. adscendens* mit zwei Staubblatttypen in monosymmetrischer Anordnung. C) Blüte von *Conostegia subcrustulata* mit nur einem Staubblatttyp, der schwach monosymmetrisch angeordnet ist, Stigma grün. D) Langes Staubblatt (wahrscheinlich mit Bestäubungsfunktion) von *A. adscendens*. E) Kurzes Staubblatt (wahrscheinlich mit Fütterfunktion) von *A. adscendens*. F), G) *M. costaricensis*, Hauptbestäuber von *A. adscendens*; die Biene krümmt sich über das gesamte Andrözium, die kurzen Staubblätter sind auf den Bauch, die langen Richtung Kopf gerichtet. H) Staubblattpore eines Bestäubungsstaubblattes von *S. reticulata*. I) Staubblattpore von *C. subcrustulata*. J) Staubblattpore des langen Staubblatts von *A. adscendens*. K) Staubblattpore des kurzen Staubblatts von *A. adscendens*. P – Befruchtungsstaubblätter. F – Beköstigungsstaubblatt. S – Stigma. st – Staminodien. Maßstabsbalken: A–E: 0,5 mm, H: 200 µm, I–K: 100 µm.

The basic and peak frequency of vibrations produced by this toothbrush are in the range of frequencies produced by buzzing carpenter bees (basic frequency 246.2 Hz, peak frequency 246 Hz, Amorim et al. 2017). Before artificial buzzing, we removed all petals from the flower. To vibrate all stamens equally, the needle was then inserted in the flower's floral base (just below the superior ovary), in a 90° angle to the flower's median plane (Amorim et al. 2017). Each flower was vibrated for 0.5 to 0.75 seconds at highest intensity (level three of the toothbrush). All ten stamens were then collected into a 1.5 ml Eppendorf tube filled with 700 µl of 70 % ethanol for later pollen counting. In addition, 10 freshly opened, unvisited (young) flowers were collected for each species and put into 70 % ethanol without further manipulation to serve as references for pollen counts (see below, referred to as "virgin" from here onwards).

Experiment 2 – Heteranthery as 'division-of-labour' (differential pollen placement)

Differential pollen placement has been shown for some *Senna* species by artificial buzzing and using surrogate bees (Amorim et al. 2017). We adopted a similar approach to testing the 'division of labour' versus the 'pollen dosing' hypothesis in heterantherous *Adelobotrys adscendens*. As for the pollen dosing experiment, we bagged inflorescences of two individuals with mesh-nets and collected freshly anthetic flowers the next morning (between 7:30 and 9:30, n = 57). Flowers were transferred to the laboratory immediately after collection. We then split flowers into three treatment classes: i) intact flowers (n = 20), ii) flowers with long stamens only (n = 19; we removed short stamens with forceps), iii) flowers with short stamens only (n = 18; we removed long stamens with forceps). We then prepared microscope slides by marking a 2 cm cross in the centre of the slide (Fig. 2A). A single flower was positioned above the slide, with the flower centre (pedicel) above the intersection of the cross and the style in the lower half of the cross (Fig. 2B, C). We then inserted the needle of the electric toothbrush in the hypanthium base as described above and artificially buzzed the flower for approximately 1 second at highest intensity (Fig. 2C). We photographed microscope slides under a stereomicroscope, always using the same settings. We prepared photos for further analyses using Photoshop (CC-Version 19.x). To improve photo quality, images were masked with an 80 % sharpening filter, converted into a negative image and any colouration of the background was removed. Further analyses were performed in the R-package *EBImage* (Pau et al. 2010): pollen images were converted into black-and-white-images and the coordinates of the black pixels, representing pollen grains, were extracted. For each treatment class, all images were combined to later obtain the average pollen grain distribution. We used the R-package *spatstat* (Baddeley et al. 2013) to convert the pixel distributions into point-pattern-datasets and calculated convex-hulls around the pixel (pollen) areas. We used Kernel-density functions with Diggle's edge correction to estimate pixel (pollen) densities in the polygons (Diggle 1985). To evaluate average pixel distributions, we drew 100 random subsets of the mean number of pixels of each treatment group (intact 14,184 pixels, short only 12,520 pixels, long only 2,419 pixels) from the combined pixel distributions and calculated the average density across these subsets. In addition, to only assess differences in pollen placement (not masked by differences in average pollen deposition), we drew another 100 random subsets of 1,000 pixels each from the three treatment groups. We then plotted and visually compared pollen grain distributions from the three treatments and the two subsets. We further tested for spatial clustering of pollen grains using the Clark-Evan's test, which compares the observed distribution of points to the expected distribution under a completely random Poisson process (Clark & Evans 1954).

We also counted pollen grains remaining in the stamens after artificial buzzing following the methods described below.

Pollen counting

We quantified the amount of pollen grains remaining in the anthers after artificial buzzing using a particle counter (Topas Particle Counter FAS362B, also see Vallejo-Marín et al. 2009). In the two heterantherous species, we randomly selected stamens of each type (one 'feeding' and two 'pollination' stamens in *Senna reticulata* and two stamens of each type in *Adelobotrys adscendens*) per flower for pollen counting. In *Conostegia subcrustulata*, we selected two stamens at random. We put single stamens into fresh 1.5 ml microcentrifuge tubes filled with 1,000 µl of purified water and squeezed them with an Eppendorf micropestle (SigmaAldrich) to rip open stamens to excise pollen. We then placed the tubes

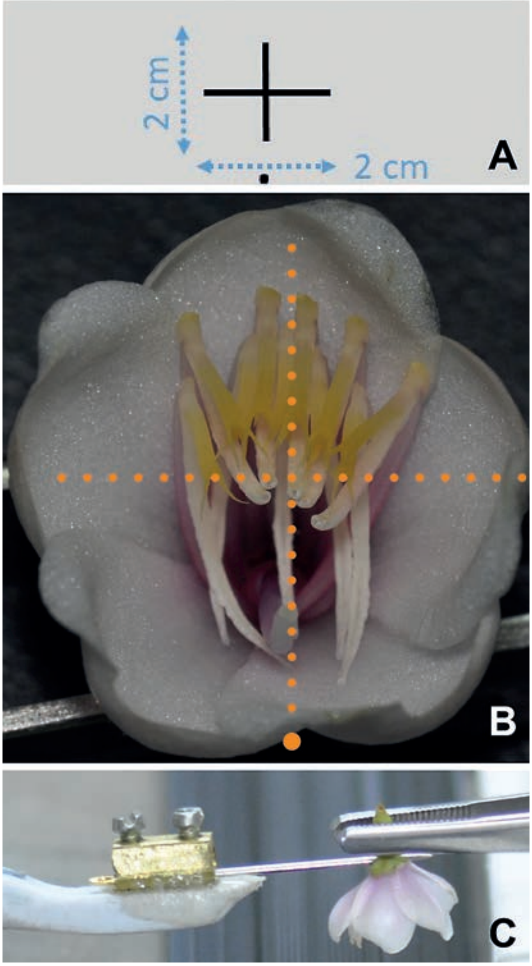

Fig. 2: Setup of experiment 2. A) Microscope slide with 2x2 cm cross marked, the flower center was arranged above the cross, the dot was used to always arrange flowers with appendages pointing to the upper side of the slide and the style towards the dot at the lower side. B) Intact flower, the orange cross indicates the flower arrangement above the cross marked on the microscope slide, the dot is shown on the side of the style. C) Setup of artificial buzzing, the flower is held upside down over the microscope slide, the needle is inserted into the hypanthium and tweezers are used to prevent the flower from turning sideways. – Abb. 2: Aufbau von Experiment 2. A) Objektträger mit 2x2 cm Kreuz, über welchem das Zentrum der Blüte positioniert wurde; der Punkt diente dazu, die Blüte stets so auszurichten, dass die Staubblattanhängsel in Richtung des oberen Randes des Objektträgers deuteten und der Griffel Richtung Punkt am unteren Rand wies. B) Intakte Blüte, das orange Kreuz zeigt, wie die Blüte über dem Kreuz am Objektträger ausgerichtet wurde, ebenso ist der Punkt markiert. C) Künstliche Vibration: Die Blüte wird mit der Kronenöffnung nach unten zeigend über den Objektträger gehalten, die Nadel der adaptierten elektrischen Zahnbürste steckt im Hypanthium, die Pinzetten dienen zum Stabilisieren der Blüte bei der künstlichen Vibration.

into an ultrasonication bath for 5 minutes to break up pollen clumps and assure complete pollen release from the stamens. We then injected 100 µl of each sample into the particle counter (duration of count per sample 10 seconds). The FAS362B counter can distinguish 62 size classes between 2 and 200 µm. In order to only analyse size classes in the size range of pollen grains (and not possible residual plant material from sample preparation), we measured pollen grain sizes under a light microscope prior to counting. We also used histograms to plot the frequency distribution of all counted particles to cross-check that the size classes representing pollen grains would be selected. Based on measurements and visual inspection of the count data, we chose the following size classes: *S. reticulata* 17.96–28.55 µm, *A. adscendens* 7.75–11.58 µm and *C. subcrustulata* 10.7–15.4 µm (starting at 9.4 µm for freshly opened, virgin stamens, see Results). We summed up particle counts for the chosen size classes to obtain total pollen number per count. The total number of pollen grains was multiplied by 10 to obtain the total amount of pollen grains per stamen.

Pore size measurements

For each species, pore sizes were measured for two stamens from five flowers for the three anthetic stages (n = 30 stamens per species). In heterantherous *S. reticulata* and *A. adscendens*, we selected one stamen per type from each flower. We prepared single stamens for Scanning Electron Microscopy (SEM) by dehydration over an ethanol series, critical point drying (CP Autosamdri-815), coating with gold (Sputter Coater (SCD 050)) and mounting them onto aluminium stubs. Samples were scanned and photographed in a JEOL JSM-6390 at 10 kV. Pore surface area was measured with the software Fiji/ImageJ (version 1.51w, Schindelin et al. 2012).

Statistical analyses

All statistical analyses were performed in R (R Developmental Core Team 2018). We first calculated the mean number of pollen grains remaining in single stamens after buzzing at different anthetic stages (Tab. 1). For *S. reticulata* and *A. adscendens*, we tested whether the two stamen types differed significantly in pollen amount of virgin flowers using GLMMs (Generalized Linear Mixed Effects Models, *lmerTest*, Kuznetsova et al. 2017), treating stamen type as fixed factor and flower ID as a random effect; data on plant individuals was not recorded.

To assess pollen dosing, we subtracted the number of pollen grains remaining after artificial buzzing in young, median or old stamens from the number initially present in virgin stamens. We used the maximum number of pollen grains detected in virgin stamens as reference for subtraction. In the heterantherous species, we used respective maximum values for the different stamen types. We chose the maximum reference value over the mean since some pollen removal estimates were negative (i.e. fewer pollen grains in virgin than in artificially buzzed flowers; for similar problems, see Muchhala & Thomson 2010). Using the maximum reference value, two buzzed samples in *A. adscendens* and *C. subcrustulata*, respectively, and eight in *S. reticulata* still had higher pollen counts than the reference maximum; these samples were excluded from analyses. For possible methodological shortcomings, see Discussion. To test for changes in pollen dosing over anthesis, we ran GLMMs on the amount of pollen removed from stamens by artificial buzzing, setting the three different anthetic stages and stamen type as fixed factors and flower ID as random effect, using a Poisson family for the model and the initial amount of pollen present in each stamen type as offset. For *C. subcrustulata*, we ran a linear model on the amount of pollen removed with the different anthetic stages as fixed factor. We calculated the proportion of removed pollen to compare pollen dosing rates between species and stamen types.

Differences in pore area between the three anthetic stages were evaluated using ANOVAS (homogeneity of variance tested by Levene's test and normality by Shapiro-Wilk-test). We did not test for changes in pore area over anthesis for the two stamen types separately due to small sample sizes.

To assess differences in pollen release between the two stamen types in *A. adscendens* (experiment 2), we ran a GLM with the initial number of pollen grains for each stamen type as offset and a quasipoisson family.

Results

Pollen grains are small in all species, largest in *S. reticulata* (20.3–28 µm) and smaller in *C. subcrustulata* (10–14.7 µm) and *A. adscendens* (8.0–11.8 µm).

We found a maximum of 100,940 pollen grains in individual virgin stamens of *S. reticulata*, 58,730 in *A. adscendens* and 32,975 in *C. subcrustulata* (Tab. 1 for averages). Virgin pollinating (large) stamens contained eight times more pollen than virgin feeding (small) stamens in *S. reticulata* (GLMM: *t-value 8.70, df = 15, p < 0.01*) and 1.4 times more pollen in *A. adscendens* (GLMM: *t-value 5.32, df = 17, p < 0.01*, Tab. 1). The staminodes did not differ significantly in pollen amount from small feeding stamens in *S. reticulata* (GLMM: *t-value –1.08, df = 19, p = 0.291*) and were not considered in subsequent analyses.

Tab. 1: Mean number (and standard deviation) of pollen grains in virgin stamens and pollen grains remaining after artificial buzzing (experiment 1) per stamen type in the different anthetic stages. – Tab. 1: Durchschnittliche Anzahl (und Standardabweichung) der Pollenkörner in unmanipulierten Staubblättern und nach künstlicher Vibration (Experiment 1) je Staubblatttyp und unterschiedlichem Anthesezeitpunkt.

species	**stamen type**	**mean (sd) no. of pollen grains**				
		virgin	**young**	**medium**	**old**	**pore area (µm²)**
S. reticulate	pollinating	63,109 (24,060)	34,819 (28,562)	16,906 (19,922)	23,693 (25,099)	1,558.4 (434.6)
	feeding	7,919 (2,478)	7,848 (2,863)	7,292 (5,502)	5,632 (4,069)	1,490.9 (895.7)
	staminodes	1,253 (732)	1,920 (774)	1,210 (729)	840 (–)	–
A. adscendens	pollinating	45,302 (7,505)	37,089 (15,326)	36,058 (11,103)	33,921 (9,471)	14,364.3 (3,479.9)
	feeding	32,069 (7,498)	31,980 (10,771)	31,611 (8,276)	27,474 (8,392)	27,178.9 (5,082.9)
C. subcrustulata	–	20,980 (7,843)	15,693 (8,746)	16,907 (6,384)	14,312 (8,278)	34,741.5 (9,690.5)

Experiment 1 – Testing the effect of flower age and heteranthery on pollen dosing

In *S. reticulata*, we found pollen dosing both through flower age and heteranthery (Fig. 3A). More than 50 % of pollen grains were released on average by artificial buzzing at any anthetic stage (young 51 %, medium 68 %, old 70 %). Pollination stamens released on average 30 % more pollen than feeding stamens. Both stamen types released significantly less pollen at the young stage than at older stages (pollination stamens: z-value –2.38, p = 0.02; feeding stamens: z-value –4.03, p < 0.001). Medium and old-stage stamens of both types did not differ in the amount of pollen released (pollination stamens: z-value –0.92, p = 0.36; feeding stamens: z-value 0.318, p = 0.75).

In *A. adscendens*, we only found pollen dosing through heteranthery, but not flower age (Fig. 3B). Long stamens released significantly more pollen (41 %) than short stamens (36 %; z-value 76.39, p < 0.01, Fig. 3B). Pollen release did not change significantly over anthesis in either stamen type, however (long stamens: young vs. medium: z-value –0.32, p = 0.75; medium vs. old: z-value 0.15, p = 0.88; short stamens: young vs. medium: z-value –1.05, p = 0.29; medium vs. old: z-value 1.19, p = 0.23).

In isomorphic *C. subcrustulata*, we did not find pollen dosing through flower age (Fig. 3C, F = 1.46, df = 2, p = 0.24). Artificial buzzing released ca. 56 % of pollen in young, 49 % in medium and 60 % of pollen in old flowers.

Pore area did not increase over anthesis in *S. reticulata* (*F 0.007, df = 2, p = 0.993*) and *A. adscendens* (F 1.08, df = 2, p = 0.35). Pore area increased 1.5 times from young to old flowers in *C. subcrustulata* (F 12.7, df = 2, p < 0.01; young 25.051 µm^2, old 39.337 µm^2). In *S. reticulata*, the two stamen types had similar pore areas while in *A. adscendens*, pore areas of short (feeding) stamens were almost twice the size of pore areas of long (pollinating) stamens (Tab. 2, Fig. 1 H-K).

Tab. 2: Average pore area (µm^2) per stamen type for all anthetic stages. – Tab. 2: Durchnittliche Porenfläche (µm^2) pro Staubblatt für alle Zeitpunkte der Anthese.

species	type	young	middle	old
S. reticulata	pollinating	1,484 (549)	1,670 (112)	–
	feeding	1,547 (1,048)	1,295 (842)	1,484 (731)
A. adscendens	pollinating	16,453 (5,433)	14,058 (2,654)	13,089 (1,479)
	feeding	26,542 (2,955)	29,659 (6,853)	24,508 (1,621)
C. subcrustullata	-	25,051 (3,370)	38,087 (9,295)	39,337 (8,027)

Experiment 2 – Heteranthery as 'division-of-labour' (differential pollen placement) or dosing strategy

Image analyses showed that pollen from *A. adscendens* flowers was deposited in similar areas on microscope slides across all treatments (intact flower, short (feeding) stamens only, long (pollination) stamens only, Fig. 3). In all treatments, we found significant clustering of pollen grains (highest pollen density) in the centre of the slide and gradual decrease in pollen density around this centre (Clark-Evan's Test: intact: *R = 0.005, p < 0.01*; long stamens only: *R = 0.002, p < 0.01*, short stamens only: *R = 0.003, p < 0.01*). Interestingly, we found that short (feeding) stamens contributed ca. 75 % of total pollen deposited while long (pollinating) stamens only contributed around 25 % of total pollen deposited, despite the latter having more initial pollen present (Fig. 4A). This observation was confirmed when testing for significant differences in the relative amounts of released pollen between the two stamen types: on average, short stamens released more than twice as much pollen as long stamens (32 % of total pollen released compared to 14 %; *t-value –3.77, df = 40, p < 0.01;* Fig. 3). When sub-setting the three treatments to 1,000 pollen grains each to only assess differences in pollen deposition density, we found that pollination stamens deposited pollen in higher densities in the centre of the slide while short stamens distributed pollen more evenly across the slide (Fig. 4B).

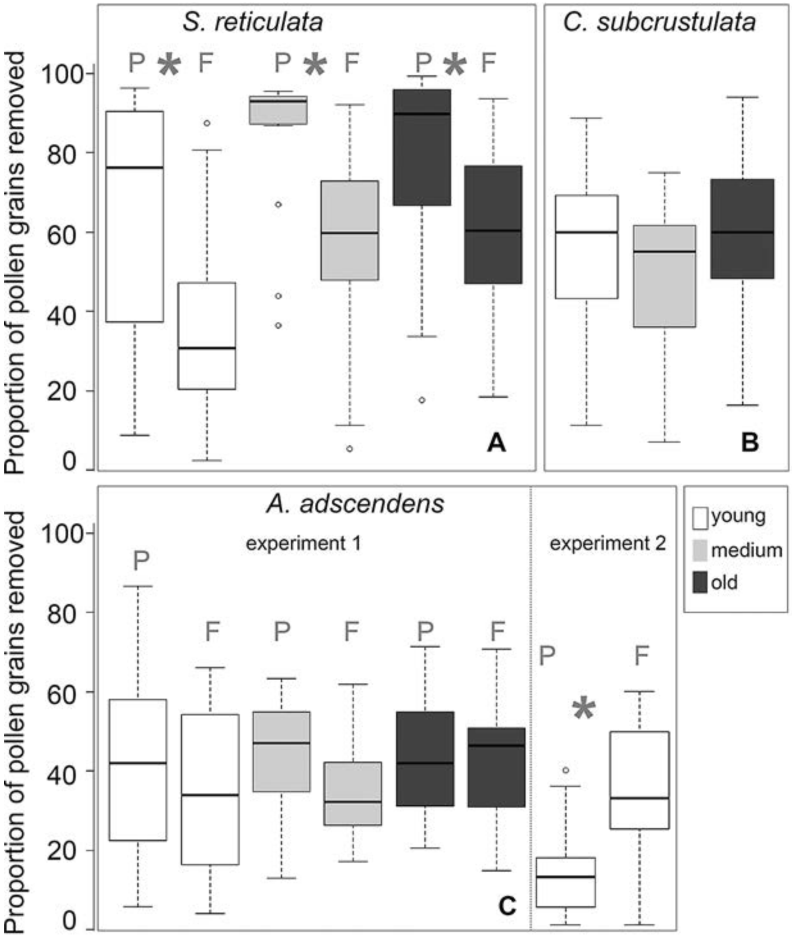

Fig. 3: Box-whisker-plots representing proportion of pollen grains removed at different anthetic stages and from different stamen types (P – pollination stamens, F – feeding stamens). A) Pollination stamens released significantly more pollen than feeding stamens in *S. reticulata*, but only feeding stamens released significantly more pollen grains at later than younger anthetic stages (see text). B) There was no significant difference in pollen release in *C. subcrustulata*. C) There was no significant difference in pollen release between anthetic stages or stamen types in experiment 1 in *A. adscendens,* but in experiment 2, short (feeding) stamens released significantly more pollen grains. P - pollination stamens, F - feeding stamens, * marks significant differences between stamen types. – Abb. 3: Die Box-Whisker-Plots zeigen die relativen Pollenmengen, die zu unterschiedlichen Zeitpunkten der Anthese aus den Blüten bzw. den unterschiedlichen Staubblatttypen entfernt wurden (P – Befruchtungsstaubblätter, F – Beköstigungsstaubblätter). A) Bei *S. reticulata* setzten die Befruchtungsstaubblätter signifikant mehr Pollen frei als die Beköstigungsstaubblätter, doch nur die Beköstigungsstaubblätter setzten zu späteren Anthesezeitpunkten signifikant mehr Pollen frei als zu früheren Anthesezeitpunkten. B) Bei *C. subcrustulata* gab es keine signifikanten Unterschiede in der Pollenfreisetzung zwischen den unterschiedlichen Anthesezeitpunkten. C) Bei *A. adscendens* wurden in Experiment 1 weder signifikante Unterschiede in der Menge des freigesetzten Pollens noch Unterschiede zwischen den Anthesezeitpunkten festgestellt. In Experiment 2 jedoch setzten die kurzen Beköstigungsstaubblätter signifikant mehr Pollen frei. P – Befruchtungsstaubblätter, F – Beköstigungsstaubblätter, * zeigt signifikante Unterschiede zwischen den Staubblatttypen an.

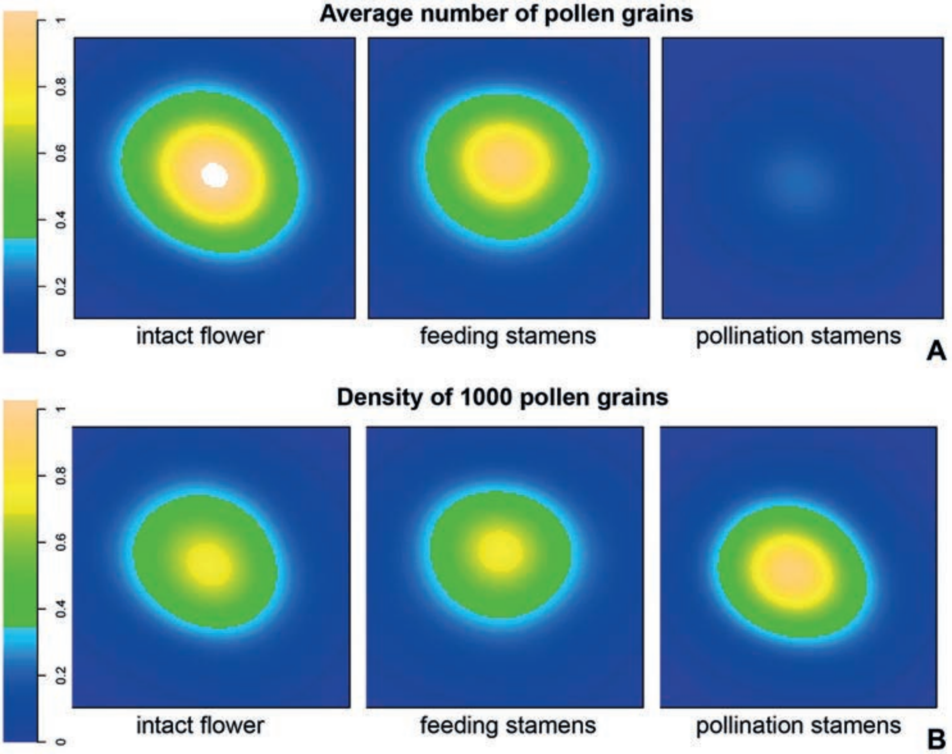

Fig. 4: Pollen densities deposited by artificial buzzing of intact *A. adscendens* flowers and flowers with feeding or pollination stamens only; the scale indicates pollen density (0 – lowest density, 1 – highest density). A) The average number of pollen grains deposited per treatment is shown, with high densities in the centre of the microscope slide by intact flowers and flowers with only feeding stamens and low densities in the same area by flowers with only pollination stamens. B) The average density of deposition of 1,000 pollen grains is shown for the three treatments, with highest densities deposited in the centre of the slide by pollination stamens. – Abb. 4: Pollendichten, die durch die künstliche Vibration von intakten und manipulierten Blüten von *A. adscendens*, auf dem Objektträger abgelagert wurden; der Maßstab zeigt die Pollendichte an (0 – niedrigste Dichte, 1 – höchste Dichte). A) The mittlere Anzahl an Pollenkörnern die je Experimentansatz (intakte Blüte, nur Befruchtungsstaubblätter, nur Beköstigungsstaubblätter) abgeladen wurde. Bei intakten Blüten und Blüten mit Beköstigungsstaubblättern befinden sich die höchsten Dichten an Pollenkörnern im Zentrum der Objektträger, während diese Zone bei Blüten nur mit Befruchtungsstaubblättern eine sehr niedrige Dichte an Pollenkörnern aufweist. B) Die mittlere Ablagerung von 1000 Pollenkörner je Experimentansatz, wiederum befindet sich die höchste Dichte im Zentrum des Objektträgers, wobei die bedeckte Fläche durch die Befruchtungsstaubblätter am größten ist.

Discussion

In this study, we investigated the functional role of poricidal anthers as pollen dosing mechanisms in relation to flower age and heteranthery in three buzz-pollinated plant species. Our results demonstrate that pollen removal from poricidal anthers is restricted as only 36 % to 70 % of pollen grains were removed when flowers were buzzed artificially. In the three species studied, we detected three different patterns: differences in dosing through heteranthery and flower age (*S. reticulata*), differences in dosing through heteranthery only (*A. adscendens*) and no differences in dosing (*C. subcrustulata*).

Following Pollen Presentation Theory (PPT), a strict dispensing mechanism such as poricidal anthers should adjust pollen release to pollinator quantity (abundance) and quality (efficiency in transferring conspecific pollen; Harder & Barclay 1994, Harder & Wilson 1994, Castellanos et al. 2006). While freshly opened virgin flowers are expected to release only small doses of pollen (provided that visitation rates are high), old virgin flowers, which are at risk of not being visited a second time, should release larger amounts of pollen. Few experimental studies have tested these ideas, but support came from buzz-pollinated *Dodecatheon* where older flowers released about 20 % more pollen than young flowers (Harder & Barclay 1994). This pattern was found in *S. reticulata* only (Fig. 3A). In this species, average pollen release was high (often more than 60 % during a single buzz, Fig. 3A) while it was generally below 50 % in *A. adscendens* and intermediate in *C. subcrustulata*. These findings suggest that *S. reticulata* doses pollen less strictly at any anthetic stage. In addition, pollen release significantly increased in later stages. This increased pollen release could be a response to relatively low pollinator visitation rates or high pollinator efficiency (Castellanos et al. 2006). We have observed irregular visits of *Xylocopa* sp. and halictid bees to *S. reticulata*, but detailed data on visitation rates and pollinator efficiency in La Gamba are lacking. Snow & Roubik (1987) reported that large bees could remove about 15–25 % of pollen grains in *S. reticulata* while halictids removed negligible amounts only. To fully understand pollen dispensing schedules in this species, field observations of natural pollinators should be combined with artificial buzzing experiments and assessments of pollen deposition on stigmas. Likewise, the lower amounts of pollen released by *A. adscendens* and *C. subcrustulata* are difficult to interpret at the current stage of knowledge. We have observed *Melipona costaricensis* as the main pollinator of *A. adscendens* in La Gamba, and preliminary results suggest that visitation frequencies are around 1.8 visits per flower per hour between 6 am and 12 pm (Dellinger, unpublished results). Thus, visits are possibly frequent enough to justify the release of relatively small doses of pollen only. In *C. subcrustulata*, we found a significant increase in anther pore size in older flowers, possibly indicating an adjustment to less strict dosing in older flowers. However, this increase in pore size was apparently not enough to significantly increase pollen release as assessed by our buzzing experiments (Fig. 3).

Significant differences in initial pollen amount (virgin stamens) and pollen release patterns between the two stamen types in *S. reticulata* and *A. adscendens*, respectively, support the hypothesis that heteranthery serves as a within-flower dosing strategy (Kay & Jogesh 2017). In *S. reticulata*, the large pollination stamens contained significantly more pollen than the small feeding stamens, and they also released significantly more pollen than the small stamens during a single buzz (Fig. 3). These results suggest that pollination pollen is released quickly and upon the first visits a flower may receive, assuring pollen export and increasing male fitness. Both male and female fitness may benefit from the more gradual pollen release from feeding stamens: the continued (and increasing) release of small doses of the pollen reward may assure further bee visits and each visit increases the chance of exporting and receiving pollen to/from different flowers or individuals (Muchhala et al. 2010). In *A. adscendens*, the long stamens contained significantly more pollen than the short stamens (Tab. 1). While we found significantly higher pollen release by the large stamens in experiment 1, results of experiment 2 are opposed: the short stamens released twice as much pollen (Fig. 3B, Fig. 4). Given that pore area of short stamens is almost twice as large as the pore area of long stamens and pollen can thus be released more easily (Fig. 1J, K, Tab. 2), we consider the significant differences detected in experiment 2 as meaningful. Also, anthers of short stamens are only about half the length (ca. 3.8 mm) of anthers of long stamens (ca. 7.6 mm) and have smooth thecal walls as opposed to corrugated thecal walls in long stamens. Thus, the distance that pollen has to travel through short anthers is not only much shorter, but the smooth thecal walls may allow for relatively unhindered passage and the bigger pore for less restricted release (Fig. 2D, E). This incongruence in results, together with the fact that some buzzed stamens contained more pollen than virgin reference stamens, point towards methodological shortcomings of our study. We cross-checked pollen counts from the particle counter by inspecting single samples under the light microscope to rule out effects of pollen clumping possibly confounding total pollen counts. We could rule out problems related to clumping as our treatment with the ultra-sonication bath effectively broke up possible pollen clumps. Single pollen grains were present in all samples inspected with the light microscope. Other reasons for the observed differences could be gradual pollen maturation with flower age. We made reference counts for virgin flowers of *S. reticulata* and *C. subcrustulata* collected at 7 am and at 12 pm. The mean number of pollen grains did not differ from the pollen counts obtained from virgin flowers collected at earlier stages. Also, we removed one stamen type in experiment 2 before buzzing. This alteration in the floral architecture may affect how vibrations are transmitted through the tissue and hence influence pollen dispensing. Finally, we cannot rule out that the application of buzzes itself has an effect on pollen grains, possibly affecting their electric charge. Further experiments are, however, required to clarify these issues. For future studies, one possible solution to the apparent variability in pollen numbers across individual flowers would be to collect a single virgin stamen from each experimental flower before the experiment, as a reference.

Kay & Jogesh (2017) have shown that dimorphic stamens in *Clarkia* function in staggered pollen dosing, with conspicuous stamens depleted first by bees and cryptic stamens only depleted at later stages. In contrast to *Senna* or *Clarkia*, bees visiting *A. adscendens* do not buzz only one stamen type but crouch above the entire androecium and buzz all stamens simultaneously. We argue that stamens in *Adelobotrys* carry mixed dosing and 'division-of-labour' functions. In our assessment of differential pollen placement on microscope slides (Fig. 4), we did not find support for differential pollen placement between the two stamen types. However, the fact that the two stamen types of *A. adscendens* differ in how densely they deposit pollen (Fig. 4B) supports the idea that pollen may indeed be deposited differently on the bee's body. Furthermore, pores differ in their orientation: pores of the long stamens are dorsally inclined and likely fire pollen at the bee's head and back (safe sites, Mesquita-Neto et al. 2017), while pores of short stamens are apically inclined and shoot pollen directly at the bee's face and belly (Fig. 1 J, K). It is possible that our approach of assessing pollen deposition on the flat surface of a microscope slide could not pick up these small scale differences in the direction of pollen expulsion between the two stamen types. Pollen expulsion movement has been shown to be highly complex (e.g. pollen reflected by petals in *Senna*, Amorim et al. 2017) and clear differential pollen placement between *Adelobotrys'* stamen types may only be picked up on surrogate or real three-dimensional bees and in completely intact flowers.

Traditional 'division-of-labour' into pollination and feeding functions of stamens has been experimentally shown for some buzz-pollinated species of *Senna, Solanum rostratum* (Solanaceae) and the melastome species *Melastoma malabathricum* (Luo et al. 2008, Vallejo-Marín et al. 2009, Mesquito-Neto et al. 2017). Taking the idea of Kay & Jogesh (2017) one step further, we believe that stamens in *Adelobotrys,* and possibly many other buzz-pollinated flowers, show even more complex 'division-of-labour' between stamen types by combining differential dosing ('shift-working') with differential pollen placement. Bees likely direct their initial collection activity to conspicuous, short stamens which deposit pollen conveniently and abundantly on their bellies (Luo et al. 2008). We hypothesize that once these (feeding/early-shift) stamens are depleted, bees may start to buzz (often less showy) longer (pollination/late-shift) stamens. In this context, it would be interesting to assess pollen viability of the two stamen types in *A. adscendens*, as studies on other heterantherous species have found mixed support for reduced viability of pollen from feeding stamens (Gross & Kukuk 2001, Luo et al. 2008, Mesquita-Neto et al. 2017). We agree with Luo et al. (2008) that viability of pollen from both stamen types may be maintained as a safeguard as long as pollen from either stamen type may be deposited on stigmas. Indeed, continued viability of pollen from both stamen types may be particularly important if stamens perform 'shift-working'. In case bees start actively buzzing pollination/late-shift stamens, they may have to re-adjust their position in the flower (e.g. move closer towards the pores in the flower centre) and thereby possibly transfer pollen deposited on different body parts (Mesquita-Neto et al. 2017). Experimental studies have shown that the size of the bee and fit with the flower has a major influence on how much pollen is transferred (Mesquita-Neto et al. 2017, Solís-Montero & Vallejo-Marín 2017). More detailed experiments are required to understand if and how mechanical fit between bees and flowers may affected the transfer of pollen of different stamen types. Furthermore, comparative studies may help to understand under which conditions selection may lead to the evolution of heteranthery and in which cases sufficient pollen dosing may be achieved by poricidal anthers only.

Taken together, our results underline the complex nature of stamen functioning in buzz-pollinated flowers. While we found some support for adjustments of pollen dosing strategies over anthesis, differential dosing between stamen types was apparent. Heteranthery may thus represent an elaborate combination of strategies maximizing reproductive success, including differential pollen placement ('division-of-labour') as well as differential dosing ('shift-working').

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Chemical diversity and richness of fungal endophytes from Costa Rican *Palicourea* and *Psychotria* species (Rubiaceae)

Wolfgang Hinterdobler & Johann Schinnerl

Screening of fungal endophytes from five Costa Rican *Palicourea* and three *Psychotria* species (Rubiaceae) growing in the surroundings of the Tropical Rainforest Station *La Gamba* resulted in the identification of strains belonging to the genera *Xylaria*, *Arthrinium, Fusarium, Clonostachys* and *Colletotrichum*. Metabolic profiles of isolated fungi were analyzed. Several cytochalasin derivatives, piliformic acid as well as the antifungal agents griseofulvin and its derivative 7-dechlorogriseofulvin were identified. Additionally, griseofulvin and its 7-dechloro form were found to be sequestered in the guttation droplets of four strains. Growth inhibiting effects against various microbial test organisms highlight the potential of the isolated fungi to produce powerful antibiotic agents.

Hinterdobler W. & Schinnerl J., 2019: Vielfalt an endophytischen Pilzen und deren chemische Diversität von Costa Ricanischen *Psychotria* und *Palicourea* Arten (Rubiaceae).
Das Ziel dieser Arbeit war die Untersuchung endophytischer Pilze aus acht *Psychotria* und *Palicourea* Arten (Rubiaceae) aus dem der Tropenstation La Gamba angrenzenden Tieflandregenwald in Costa Rica. Insgesamt wurden 102 Pilzkulturen aus Blättern, Sprossen, Früchten und Samen isoliert und dabei konnten Pilze der Gattungen *Xylaria*, *Arthrinium*, *Fusarium*, *Clonostachys* und *Colletotrichum* identifiziert werden. Cytochalasin-Derivate, Piliformic acid sowie Griseofulvin und 7-Dechlorogriseofulvin konnten als Inhaltsstoffe identifiziert werden. Zusätzlich wurde die chemische Zusammensetzung von Guttationstropfen einiger Isolate mit den dazugehörigen Rohextrakten verglichen sowie auch die medium-abhängige Produktion von Sekundärmetaboliten dieser Pilze getestet. Antimikrobielle Eigenschaften der isolierten Endophyten wurden an *Bacillus subtilis*, *Escherichia coli*, *Candida albicans* und *Saccharomyces cerevisiae* getestet. Diese Ergebnisse dokumentieren das Vorkommen endophytischer Pilze in *Psychotria* und *Palicourea* Arten und deren Potential, antimikrobielle Substanzen zu bilden.
Keywords: *Palicourea*, *Psychotria*, fungal endophytes, *Xylaria*, cytochalasin D.

Introduction

Fungi and plants interact in numerous distinct and fascinating ways. Due to the close connection of plants and fungi in the rhizosphere, many fungal species are also capable of growing entirely inside of plant tissues. All plant organs including flowers and seeds are suitable habitats. Besides fungi, also bacteria including actinomycetes are found as colonizers of plants. The ability of microorganisms to completely reside in a living host plant, for at least a part of their life cycle, without triggering visible infection symptoms characterizes them as endophytes (Petrini 1991, Wilson 1995). Growing inside their host, endophytes encounter a niche with reduced environmental stress and sufficient supply of nutrients (Schulz & Boyle 2005). Presence of endophytic fungi has been reported from higher plants, ferns and mosses, with the highest diversity observed in the tropics (Arnold & Lutzoni 2007, Arnold et al. 2001, Davis et al. 2003). The adaptation to a life within plants lead to a loss of reproduction via spores in some endophytic species. In order to persist in the next generation, these fungi are distributed within or on the plant seeds and pollen (Hodgson et al. 2014).

Research on endophytic fungi comprises a wide array of scientific fields. The strong impact of endophytes on plant survival and interaction with the environment has been investigated extensively. Contribution to plant defense mechanisms and increased resistance against pathogens were reported for some prominent and agriculturally important species, such as *Theobroma cacao* L. (cacao tree) (Malvaceae), *Hevea brasiliensis* (Willd. ex A.Juss.) Müll.Arg. (rubber tree) (Euphorbiaceae), and species of the coffee plant genera *Coffea* sp. (Rubiaceae) (HANADA et al. 2010, GAZIS & CHAVERRI 2010, VEGA et al. 2010). Being in constant contact and competition with various other fungi and bacteria sharing the limited habitat within their host, fungal endophytes have been highlighted as a promising source for the isolation of novel secondary metabolites, often with pronounced biological activities (ALY et al. 2010, SCHULZ et al. 2002, STROBEL 2003). The discovery of paclitaxel (Taxol®), an effective medication in cancer therapy, in the fungal endophyte *Taxomyces andreana* isolated from *Taxus brevifolia* Nutt. (Taxaceae) (STIERLE et al. 1993), resulted in extensive research on endophytes of pharmaceutically important plants, and their bioactive metabolites. The indole alkaloid vincristine from *Catharanthus roseus* (L.) G.Don (Apocynaceae) and the quinoline alkaloid camptothecin from *Camptotheca acuminata* Decne. (Cornaceae), both used for cancer treatment, were discovered to be also produced by plant inhabiting fungal endophytes (ALY et al. 2013). Beside these metabolites previously only known from plants, numerous substances with antibiotic activity have been isolated from cultivated endophytes (MOUSA & RAIZADA 2013).

Fungal endophytes isolated from plant species of the genera *Psychotria* and *Palicourea* (Rubiaceae) growing in Costa Rica are in the focus of this study. These two plant genera exhibit an interesting diversity of secondary plant metabolites, e.g. iridoids, alkaloids, terpenoids and flavonoids from specific biosynthetic pathways (BERGER et al. 2012, 2016, 2017). To date, a variety of endophytic fungi and bacteria were isolated from *Psychotria* (GOVINDA RAJULU et al. 2013, LEMAIRE et al. 2012b) and *Palicourea* species (CAFÊU et al. 2005, Souza et al. 2004). In continuation of a preliminary screening for the presence of endophytic fungi in *Psychotria* species growing near the tropical field station of *La Gamba* in Costa Rica (SCHINNERL unpublished), a closer look was taken at the richness and capacity of secondary metabolite production of endophytic fungi from eight species of the genus *Psychotria* and the closely related genus *Palicourea*. To the best of our knowledge, this work is the first investigation of endophytic fungal diversity from the selected *Psychotria* and *Palicourea* species.

Results

Richness and organ specificity of endophytic fungi

Fungal endophytes were isolated from 15 *Psychotria* and *Palicourea* individuals comprising eight species. Leaves, basal and central shoots, mature and immature fruits as well as seeds were used for isolation of endophytes. A total of 102 fungal strains were isolated (Tab. 1a). From the standard, malt extract-based isolation medium, 92 fungal strains were recovered from 68 plant samples, leading to an isolation frequency of 1.35 fungal isolates per sample. Selective media supplemented with benomyl or cycloheximide led to the isolation of six and four fungal strains out of 18 and 20 plant samples, respectively. For these selective media the isolation frequency reached 0.33 strains per sample for benomyl and 0.2 for cycloheximide. For *Palicourea solitudinum* (Standl.) Borhidi, *Palicourea elata* (Sw.)

Borhidi, *Palicourea acuminata* (Benth.) Borhidi and *Palicourea pilosa* (Ruiz & Pav.) Borhidi, between 40 to 65 % of the isolated endophytes were collected from leaves followed by 25 to 40 % collected from stems. For *Palicourea tomentosa* (Aubl.) Borhidi, 20 % were isolated from leaves and 60 % from stems. Fruits and seeds account for 15 to 20 % of the total isolates per plant species (Fig. 1). Ten fungal strains were identified at least at the family level by comparison of ITS sequences to the publicly available NCBI database (Tab. 1b).

Tab. 1a: Number of isolated endophytic fungal strains and sampled individuals per plant species. – Tab. 1a: Anzahl an untersuchten Pflanzen je Pflanzenart sowie die Anzahl daraus isolierter Pilze. Tab. 1b: Identified fungal isolates. – Tab. 1b: Identifizierte Pilzgattungen und Arten.

Tab. 1a

Host species	**Sampled individuals**	**Number of fungal isolates**						
		Leaf	**Shoot B**	**Shoot C**	**Fruit**	**Fruit I**	**Seed**	**Total**
Pal. solitudinum	3	8	7	1			4	20
Pal. elata	3	10	7				3	20
Pal. acuminata	2	12	2	2	3			19
Pal. tomentosa	2	3	2	7	2		1	15
Pal. pilosa	2	5	3				2	10
Pal. winkleri Borhidi	1		4			4		8
Psy. cooperi Standl.	1	2						2
Psy. tsakiana Taylor	1		6				2	8
								Total 102

Basal shoots (B), central shoots (C) and immature fruits (I)
Basale Sprossachsen (B), zentrale Sprossachsen (C) und unreife Früchte (I)

Tab. 1b

Isolate	**Identification**	**Host species**	**Organ**	**GenBank accession no.**
whc1	*Arthrinium* sp.	*Pal. solitudinum*	Seed	MH465392*
C5	*Xylaria* sp.	*Pal. solitudinum*	Leaf	KY192281
whd4	*Fusarium* sp.	*Pal. elata*	Leaf	MH465393*
E3	Xylariaceae	*Pal. acuminata*	Fruit	KY192283
I8	*Arthrinium arundinis*	*Pal. tomentosa*	Leaf	KY192275
I9	*Fusarium proliferatum*	*Pal. tomentosa*	Shoot	KY192276
R5	*Clonostachys* sp.	*Pal. elata*	Shoot	KY192277
S1	*Fusarium proliferatum*	*Pal. pilosa*	Shoot	KY192278
T2	*Arthrinium arundinis*	*Psy. tsakiana*	Seed	KY192279
W8	*Colletotrichum* sp.	*Psy. solitudinum*	Shoot	KY192280

* Hinterdobler et al. in prep..

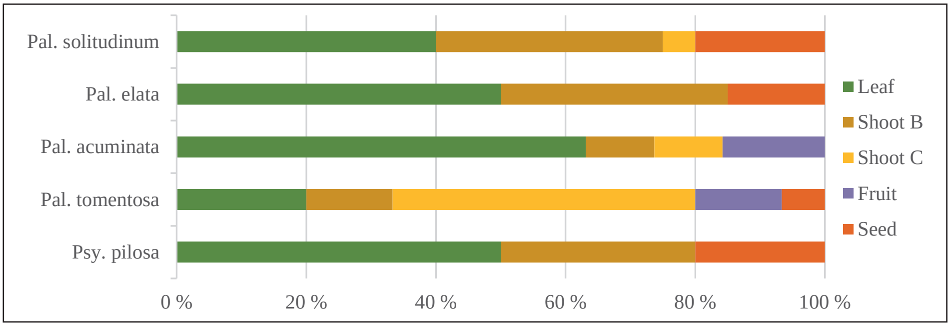

Fig. 1: Proportion of fungal isolates from plant organs (leaves, basal (B) and central (C) shoots, fruits and seeds). Plant species with more than ten fungal isolates are shown. – Abb. 1: Mengenverhältnisse von Pilzisolaten von Blättern, Sprossachsen, Früchte und Samen von Pflanzenarten mit mehr als zehn Isolaten.

Identification and distribution of fungal secondary metabolites

Analysis and extraction of fermentative cultures of a preselected subset of endophytic strains led to the identification of eight compounds (Hinterdobler et al. in prep.). The fungal isolate whc1 produced the co-chromatographically identified griseofulvin and 7-dechlorogriseofulvin on solid MEA2 medium (Fig. 2). Under fermentative conditions, piliformic acid and cytochalasin D (Fig. 2) were produced in sufficient amounts for isolation and structure elucidation. Liquid cultivation and extraction of the endophytic strain whd4 led to the isolation of four novel leucin-derived cytochalasins with one containing a rarely described 5/6/5/8-ring system (Hinterdobler et al. in prep.). The fungal metabolite griseofulvin was identified in 35 out of 102 (34.3 %) fungal isolates growing on MEA2 medium. 7-dechlorogriseofulvin was present in 30 isolates containing griseofulvin.

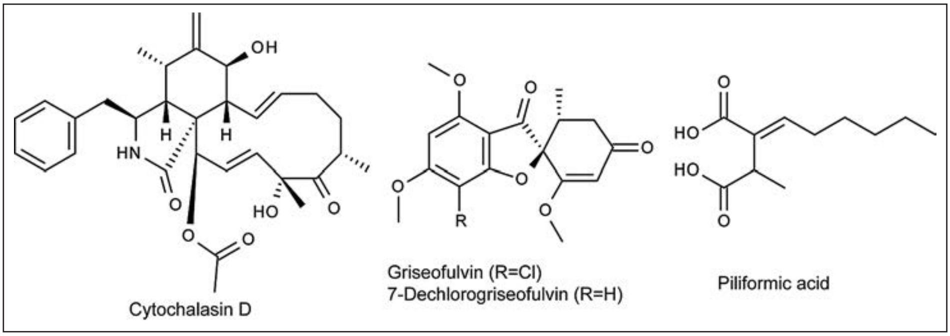

Fig. 2: Structures of cytochalasin D, griseofulvin, 7-dechlorogriseofulvin and piliformic acid. – Abb. 2: Strukturformeln von Cytochalasin D, Griseofulvin, 7-Dechlorogriseofulvin und (2*E*)-2-Hexyliden-3-methylbutandisäure.

Shifts in secondary metabolite production on different growth media was observed in the fungal isolates S1 and whc1. Fungi for upscale cultivation were chosen by their ability to produce HPLC-DAD detectible substances on MEA medium without a match in our spectral library. In order to gain a sufficient amount of the compounds for structure elucidation, fungi were grown under liquid fermentative conditions. Comparative measurements of solid and liquid cultures have shown griseofulvin and 7-dechlorogriseofulvin no longer to be produced by whc1 growing in liquid medium (Hinterdobler et al. in prep.). A similar change in the metabolite profile was observed for the isolate S1. This strain produced three substances with similar UV spectrum but different retention times growing on MEA2. Cultivated under fermentative conditions in liquid medium (ME), these metabolites were no longer detectable and therefore the extract not further fractionated (data not shown).

For comparative analysis of plant and fungal secondary metabolites, fungal cultures and the corresponding host plant organs were analyzed by HPLC. Subsequently, plant organ extracts were cross checked for the production of substances known from fungal cultures. None of the fungal metabolites produced in culture were found in the plant crude extracts.

Analyses of aerial guttation droplets

Guttation droplets were collected from four fungal isolates (whc1, D1, L1, W2) and their secondary metabolite composition was qualitatively compared to the corresponding fungal crude extracts (Fig. 3). Griseofulvin and 7-dechlorogriseofulvin, produced by these strains growing on the agar medium MEA1, were deposited in the guttation droplets. In addition, hydrophilic medium ingredients and the antibiotic additive chloramphenicol were also identified as part of the guttation liquid.

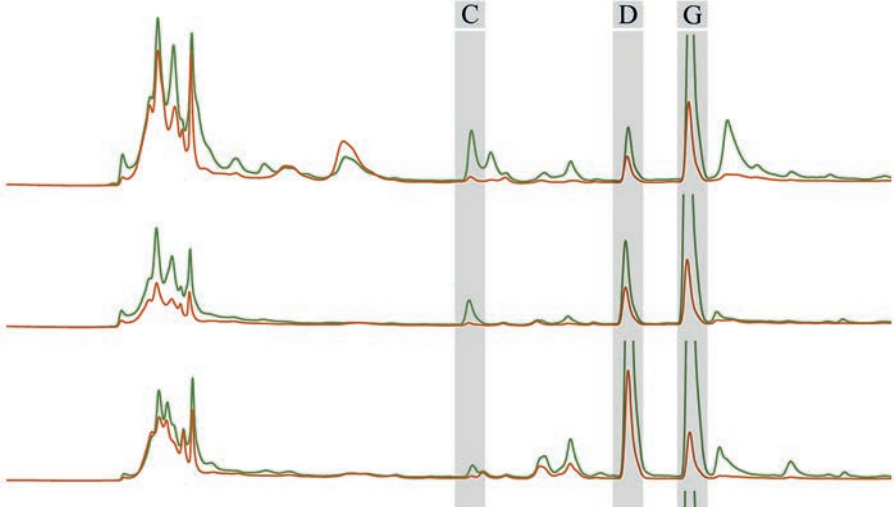

Fig. 3: Qualitative comparison of HPLC chromatograms at 230 nm of collected guttation droplets (orange) and the crude extracts of the corresponding fungus (green). From top to bottom: whc1, D1, L1, W2. Pile of peaks on the left represents hydrophilic components from the growth medium MEA1. Highlighted peaks: Chloramphenicol (C), 7-dechlorogriseofulvin (D) and griseofulvin (G). – Abb. 3: Vergleichende HPLC Chromatogramme von Guttationstropfen mit den mit den Rohextrakten der korrespondierenden Pilze. Von oben nach unten: whc1, D1, L1, W2. Markierte Peaks entsprechen Chloramphenicol (C), 7-Dechlorogriseofulvin (D) und Griseofulvin (G).

Bioassays

Metabolites that are constitutively sequestered into the growth medium were tested for their anti-microbial effects on *Escherichia coli* and *Bacillus subtilis*. Additionally, a subset of fungi was tested against *Saccharomyces cerevisiae* and on *Candida albicans* (Tab. 2). Due to further diffusion into the bacterial growth medium, a zone of inhibition was visible around the agar plug if an antibiotic substance was present. For *E. coli*, only the fungus C5 showed bacterial growth inhibition around the plated agar plug. The growth of *B. subtilis* was inhibited by seven fungal isolates. The isolates I8 and N1 inhibited growth of *S. cerevisiae* whereas none of the tested strains showed inhibitory effects against *C. albicans*.

Tab. 2: Fungal isolates with anti-microbial effects on applied test organisms. – Tab. 2: Pilzisolate mit antimikrobieller Wirkung gegen die verwendeten Testorganismen.

		Microbial test organisms			
Fungal isolates		***E. coli***	***B. subtilis***	***S. cerevisiae***	***C. albicans***
C5	*Xylaria* sp.	x	-	-	
E3	*Xylaria* sp.	-	~~X~~		-
I3	-	-	~~X~~		-
I8	*Arthrinium arundinis*	-	-	x	
I9	*Fusarium proliferatum*	-	x	-	
N1	-	-	-	x	
R5	*Clonostachys* sp.	-	x	-	
S1	*Fusarium proliferatum*	-	x	-	
T2	*Arthrinium arundinis*	-	x	-	
W8	*Colletotrichum* sp.	-	~~X~~		-

No inhibition (-), inhibition growing on MEA2-C (x) and MYEA (~~X~~) medium
Keine Aktivität (-), Aktivität, bei Wachstum auf MEA2-C (x) und MYEA(~~X~~) Medium

Discussion

Richness and organ specificity of endophytic fungi

Multiple fungal species exist side by side inside the plant tissue. In order to increase the chance of harvesting a high diversity of cultivable strains, repetition of the isolation process is necessary. Faster-growing or more competitive species dealing best with the offered medium, tend to overgrow slower-growing or later-emerging ones. In order to overcome this phenomenon, selective isolation media containing fungal growth-inhibiting substances were applied, as used for the isolation and *a priori* classification of pathogenic fungi (Tsao 1970).

The inhibitory effect of these additives favors a subset of resistant, stress-tolerant or slow-growing fungi, increasing the species output. Beside this well-known technique, the plant fragment size for isolation also influences recovered fungal diversity. The reduction of fragment size comes with an increased cutting margin where the hyphae have access to the provided medium. It is estimated that one leaf fragment of 4 cm² harbors half of the leaf-inhabiting fungal diversity (Gamboa et al. 2002). With the used fragment size and repetition of the process in the present work, 1.35 fungal strains per plant sample were isolated. Gamboa & Bayman (2001) described an isolation frequency of 1.4 fungal species per sample for *Guarea guidonia* (L.) Sleumer (Meliaceae) with 20 mm² fragments and increased the frequency to 2.9 by the use of 4 mm² pieces. The time between collection work in Costa

Rica and axenic cultivation of single strains is also considered a major factor influencing the isolation frequency. Growing together in one petri dish, representing an artificial environment, more competitive fungi can overgrow others, leading to a less representative fungal composition. The use of selective media reduced the isolation frequency to a fraction in comparison to the standard medium. The low output indicates a strong suppression of growth for most fungal species emerging on the standard medium. For isolation work in Costa Rica, benomyl and cycloheximide were prior added to the isolation medium. Benomyl was shown to inhibit ascomycetous fungi but has minor effects on basidiomycetes (Summerbell 1993). Cycloheximide is an antifungal antibiotic and, like benomyl, is used for fungal classification (Salkin 1975). The successful increase of diversity by the addition of selective agents to the isolation medium depends on the fungal species present as endophytes and the processed plant organ. The latter is argued by Bills & Polishook (1992) as a possible result of higher diffusion of the selective agent into small leaf fragments. Despite an increased isolation of fungal endophytes from *Carpinus caroliniana* Walter (Betulaceae) bark disks using cycloheximide medium, the isolation using cycloheximide and benomyl for *Chamaecyparis thyoides* (L.) Britton, Sterns & Poggenb. (Cupressaceae) leaves hindered fungal growth and isolation (Bills & Polishook 1991, Bills & Polishook 1992). An optimization of the isolation process regarding the plants' properties and different organs might increase the output of cultivable endophytes.

The identified fungal genera and species fit well in the expected diversity of a tropical endophyte community. Fungal species belonging to the Xylariaceae family are regularly encountered endophytes and saprobes in the tropics (Rogers 2000). The genus *Arthrinium* harbors typical endophytic species as well, but also human- and phytopathogens. *Arthrinium* species have further been proven a good source for novel anti-microbial metabolites (Crous & Groenewald 2013, Ramos et al. 2010). *Fusarium proliferatum* on the other hand is a typical crop pathogen but is also found frequently as an endophyte in healthy leaves of various plant species (Stępień et al. 2011). Like *Fusarium*, also *Colletotrichum* species were described as endophytes in *Theobroma cacao* and *Taxus* × *media* (Taxaceae) (Rubini et al. 2005, Xiong et al. 2013). One *Colletotrichum* species similar to the strain W8 was isolated previously from *Trichilia tuberculata* (Triana & Planch.) C. DC. (Meliaceae) from Costa Rica (GenBank accession number KU204655). One isolate could be assigned to the genus *Clonostachys*. This genus harbors also entomopathogenic species that colonize leafhoppers and nematodes (Toledo et al. 2006, Zhang et al. 2008). The relatively small sample size in this study already shows the huge diversity of species and ecotypes that exist side by side in such an extraordinary niche as the inside of the plant body. From the plant's perspective, the genera and species identified here range from real mutualists providing an additional chemical reservoir against pathogens to temporarily silent saprobes and plant pathogens. The endophytic lifestyle provides an advantage for saprobes in early colonization and degradation of dying plant parts. The entomopathogenic fungi use their endophytic state as an effective way to colonize insects feeding on these plants and therefore can also be seen as mutualists against herbivores.

The ubiquity of fungal endophytes encountered in seeds is striking. Vertical transmission of endophytes within plant seeds in contrast to horizontal transmission via spores was highlighted for several herbaceous eudicots. Endophytes found within and on pollen were also collected from seeds, leading to the assumption of an infection of seeds via the pollen tube (Hodgson et al. 2014). Some vertically transmitted fungi have lost their ability to produce spores and thus rely on distribution via the plant (Steiner et al. 2006, Steiner & Leistner 2012). Bacterial endophytes collected from *Psychotria* were also found to be partly transferred vertically (Lemaire et al. 2012a). The early infection of the plant seedling is thought to be a first ecological defense mechanism against pathogens as it was shown for the tropical tree *Theobroma cacao* (Hodgson et al. 2014, Arnold et al. 2003). The expansion of the screening process to anthers and pollen in future studies as well as the controlled infection of *in vitro* cultivated plants with endophytes might give insights into early fungal colonization tendencies and potential protection mechanisms for the seedling.

Identification and distribution of fungal secondary metabolites

The identified griseofulvin is an antifungal secondary metabolite produced by various fungal genera and was first isolated from *Penicillium griseofulvum* (Petersen et al. 2014, Oxford et al. 1939). The so-called *curling factor* produced by *P. janczewskii*, which leads to abnormal growth of hyphae in co-cultured fungi was identified as griseofulvin by Brian et al. (1949). Griseofulvin was isolated from an endophytic *Xylaria* species from *Palicourea marcgravii* A.St.-Hil. and was shown to be active against *Cladosporium cladosporioides* and *C. sphaerospermum* (Cafêu et al. 2005). Furthermore, this compound is produced *in vitro* by *Xylaria cubensis*, an endophyte of *Asimina triloba* (L.) Dunal (Annonaceae) and *Silybum marianum* (L.) Gaertn. (Asteraceae), and by a *Nigrospora* species isolated from *Moringa oleifera* Lam. (Moringaceae) (Sica et al. 2016, Zhao et al. 2012). Piliformic acid is frequently found in xylariaceous fungi and close relatives (Chesters & O'Hagan 1997). It was further identified to be part of the chemical profile of one *Xylaria* strain isolated from mangrove trees in South China and the marine fungus *Halorosellinia oceanica* from Thailand (Liu et al. 2006, Chinworrungsee et al. 2001). Cytochalasin D is another well-known fungal metabolite with actin-polymerization-inhibiting properties (Casella et al. 1981). Cytochalasins are produced by a wide range of ascomycetous and basidiomycetous fungal genera (Scherlach et al. 2010). Cytochalasin derivatives have been isolated from various fungal endophytes belonging to the genera *Aspergillus*, *Chaetomium* and *Xylaria* (Lin et al. 2009, Ming Ge et al. 2008, Espada et al. 1997). The cytochalasin D described here was identified in one *Tubercularia* isolate from *Taxus mairei* (Lemée & H.Lév.) S.Y.Hu (Taxaceae) and one *Xylaria* species found in *Palicourea marcgravii* (Li et al. 2009, Cafêu et al. 2005).

Severe media-dependent changes in the metabolite pattern were observed in the fungal strains whc1 and S1. Paranagama et al. (2007) reported a shift from the production of the polyketide chaetochromin A in liquid medium to radicicol as the main metabolite produced on solid medium by *Chaetomium chiversii*. Furthermore, an addition of six substances to the metabolic profile of *Paraphaeosphaeria quadriseptata* was observed by adding tap water instead of distilled water to the medium. The screening for a broad fungal metabolite spectrum by a systematic change of culture conditions is highlighted in the OSMAC approach (one strain many compounds) by Bode et al. (2002). Using this approach, novel substances were described for *Streptomyces* species, sponge-associated and endophytic fungi (Rateb et al. 2011, Christian et al. 2005, Hewage et al. 2014). Considering the effects of slight modifications of the medium composition, oxygen availability or temperature to the production of metabolites, the output of novel structures can be increased at a small scale. Regarding the high variability of fungal secondary metabolite production *in vitro*, the presented results are viewed as preliminary due to limited variation of media applied.

The total loss or appearance of single substances in the metabolite profile gives a hint to the vast, unseen biochemical potential of these fungi.

Analysis of aerial guttation droplets

The excretion of liquid exudates of basidiomycetous fruiting bodies in their natural habitat is known to most mushroom collectors and connoisseurs. Under axenic *in vitro* conditions some ascomycetous fungal species also tend to produce exudate droplets on top of their aerial mycelium. These sequestrations are well documented in literature and their chemical constituents have been investigated for a variety of fungal species (Gareis & Gareis 2007, Gareis & Gottschalk 2014, Hutwimmer et al. 2010). In analogy to a similar plant phenomenon, these excretions are called guttation droplets. Regarding their function, external storage of secondary metabolites and water to cope with the unfavorable environment of the growth media were discussed (McPhee & Colotelo 1977, Jennings 1991). The presence of griseofulvin in guttation droplets at an even higher concentration than in the mycelium was observed by Sica et al. (2016) for *Xylaria cubensis*. External storage of antifungal substances like griseofulvin might be used as a backup defense system to hold on to the colonized, limited medium. The medium ingredients present in the droplets seem to be a byproduct of the sequestration of water. The occurrence of small amounts of chloramphenicol in the droplets is direct evidence that this substance is taken up by the fungus. This raises the question of whether the use of chloramphenicol in the growth medium influences growth or even secondary metabolite production. However, the deposition of chloramphenicol indicates that it is not entirely metabolized until it reaches the guttation droplets. Its function as a trigger for substance production or being partly used as substrate cannot be excluded.

Screening for anti-microbial effects

Endophytic fungi have been reported as a promising source for anti-microbial compound isolation (Mousa & Raizada 2013). In total, 9.8 % of the tested fungal isolates showed growth-inhibiting effects against one of the applied test organisms. Antibacterial activity against *B. subtilis* was earlier reported for one *Xylaria* strain isolated from *Psychotria bisulcata* Wight & Arn. (Govinda Rajulu et al. 2013). Regarding the close interaction of endophytes with their host and other microorganisms residing within the same limited space, the ability to produce antibacterial and antifungal substances is essential. The defense of the colonized fungal habitat comes hand in hand with an increased defense of the host plant against pathogens (Arnold et al. 2003). These results reveal fungal endophytes from *Psychotria* and *Palicourea* species as a promising source for the discovery of antimicrobial compounds. As mentioned before, secondary metabolite production is highly dependent on the applied cultivation medium. The agar plug diffusion assay is a basic assay for the evaluation of *a priori* produced defense chemicals. As the active principle of the inhibition is not known, this screening underlines the potential for the discovery of antimicrobial substances from the examined endophytes but leaves open further questions on the structure and media-dependency of the involved metabolites.

Evaluation of applied methods

Research on endophytic fungi is highly dependent on the methods used for isolation and cultivation. Several surface sterilization procedures using sodium hypochlorite (NaOCl), ethanol or formaldehyde for plant samples have been evaluated and proven to be suitable for endophyte isolation by Schulz et al. (1993). Tropical environments are known for their commonly occurring epiphytic mosses, ferns and algae. Considering the highly colonized surfaces of tropical plants, a rather strong sterilizing protocol was used for the isolation process in Costa Rica. Highly sensitive endophytes might be harmed also inside the plant tissues by the applied disinfectants. This effect has to be taken into account regarding the cultivated endophytic diversity observed after isolation. The diversity is also strongly affected by the ability of the present fungi to grow on the isolation media thus leading to many fungal strains and species staying undetected if the given medium does not fit their needs (Schulz & Boyle 2005). Beside these limitations of the isolation process, one must reconsider the definition of endophytes. In this study, all fungi growing within the sampled, healthy plant organ are considered endophytes. This may also include latent pathogens which do not trigger any visible virulence symptoms in the plant at the moment of harvesting. The used antifungal additives in the selective isolation media were chosen based on availability and literature search. Besides cycloheximide, benomyl and the antibacterial chloramphenicol, several other antifungal and antibacterial additives (e.g. cyclosporine A, natamycin and rose bengal) are commonly used for pre-selective isolation of fungal endophytes (Stone et al. 2004). Regarding fungal endosymbionts, the use of antibiotics in the isolation and cultivation medium can lead to a loss of endohyphal bacteria in fungal cultures (Hoffman & Arnold 2010) and thereby further influence the production of secondary metabolites *in vitro*.

Experimental

Plant and fungal material

Plant and fungal material was collected during the course of a field trip to the Golfo Dulce Region of Costa Rica in November and December 2015. The tropical lowland forest of the *Piedras Blancas* national park around the field station *La Gamba* of the University of Vienna was selected as an ecologically intact area for collection work (Weber & Baumgartner 2001). The nearby laboratory facilities were used for fast processing of the collected material. In the course of three weeks, 15 individuals of eight *Psychotria* and *Palicourea* species were successfully sampled. Altogether 102 fungal strains were isolated and further cultivated. For each plant individual collected, two specimens as well as leaves, shoots and – if present – inflorescences were sampled. For the purpose of later phytochemical analysis, the plant material was dried at room temperature using an air dehumidifier. For fungal endophyte isolation, whole, apparently healthy leaves, central and basal grown branches and – if available – fruits were placed in clean plastic bags until further processing. Plant specimens were deposited in the herbarium of the University of Vienna (WU) and in the national herbarium of Costa Rica (CR) – one copy in each.

Isolation and cultivation of endophytic fungi

Fungal endophytes were isolated the same day of collection of plant material. Plant samples were washed under running tap water and obvious dirt, epiphytic mosses and algae cautiously removed. Pieces of approximately 3 cm of shoot and 2 cm^2 of leaves including the midrib were prepared for surface sterilization. Seeds were extracted from the fruits and cleaned with tap water and paper tissues. Samples were surface-sterilized by immersion in 70 % ethanol for 1 min, followed by 5 min 3 % NaOCl solution and again 70 % ethanol for one min to remove excess hypochlorite solution. To guarantee successful surface sterilization, shoots, seeds and fruits were rolled over and both sides of the leaf cuttings were imprinted on petri dishes containing the isolation medium MEA3, respectively (Petrini, 1984). Petri dishes with imprints were incubated at room temperature. If no fungal colonies were visible after one week, sterilization was considered successful. After surface sterilization, margins of leaf and shoot samples damaged by NaOCl were removed using sterile razor blades. Shoots, fruits and seeds were divided lengthwise and plated with the cut surface onto the isolation medium. For each collected plant organ, several pieces were used for isolation (one per petri dish). Petri dishes were stored in darkness at room temperature. All isolates linked to a positive imprint test were disposed.

The standard cultivation media contained 20 g/L glucose, 2 g/L peptone, 12 g/L agar, 100 mg/L chloramphenicol and 20 g/L malt extract for MEA1 and 5 g/L malt extract for MEA2. For anti-microbial screening, the MEA2 recipe without chloramphenicol (MEA2-C) and one with additional 0.5 g/L of yeast extract (MYEA) were used. The medium for isolation of endophytes in Costa Rica (MEA3) was low in nutrients for slow growth regarding the transportation time: 10 g/L malt extract, 20 g/L glucose, 1 g/L peptone, 12 g/L agar and 100 mg/L chloramphenicol. The liquid medium for fermentative cultivation (ME) contained 20 g/L malt extract, 38 g/L glucose, 1.25 g/L peptone and 100 mg/L chloramphenicol. For the selective isolation media, 4 mg/L benomyl and 200 mg/L cycloheximide were added to the MEA3 recipe. Benomyl was added to the medium after autoclaving and cooling down to approximately 50° C (Hutchison 1990, Summerbell 1993). A stock solution dissolved in acetone was prepared and added to the medium after sterile filtration through a 0.22 µm syringe filter. Excess acetone was evaporated under lamina flow. Emerging fungi from plated plant samples were separated by transferring small agar plugs to new petri dishes. Morphologically distinct strains were separated again until isolates were morphologically homogenous. Five fungal isolates were assigned to fermentation after first analytical screenings. Agar plugs with mycelium were cut from cultures on MEA1 and used to inoculate the liquid (ME) medium. S1 and whd4 were cultivated in 280 mL ME medium (2 × 140 mL) in darkness at 27° C for 49 days and additionally in 250 mL medium for 24 days (Hinterdobler et al. in prep.). Whc1 and C4 were cultivated in 280 mL ME medium (2 × 140 mL) in darkness at 27° C for 68 days and additionally in 250 mL medium for 43 days (ibid.).

Species identification

A subset of fungal isolates was identified by Sanger sequencing and comparison to the NCBI database by BLAST search (see also Hinterdobler et al. in prep.). Internal transcribed spacer (ITS) regions of fungal ribosomal DNA were amplified using the standard ITS5 forward primer for fungal isolates C5 and E3, and ITS1F primer for the remaining isolates (I8, I9, R5, S1, T2, W8). ITS4 was used as reverse primer. Consensus sequences were generated in SeqMan Pro 14 (DNASTAR®). Fungal DNA was extracted with the help of Dr. Alexander Urban and sequenced at the Division of Systematic and Evolutionary Botany, University of Vienna.

Secondary metabolite analysis and extraction

For isolation procedure of whc1 and whd4 crude extracts see Hinterdobler et al. (in prep).

Agar plug diffusion assay

Fungi were grown on MEA2-C or MYEA medium until full colonization. Agar plugs of 5 × 5 mm were transferred to petri dishes covered with test organisms. Results of the screenings were evaluated the next day. Endophytic fungi were tested against *Bacillus subtilis*, *Escherichia coli* DH5α, *Candida albicans* and *Saccharomyces cerevisiae*. The first selected subset of 60 fungal strains was chosen by their ability to produce HPLC-DAD detectable substances in culture and good growth. For the screening of the remaining fungal isolates, *S. cerevisiae* was replaced by *C. albicans*. The change of growth medium from MEA2-C to MYEA was due to a possible increase of secondary metabolite production by the addition of yeast extract to the medium (Zotchev pers. comm.). Tests were performed in the laboratory of Dr. Sergey B. Zotchev at the Department of Pharmacognosy, University of Vienna.

Conclusion

Psychotria and *Palicourea* species growing in Costa Rica were shown to be a rich source for fungal endophyte research. The area of sampling at the Pacific side of Costa Rica provides a suitable habitat for fungi of all kinds (Weissenhofer & Huber 2008, Piepenbring & Ruiz-Boyer 2008). The results showed that endophytic fungi occur in leaves, shoots, fruits and seeds of the studied plant species. The identified endophytic fungi belong to the frequently isolated genus *Xylaria* (Xylariaceae) and related genera. Production of plant-like secondary metabolites in fungal cultures could not be observed during this study but the isolated fungal endophytes have the ability to produce anti-microbial defense chemicals against applied test organisms. These defense chemicals could not be detected in previous studied plant material (Berger et al. 2012, 2016, 2017). Further studies are required to illuminate the symbiotic interactions between plants and their endophytes.

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Taxonomical and phytochemical diversity of Costa Rica Palicoureeae and Psychotrieae (Rubiaceae)

Andreas Berger & Johann Schinnerl

Species of the large and complex coffee family (Rubiaceae) are frequent elements in any tropical rainforest. The two hyperdiverse sister tribes Palicoureeae and Psychotrieae represent some of the largest radiations within the family as well as in Costa Rica. Although these groups are of great ecological and ethnopharmacological importance, there are only few studies dealing with their local diversity, taxonomy and life history traits. Some of these topics are addressed in an ongoing project at the Field Station La Gamba and recent results are briefly summarized here. Using modern generic concepts, initial taxonomic studies of Central American representatives revealed that a large number of species traditionally placed in *Psychotria* actually belong to the genus *Palicourea*, which necessitated several new nomenclatural combinations. In the new circumscription, *Palicourea* becomes one of the largest plant genera and includes prominent species such as the 'kissing lips' (*P. elata*, *P. tomentosa*), which are easily spotted in the forests around La Gamba. Extensive floristic studies and herbarium revisions revealed the occurrence of six genera and 45 species in the Piedras Blancas National Park and the adjacent Golfito National Wildlife Refuge. Subsequent studies have addressed the phytochemical diversification and differentiation of both tribes and genera, which is of putative importance in defending plants against herbivores and pathogens. Interestingly, each genus shows a characteristic chemical composition with various types of indole alkaloids as chemosystematic markers. In addition, many new alkaloids such as the structurally remarkable lagamboside were first described from plants collected in this region.

Berger A. & Schinnerl J., 2019: Taxonomische und Phytochemische Diversität von Palicoureeae und Psychotrieae (Rubiaceae).
Arten der großen und taxonomisch komplexen Familie der Kaffeegewächse (Rubiaceae) sind in tropischen Regenwäldern häufig anzutreffen und stellen einen wichtigen Anteil der pflanzlichen Biodiversität dar. Eine der artenreichsten Gruppen dieser Familie sowie der Flora von Costa Rica stellen die extrem diversen Schwestergruppen Palicoureeae und Psychotrieae dar. Obwohl deren Arten von großer ökologischer und ethnobotanischer Bedeutung sind, ist vergleichsweise wenig über deren lokale Diversität, Taxonomie und phänotypische Merkmale bekannt. Um einige dieser Lücken zu schließen, wird diese Gruppe im Rahmen eines laufenden Projektes an der Tropenstation La Gamba ausführlich untersucht, und einige Ergebnisse werden im Folgenden kurz zusammengefasst. Taxonomische Studien konnten zunächst zeigen, dass zahlreiche Arten, welche traditionell zu *Psychotria* (Psychotrieae) gestellt wurden, phylogenetisch zur Gattung *Palicourea* (Palicoureeae) gehören. In ihrer neuen bzw. modernen Umschreibung wird *Palicourea* somit zu einer der größten Pflanzengattungen weltweit, und beinhaltet einige prominente costa-ricanische Arten, wie die in der Gegend von La Gamba häufig vorkommenden 'kissing lips' (*P. elata*, *P. tomentosa*), welche durch ihre leuchtend roten Hochblättern sehr auffällig sind. Umfangreiche Feldarbeit in Kombination mit Herbariumsstudien konnten bisher 45 Arten aus sechs Gattungen der Palicoureeae und Psychotrieae für den Piedras Blancas Nationalpark und das Benachbarte Golfito National Wildlife Refuge nachweisen. Phytochemische Studien an diesen Arten ergaben schließlich eine bemerkenswerte chemische Differenzierung einzelner Arten, welche in Bezug auf Herbivorie und Verteidigung gegen Pathogene von großer Bedeutung sein kann. Jede der untersuchten Gattungen und Triben besitzt hierbei charakteristische Sekundäre Inhaltsstoffe, wobei verschiedene Klassen von Indolalkaloiden sich als wichtige chemische Merkmale erwiesen haben. Im Zuge dieser Untersuchungen wurde zudem eine Vielzahl neuer Verbindungen beschrieben, von denen einige, wie das nach der Tropenstation benannte Alkaloid Lagambosid, außergewöhnliche strukturelle Eigenschaften besitzen.

Keywords: *Palicourea, Psychotria,* Rubiaceae, chemosystematics, chemodiversity, phytochemistry.

Taxonomy of the *Psychotria* alliance – an introduction

The scientific study of Costa Rican Rubiaceae – better known as the coffee-family – follows a long tradition. Not least due to the establishment of the La Gamba Field Station in the Piedras Blancas National Park, Puntarenas Province, part of these studies are connected to the University of Vienna. The diversity of previous research projects spans from cytological studies (Kiehn 1995, Kiehn 2010, Kiehn & Berger under review), floristic surveys (Will & Kiehn 2008) to pollination ecological studies (Csekits 2008). Since the first visit to La Gamba by Harald Greger and Martin Bernhard in 2008, our "Chemodiversity Research Group" has contributed to the study of this fascinating family, and will continue to do so in the future.

The focus of our studies are the two sister tribes Psychotrieae and Palicoureeae, herein termed as the *Psychotria* alliance. This hyperdiverse group is the largest radiation within the family Rubiaceae, and consists of more than 3,100 closely related and morphologically similar species. In the Neotropics, the group is currently subdivided into the genera *Carapichea*, *Eumachia*, *Geophila*, *Notopleura*, *Palicourea*, *Psychotria* and *Rudgea*, and these are placed in two sister tribes Psychotrieae (*Psychotria*) and Palicoureeae (all others) (Razafimandimbison et al. 2014). The whole group is renowned for its ethnobotanical importance (e.g. Rivier & Lindgren 1972) and species richness including one of the largest genera of higher plants, *Psychotria*. Furthermore, some species such as the stunning kissing lips plants *Palicourea elata* (Sw.) Borhidi (syn. *Psychotria elata* (Sw.) Hammel) and *P. tomentosa* (Aubl.) Borhidi (syn. *Psychotria poeppigiana* Müll. Arg.) feature prominently among tourists and naturalists (Fig. 1).

Most species of the alliance are evergreen shrubs and treelets, and contribute an important part to rainforest understory diversity and biomass (Gentry 1990). Their fruits provide an important food source for frugivorous birds (Snow 1981), and hundreds of Lepidopteran species were observed feeding on them (see below; Janzen & Hallwachs 2018). Other-

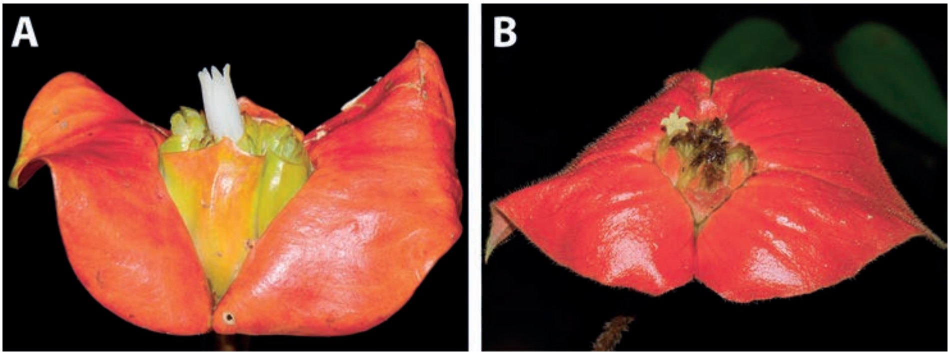

Fig. 1: Inflorescences of *Palicourea elata* (A) and *Palicourea tomentosa* (B, both Palicoureeae), two common shrubs or treelets in the Piedras Blancas National Park, Costa Rica. Both feature showy, red, involucral bracts advertising flowers to pollinators. Note differences in bract pubescence and flower color, which distinguish these two frequently confused species. Photos: A. Berger. – Abb. 1. Blütenstände von *Palicourea elata* (A) und *Palicourea tomentosa* (B, beide Palicoureeae), zwei häufige Sträucher bzw. Kleinbäume im Piedras Blancas Nationalpark, Costa Rica. Beide besitzen auffällige rote Brakteen, welche zur Anlockung von Bestäubern dienen. Beide Arten werden oft verwechselt, unterscheiden sich jedoch in Behaarung und Blütenfarbe. Fotos: A. Berger.

wise, little is known about their natural history, phylogeny and trait diversity, but many species are diverse in chemistry, accumulating alkaloids, flavonoids and iridoids of various kinds (e.g. Berger 2017b). In addition, some species were shown to be toxic to mammals (Cook et al. 2014) and to trigger antifeedant activities in insect pests in laboratory studies (see below), leading one to expect some degree of functional importance of their chemical composition.

Changing generic concepts

Palicourea and *Psychotria* have long been classified in the tribe Psychotrieae. Recent DNA-phylogenetic studies in combination with a re-evaluation of morphological characters have finally challenged the long-standing circumscription of the genus *Psychotria* and the tribe Psychotrieae. Views shifted towards a narrower concept of both groups, which led to the establishment of the new tribe Palicoureeae and the ongoing transfer of hundreds of species to various segregate genera now placed in the Palicoureeae (e.g. Berger 2018, Nepokroeff et al. 1999, Razafimandimbison et al. 2014). For species belonging to the rather small genera *Carapichea*, *Eumachia* and *Notopleura*, corresponding species have already been identified and transferred (Taylor & Gereau 2013, Taylor et al. 2017, Taylor 2001). Taxonomic problems remain with the delimitation of the large genus *Palicourea*, which is now understood to include *Psychotria* subg. *Heteropsychotria*, necessitating major nomenclatural changes.

Species of both groups cannot be distinguished by vegetative or fruit characters and deviate only in a set of traits associated with pollination syndromes (e.g. Taylor 1996). The traditional concept of the genus *Palicourea* included all species adapted for hummingbird-pollination. Usually, these have open and long-pedunculate inflorescences with colored inflorescence axes and vividly colored corollas possessing a long tube and a gibbous nectar-accumulating base. By contrast, species of subg. *Heteropsychotria* included only bee- and hawkmoth-pollinated species. These usually have grouped or densely capitate flowers, born on inconspicuously colored inflorescence axes and often show conspicuous floral or involucral bracts. In bee-pollinated species, the corollas possess a short and straight tube, whereas these are white and long-tubed in moth-pollinated species (e.g. Taylor 1996). Pollination syndromes in the group are now understood to have evolved repeatedly and do not show much phylogenetic signal (e.g. Nepokroeff et al. 1999, Razafimandimbison et al. 2014).

In order to render both *Palicourea* and *Psychotria* monophyletic groups, species of *Psychotria* subg. *Heteropsychotria* need to be identified and transferred to an expanded *Palicourea*, which is the oldest available name for the group. The combined group is variable in floral characters, but well supported by vegetative and fruit characters, DNA-phylogenetic data and accumulation patterns of secondary metabolites (see below).

A new circumscription of *Palicourea* and *Psychotria*

As a prerequisite of any study on plant diversity, ecology and traits such as secondary metabolites, the generic position of each species of *Psychotria* needs to be ascertained according to morphological characters proven to be phylogenetically informative (see above). Fortunately, the genera *Palicourea* and *Psychotria* are readily differentiated by a set of such characters.

1) Leaves of the genus *Palicourea* show a greenish drying color, in contrast to a reddish-brown or blackish color in *Psychotria*. 2) In *Palicourea*, the stipules are persistent and usually fused to form a sheath that bears two lobes or awns on each side. In contrast, stipules in *Psychotria* are interpetiolar, triangular and caducous. 3) Fruits of *Palicourea* species are metallic blue or purple-black when mature. In contrast, fruits of *Psychotria* usually pass through a yellowish and orange stage, becoming red at maturity. 4) In *Palicourea*, pyrenes show preformed germination slits and seeds without an alcohol-soluble red seed coat pigment, while *Psychotria* largely features the opposite character states.

Diagnosed as such, *Palicourea* includes around 800 species and these are found from Southern Florida to Northern Argentina as well as in the Greater and Lesser Antilles (e.g. Razafimandimbison et al. 2014). For Central American species, the respective combinations transferring species of *Psychotria* subg. *Heteropsychotria* to *Palicourea* have recently been provided by a number of studies (e.g. Berger 2017a, Berger 2018, Borhidi 2017, Taylor et al. 2010, Taylor 2015). Hence, these studies have established a taxonomic framework for studying plant traits such as in the field of phytochemistry.

Floristic diversity in the Piedras Blancas National Park

Due to the progress in the delimitation of the genus *Palicourea* in respect to *Psychotria*, it is finally possible to assess the Costa Rican diversity of the large tribes Psychotrieae and Palicoureeae in a modern circumscription. Whilst the *Manual de Plantas de Costa Rica* (Taylor 2014) lists 44 species of *Palicourea* for Costa Rica, the recent enumeration of Central American *Palicourea* (Berger 2018) has expanded the number of species to 91. Consequently, *Palicourea* now outnumbers *Psychotria* with 45 described species and becomes one of the largest plant genera in Costa Rica.

Whilst plant diversity of the country level is sufficiently well known due to centuries of floristic inventories in the context of the *Flora Costaricensis*, the *Manual de Plantas* and others, data on the regional biodiversity is scarcer. The Golfo Dulce region in Southern Costa Rica harbors one of the last remnants of tropical lowland rainforest in Central America, but has long suffered from limited research infrastructure. Weber et al. (2001) were the first to publish a checklist and an introductory field guide for the Golfo Dulce area. Furthermore, they showed that the region harbors one of the highest plant diversities in Central America as well as worldwide. In addition, Will & Kiehn (2008) published a first checklist of Rubiaceae occurring in the region. Whilst the flora of the Osa Peninsula and its world-famous Corcovado National Park is comparably well studied and provides the bulk of floristic data for the above-mentioned publications, few studies have yet dealt with the Piedras Blancas National Park and the Esquinas Rainforest, in which the Field Station La Gamba is located.

With the aim to fill the gap and to provide data for a planned *Flora of the Piedras Blancas National Park*, extensive fieldwork and herbarium studies have been performed. Six field trips to the La Gamba Field Station and the Esquinas Rainforest have yielded many interesting floristic records. In addition, hundreds of herbarium specimens from decades of collection efforts at La Gamba were revised and databased in two herbaria: specimens from the herbarium of the Biologiezentrum Linz (LI) are accessible via their database ZOBODAT (https://www.zobodat.at). Specimens from the herbarium of the University of Vienna (WU) are accessible using the international herbarium database system JACQ (https://herbarium.univie.ac.at).

Although fieldwork and herbarium studies for the *Flora of the Piedras Blancas National Park* are far from complete, the study of several large families is almost finished and respective data is available online. As a focus of our research group, this applies particularly to the rubiaceous tribes Psychotrieae and Palicoureeae. To date, six genera and 42 species of the group are known from the Piedras Blancas National Park. The list is supplemented by three additional species reported from around Golfito, which is located just a few kilometers outside the limits of the national park and is likewise protected by the adjacent Golfito National Wildlife Refuge. Due to similarities in habitat characteristics, all of these species are expected to be found in the Piedras Blancas National Park upon further fieldwork.

In total, this raises the number of species known or expected in the Piedras Blancas National Park to 45, which corresponds to about 26 % of the overall Costa-Rican diversity in the group (Taylor 2014). An updated checklist and selected voucher specimens are presented in table 1. Three species have only been recorded once, and the occurrence of *Palicourea brachiata* appears doubtful and needs confirmation by further fieldwork.

Chemodiversity and chemosystematic studies

Due to a growing interest in the discovery of bioactive secondary metabolites and new leads for drug development, studies on chemodiversity and chemosystematics are of increasing importance in providing such data. It is important to note that, from a plant's perspective, these metabolites are not at all secondary and have not evolved to combat human diseases, but are of utmost importance for plant defense and survival in challenging ecosystems (see below). Hence, one should expect a non-random distribution of these compounds across the plant kingdom. The study of such diversification patterns is called chemosystematics. Using recent phylogenetic studies as a backbone for the interpretation of phytochemical data, our working group has focused on studying the chemical diversity of Costa Rican Psychotrieae and Palicoureeae.

Methodology

The study of secondary metabolites requires a highly interdisciplinary approach and a combination of fieldwork and various laboratory techniques. Briefly summarized, plants were collected throughout Costa Rica, identified and vouchered for permanent documentation. The respective specimens were stored in the herbaria of the Museo Nacional de Costa Rica (CR), the Missouri Botanical Garden (MO) and the University of Vienna (WU). For phytochemical analyses, plants were separated into different organs (leaves/stem/roots), air-dried, ground and extracted with methanol (2 × 3 d) at room temperature. After filtration and removal of the solvent in vacuo, comparative profiling of the crude methanolic extracts was done by thin layer chromatography (TLC) and high performance liquid chromatography (HPLC) coupled with UV diode array detection.

Using retention time and UV spectra, the latter technique allows peaks to be assigned to compound classes or known compounds. For identification of unknown peaks, various chromatographic techniques were used to isolate individual compounds for subsequent structure elucidation. These techniques include liquid-liquid extraction, column chromatography (CC) with silica gel and Sephadex LH-20 as chromatographic media, medium pressure liquid chromatography (MPLC) and preparative thin layer chromatog-

Tab. 1: Checklist of Palicoureeae and Psychotrieae occurring in the Piedras Blancas National Park and the adjacent Golfito National Wildlife Refuge, Puntarenas Province, Costa Rica. – Tab. 1: Liste der Palicoureeae and Psychotrieae, die im Piedras Blancas Nationalpark und dem angrenzenden Golfito National Wildlife Refuge, Provinz Puntarenas, Costa Rica, vorkommen.

Species	Selected voucher specimen
Carapichea	
Carapichea affinis (Standl.) L. Andersson	A. Berger 1838 (WU!)
Geophila	
Geophila repens (L.) I.M. Johnst. [1]	M. Kiehn et al. MK-920207-1/2 (WU!)
Geophila macropoda (Ruiz & Pav.) DC.	A. Berger 2247 (WU!)
Notopleura	
Notopleura anomothyrsa (K. Schum. & Donn. Sm.) C.M. Taylor	W. Huber & A. Weissenhofer 1579 (WU!)
Notopleura capacifolia (Dwyer) C.M. Taylor	M. Bernhard & H. Greger HG 2907082 (WU!)
Notopleura epiphytica (K. Krause) C.M. Taylor [1]	G. Herrera Chacon 5046 (MO n.v.)
Notopleura polyphlebia (Donn. Sm.) C.M. Taylor	A. Berger 1349 (WU!)
Notopleura uliginosa (Sw.) Bremek.	A. Berger 1317 (WU!)
Palicourea	
Palicourea acicularis (C.M. Taylor) Borhidi	A. Berger 1827 (WU!)
Palicourea acuminata (Benth.) Borhidi	A. Berger 1339 (WU!)
Palicourea brachiata (Sw.) Borhidi [2]	W. Huber & A. Weissenhofer 9 (MO!)
Palicourea calidicola (C.M. Taylor) C.M. Taylor	A. Berger 1812 (WU!)
Palicourea cyanococca (Dombrain) Borhidi	A. Berger 1811 (WU!)
Palicourea deflexa (DC.) Borhidi	A. Berger 1338 (WU!)
Palicourea elata (Sw.) Borhidi	A. Berger 1395 (WU!)
Palicourea glomerulata (Donn. Sm.) Borhidi [2]	G. Induni 204 (MO!)
Palicourea grandifructa (C.M. Taylor) C.M. Taylor	A. Berger 1863 (WU!)
Palicourea guianensis Aubl.	A. Berger AB 16021010 (WU!)
Palicourea hoffmannseggiana (Roem. & Schult.) Borhidi	A. Berger 1763 (WU!)
Palicourea hondensis (Standl.) C.M. Taylor	A. Berger 1849 (WU!)
Palicourea longiinvolucrata A.C. Berger	A. Berger 1418 (WU!)
Palicourea mortoniana (Standl.) Borhidi	M. Kiehn & A. Weissenhofer MK 961119-2/1 (WU!)
Palicourea pilosa (Ruiz & Pav.) Borhidi	A. Berger 1397 (WU!)
Palicourea pseudaxillaris (Wernham) C.M. Taylor	A. Berger 1301 (WU!)
Palicourea racemosa (Aubl.) G. Nicholson	A. Berger 1736 (WU!)
Palicourea solitudinum (Standl.) Borhidi	A. Berger 1392B (WU!)
Palicourea suerrensis (Donn. Sm.) Borhidi	A. Berger AB 16021005 (WU!)
Palicourea tetragona (Donn. Sm.) C.M. Taylor & Lorence	A. Berger 1852 (WU!)
Palicourea tomentosa (Aubl.) Borhidi	A. Berger 1394 (WU!)
Palicourea triphylla DC.	A. Berger 1738 (WU!)
Palicourea tsakiana (C.M. Taylor) C.M. Taylor	M. Bernhard & H. Greger HG 31070812 (WU!)
Palicourea violacea (Aubl.) A. Rich.	A, Berger AB 16021009 (WU!)
Palicourea winkleri Borhidi	A. Berger 1369 (WU!)
Psychotria	
Psychotria chagrensis Standl.	A. Berger 1398 (WU!)
Psychotria chitariana Dwyer & C.W. Ham.	A. Berger 1316 (WU!)
Psychotria costivenia Griseb. [2]	S. Will 87 (MO n.v.)
Psychotria grandis Sw.	W. Huber & A. Weissenhofer 2447 (WU!)
Psychotria limonensis K. Krause [1]	A. Berger 1757 (WU!)
Psychotria marginata Sw.	A. Berger 1410 (WU!)
Psychotria micrantha Kunth	A. Berger 1776 (WU!)
Psychotria panamensis Standl.	A. Berger 2137 (WU!)
Psychotria psychotriifolia (Seem.) Standl.	A. Berger 2249 (WU!)
Rudgea	
Rudgea cornifolia (Kunth) Standl.	A. Berger 1368 (WU!)
Rudgea raveniana W.C. Burger	A. Berger 1420 (WU!)
Rudgea skutchii Standl.	A. Berger 1820 (WU!)

[1] Currently known only from the forests around Golfito, Golfito National Wildlife Refuge, just a few km outside the limits of the adjacent Piedras Blancas National Park. Due to similarities in habitat characteristics, these species are expected to occur in the adjacent National Park as well; [2] currently known only from a single record, and probably in need of confirmation.

raphy (prTLC). Structures were elucidated by our collaborator Lothar BRECKER (Faculty of Chemistry, University of Vienna) by employing various NMR spectroscopic and MS spectrometric techniques.

Chemical characters and generic differentiation

In the course of several years, our group worked on the phytochemistry of many previously unstudied species. This led to the discovery of several new secondary metabolites, some of which possessing new and remarkable structural and functional features (e.g. BERGER et al. 2012, BERGER et al. 2015, BERGER et al. 2017). Together with literature data, these studies enabled new insights into the diversity and evolution of biosynthetic capabilities as putative ecological and defensive agents.

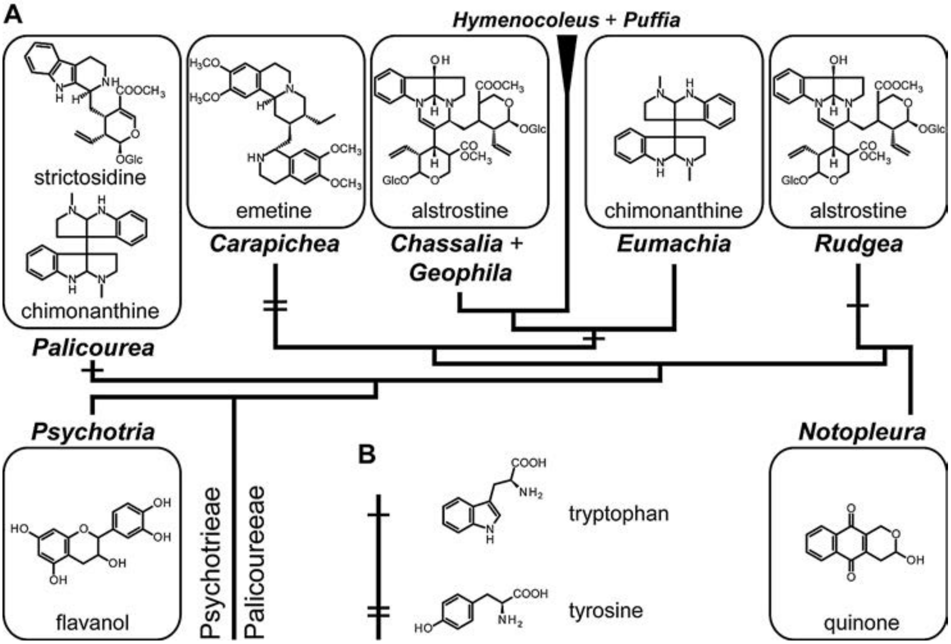

Fig. 2: Major groups of secondary metabolites mapped on a phylogeny of the *Psychotria* alliance (Palicoureeae + Psychotrieae). A: Boxes with representative chemical formulae illustrate the compound classes here understood to characterize each genus. Branches and boxes of lineages showing alkaloid accumulation face upward, others face downward. B: In alkaloid-accumulating clades, crossbars on the branches of the tree indicate the biosynthetic origin of the alkaloid-defining nitrogen, either from the amino acid tryptophan or tyrosine. Note that all compounds have previously been ascribed to the genus *Psychotria*. Graphic from BERGER (2017). – Abb. 2: Großgruppen sekundärer Pflanzeninhaltsstoffe aufgetragen auf einem phylogenetischen Stammbaum der *Psychotria*-Verwandtschaft (Palicoureeae + Psychotrieae). A: Boxen mit Formeln repräsentativer Inhaltsstoffe zeigen die charakteristischen Inhaltsstoffmuster der einzelnen Gattungen. Boxen von Gattungen welche durch Alkaloide gekennzeichnet sind, zeigen nach oben, andere nach unten. B: In Alkaloid-beinhaltenden Gruppen zeigen die Querstriche am Stammbaum die biosynthetische Herkunft des Stickstoffs aus den Aminosäuren Tryptophan oder Tyrosin. Alle hier gezeigten Stoffgruppen wurden früher einer breit gefassten Gattung *Psychotria* zugeschrieben. Grafik aus BERGER (2017).

As hypothesized, accumulation of secondary metabolites is not random within the Palicoureeae/Psychotrieae. Whilst some metabolite classes such as iridoids and flavonoids are widespread across the plant kingdom as well as within the alliance, each genus is chemically characterized by the accumulation of specific compound classes such as alkaloids (see Berger 2017b). Put briefly, alkaloids appear to be restricted to various genera of the tribe Palicoureeae and are almost absent in the Psychotrieae. So far, the only exception is the hallucinogenic *N*,*N*-dimethyltryptamine (DMT) known only from *Psychotria viridis* Ruiz & Pav. (Rivier & Lindgren 1972). Hence, the here-adopted new view of the chemistry of *Psychotria* as a largely alkaloid-free and tannin-accumulating genus is in sharp contrast to the previous understanding of the genus. Simplified accumulation patterns mapped on a phylogeny are shown in Figure 2, and some important genera and classes of secondary metabolites are discussed below.

Strictosidine type-alkaloids in *Palicourea* species

Alkaloids are a diverse class of nitrogen-containing secondary metabolites, and are well known due to their manifold bioactivities and as leads in drug discovery. Alkaloids are found in bacteria, fungi and plants, and include many important compounds such as the antiprotozoic agent quinine, the analgesic drug morphine, the stimulant nicotine or the highly toxic strychnine. Likewise, many alkaloids fulfill important biological functions and play a role in defending plants against herbivores, pathogens or parasites (e.g. O'Connor & Maresh 2006).

Monoterpene-indole i.e. tryptamine iridoid alkaloids are a subclass of alkaloids comprising more than 2,000 structurally highly diverse compounds which are mainly accumulated in members of the order Gentianales, including large and important families such as Apocynaceae and Rubiaceae. Chemically, all these alkaloids are derived from a Pictet–Spengler condensation of the amino acid-derivative tryptamine and the iridoid glucoside secologanin. *In planta*, the reaction is catalyzed by the enzyme strictosidine synthase and leads to strictosidine (**1**), the main precursor of all the other tryptamine-irdidoid alkaloids (O'Connor & Maresh 2006, Panjikar et al. 2012).

Basically, all alkaloids that our group isolated from *Palicourea* species are derived from or are structurally related to strictosidine (Fig. 3), but show considerable variation in the linkage between the iridoid and tryptamine moieties (A), in derivatization of the iridoid or tryptamine moieties (B) or deglucosylation of the iridoid followed by downstream modifications and reorganizations of the whole core (C). Some examples of the respective groups are discussed below.

(A). Alteration in the linkage between tryptamine and secologanin such as via the formation of a six-membered heterocycle leads to strictosamide (**2**) and its derivative deoxostrictosamide (**3**), which was recently described from *Palicourea winkleri* Borhidi (Berger et al. 2017). Structurally, strictosamide (**2**) links to the important anticancer drug camptothecin, first isolated from the Chinese medicinal plant *Camptotheca acuminata* Decne. (Cornaceae).

(B). Several alkaloids show variation in their **tryptamine moiety**. For example, β-carboline alkaloids such as lyaloside (**4**) are frequently found dehydrated derivatives of strictosidine (**1**) and occur, e.g., in *Palicourea acuminata* (Benth.) Borhidi and *Palicourea cyanococca*

(Dombrain) Borhidi (Berger et al. 2017). Other types of derivatization are much rarer, and only few species possess compounds with *N*-alkylations. Examples include palicoside (*N*-methyl strictosidinic acid) from *Palicourea tsakiana* (C.M. Taylor) C.M. Taylor as well as bahienoside B (**5**, *N*-secologanyl strictosidine) from *Palicourea acuminata*. The latter alkaloid possesses a second secologanin moiety leading to the rarely found tryptamine/iridoid ratio of 1:2 (Berger et al. 2012). In addition, the latter species also contains 5-carboxy derivatives such as desoxycordifoline and 5α-carboxystrictosidine (**6**). The occurrence of such compounds indicates that strictosidine synthase found in Rubiaceae has a different substrate specificity than its apocynaceous counterpart in accepting the amino acid tryptophan (Treimer & Zenk 1979), which, however, remains unstudied to date.

Variation in the **iridoid moiety** leads to a small number of derivatives, which feature a loganin instead of a secologanin moiety. Such compounds were first described from South American collections of *Palicourea crocea* (Sw.) Schult. (Düsman et al. 2004). Analyses of several Costa Rican accessions of this species led to the isolation and description of the new

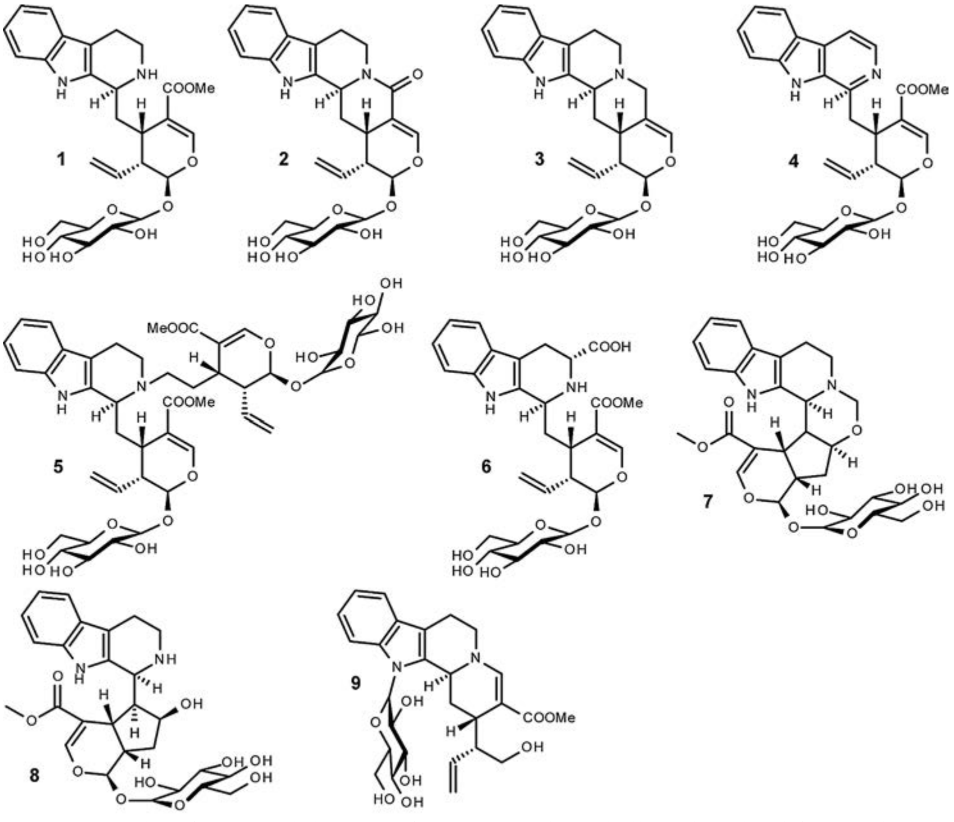

Fig. 3: Examples of structural diversity of tryptamine-derived alkaloids isolated from *Palicourea* species. Strictosidine (**1**), strictosamide (**2**), deoxostrictosamide (**3**), lyaloside (**4**), bahienoside B (**5**), 5α-carboxystrictosidine (**6**), palicroceaine (**7**), brachycerine (**8**), lagamboside (**9**). – Abb. 3: Beispiele der strukturellen Diversität der von Tryptamin abgeleiteten Alkaloide aus Arten der Gattung *Palicourea*. Strictosidin (**1**), Strictosamid (**2**), Deoxostrictosamid (**3**), Lyalosid (**4**), Bahienosid B (**5**), 5α-Carboxystrictosidin (**6**), Palicroceain (**7**), Brachycerin (**8**), Lagambosid (**9**).

alkaloid palicroceaine (7), a derivative of brachycerine (**8**) featuring an unusual hexacyclic core with an additional six-membered heterocyclic ring. Based on these results, we showed that historic herbarium specimens (< 150 yr.) may be used for large-scale HPLC screenings of alkaloid accumulation patterns. Samples from various Caribbean, Central and South America countries were obtained from the herbaria of the Natural History Museum, Vienna (W) as well as from the herbaria MO and WU. These screenings first evidenced the presence of chemotypes in the complex species *Palicourea crocea* and *Palicourea croceoides* Desv. ex Ham., which are probably indicative of cryptic species (Berger et al. 2015).

(C). Upon deglucosylation of strictosidine (**1**) by a dedicated strictosidine β-glucosidase, the iridoid moiety converts to a reactive dialdehyde intermediate, that spontaneously rearranges to precursors leading to an enormous number of complex alkaloids with pronounced bioactivities (De Luca et al. 2014, Fadaeinasab et al. 2015, Zeng et al. 2017). Well-studied examples include the anti-cancer drugs vinblastine and vincristine found in the Madagascar periwinkle *Catharanthus roseus* G. Don (Apocynaceae), the antiarrhythmic agent ajmaline from *Rauvolfia serpentina* (L.) Benth. ex Kurz (Apocynaceae), the anti-malaria drug quinine from *Cinchona* species (Rubiaceae) or alkaloids containing complex cage-structures (e.g. Fadaeinasab et al. 2015, O'Connor & Maresh 2006, Panjikar et al. 2012, Qu et al. 2016).

Lagamboside (**9**) is one of few examples of alkaloids with a rearranged core found in the genus *Palicourea*. The compound was first isolated from *Palicourea acuminata* collected near the La Gamba Field Station and was named after the station (Berger et al. 2012). Besides an unusual *N*-glucosylation, the compound is characterized by a cleaved and modified iridoid ring system. It appears likely that lagamboside originates from the activity of a strictosidine β-glucosidase (Fig. 3), but the respective reactions leading to lagamboside (**9**) remain unstudied. Interestingly, more complex alkaloids appear to be lacking in *Palicourea*, which suggests that downstream rearrangement of strictosidine follows different biosynthetic routes in Rubiaceae and Apocynaceae.

Emetine-alkaloids from *Carapichea* species

The small neotropical genus *Carapichea* (23 species) is well known for including the source-plant of the powerful emetic and antiamoebic drug Ipecac, *Carapichea ipecacuanha* (Brot.) L. Andersson. Apart from this important and well-studied species, the phytochemistry of the genus remains poorly known. The active principles of Ipecac are the alkaloids emetine and cephaeline (**10**), which are structurally classified as tetrahydroisoquinoline alkaloids (e.g. Lee 2008). Biosynthetically, they are derived from a Pictet-Spengler condensation of tyrosine-derived dopamine and secologanin analogous to the formation of tryptamine-secologanin alkaloids (O'Connor & Maresh 2006).

Besides the above-mentioned *C. ipecacuanha*, a second species is found in Costa Rica. *C. affinis* (Standl.) L. Andersson occurs mainly in the Amazon basin, but has disjunct populations in Southern Costa Rica, and in particular in the Golfo Dulce region. Two studies using plants from the Esquinas Rainforest have shown that the species likewise accumulates Ipecac-alkaloids, including a few hitherto unknown derivatives (**10–14**). In addition, these showed pronounced insecticidal activity and may take part in protecting plants against herbivores (Bernhard et al. 2011, Kornpointner et al. 2018). As seen in Figure 4, structural variation is found in the basic structure of the ring system, patterns of methoxylation and the presence of *N*-acetyl or *N*-cinnamoyl groups.

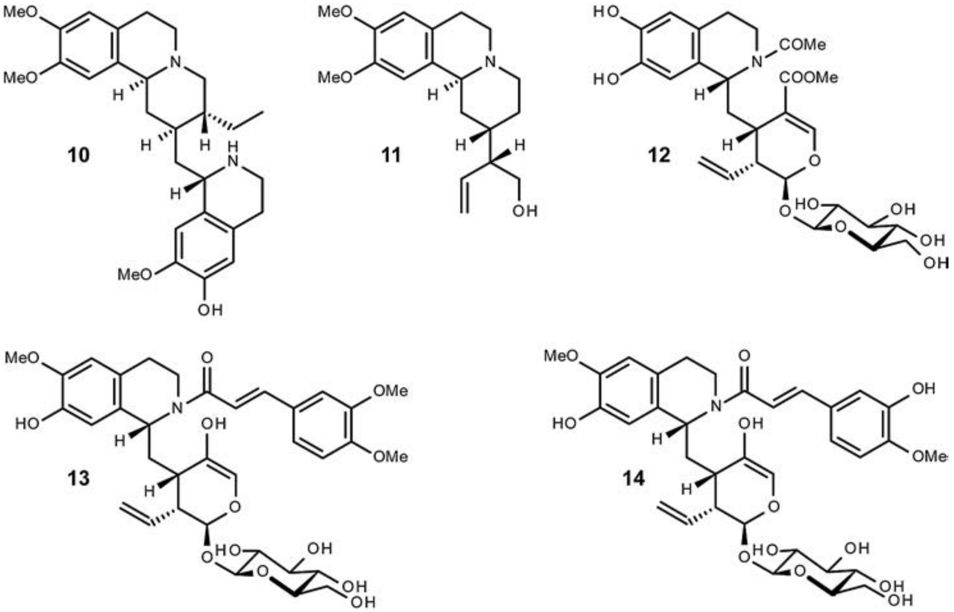

Fig. 4: Structural variation of dopamine-derived alkaloids isolated from *Carapichea affinis*. Cephaeline (**10**), 7-*O*-methyl-alangine (**11**), ipecoside (**12**), borucoside (**13**), 3‴-*O*-demethyl-borucoside (**14**). – Abb. 4: Diversität der von Dopamin abgeleiteten Alkaloide der Art *Carapichea affinis*. Cephaelin (**10**), 7-*O*-Methyl-alangin (**11**), Ipecosid (**12**), Borucosid (**13**), 3‴-*O*-Demethyl-borucosid (**14**).

Tannins from *Psychotria* species

Comparative HPLC analyses of *Psychotria* species revealed a lack of alkaloids that characterize the genera *Carapichea* and *Palicourea* (see above). Instead, chromatograms of all studied species showed a similar broad hump of coalescent peaks with UV spectra similar to that of catechin, a widespread polyphenol and one of the monomers involved in the formation of condensed tannins. These tannins occur as oligo- to polymers with complex linkage patterns and stereochemistry, and are virtually impossible to separate using conventional chromatographic techniques. In order to confirm the assumption of tannin content in *Psychotria*, we first applied elemental analyses of CC-fractions containing these compounds. These revealed the presence of only carbon (C), hydrogen (H) and oxygen (O), which is in accordance with the presence of condensed tannins, and agrees with a lack of alkaloids. Finally, we applied HPLC-MS for identification of these compounds, which likewise showed mass spectra of catechin and its oligomers (data not shown).

Catechins as well as condensed tannins are among the most abundant secondary metabolites and play an important role in defending plants against herbivores. Tannins show deterrent as well as toxic effects, which are caused by reducing protein digestion and by creating reactive oxygen species such as quinones and semiquinone radicals. In addition, costs and trade-offs of such a “carbon-based” defense are considered as minor, when compared to “nitrogen-based” alkaloids, allowing plants to allocate resources to growth instead of defense (Barbehenn & Constabel 2011).

Iridoids and megastigmanes in the *Psychotria* alliance

Terpenes are a large class of lipophilic secondary metabolites derived from C5 isoprene units with ubiquitous distribution in the plant kingdom. Depending on the number of these C5-building blocks, mono- (C10), sesqui- (C15), di- (C20), tri- (C30) and tetraterpenes (C40) are formed. Terpenes fulfill important biological functions such as the attraction of pollinators by monoterpenes in floral scent, or the deterrence of herbivores by mono- and sesquiterpenoids in essential oils. In addition, tetraterpenes such as lycopene and carotene are important plant pigments that are involved in photosynthesis, and advertise fruits to seed-dispersing animals (e.g. Tholl 2015).

Terpenoids are modified terpenes featuring oxygen-containing functional groups which result in hydrophilic properties. Biosynthetically, they are derived from terpenes and constitute one of the largest groups of secondary metabolites. Among the most important terpenoids is a group of monoterpenoids termed iridoids, which were named after a defensive chemical in the secretion of *Iridomyrmex* ants (Cavill et al. 1956). Iridoids are largely characterized by an oxidized bicyclic cyclopenta[*c*]pyran ring, and are often stored as glycosides. Many of them are highly bioactive, act as antimicrobial agents, and have a bitter taste that deters herbivores.

Within Rubiaceae, iridoids are widespread, found in many species, and may be used as chemosystematic markers (Fig. 5). For example, the genus *Ronabea*, recently excluded from the Psychotrieae and placed in the Lasiantheae, was shown to be characterized by iridoids including asperuloside (**15**), 6α-hydroxygeniposide (**16**) and sweroside (**17**; Berger et al. 2011). Within the *Psychotria* alliance, iridoids appear to be of scattered occurrence, and are thus of minor importance when compared to other compound classes such as alkaloids (Berger 2012, 2017, Berger et al. 2017, Gruber 2015). In part, the apparent lack of iridoids may be explained by their incorporation in tryptamine-iridoid alkaloids (see above).

Megastigmanes are likewise scattered within the alliance. Biosynthetically, these are breakdown products of tetraterpenoid carotinoids, but are usually classified as C13 norisopre-

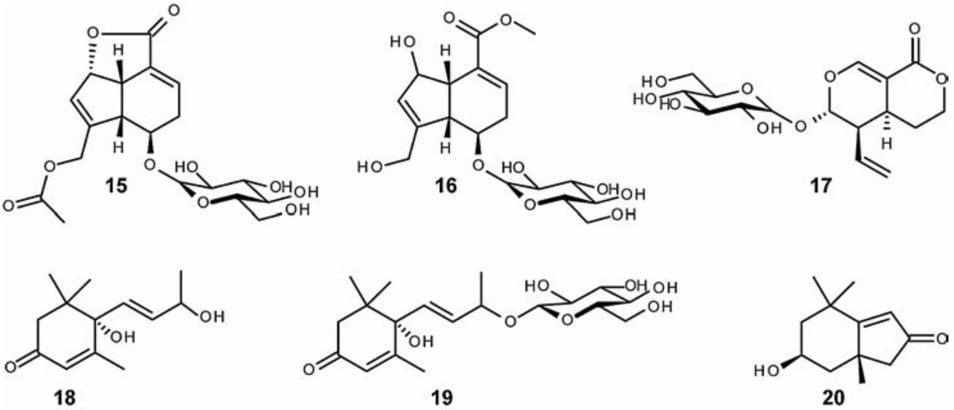

Fig. 5: Iridoids (**15**–**17**) and megastigmanes (**18**–**20**) isolated from various species of the *Psychotria* alliance. Asperuloside (**15**), 6α-hydroxygeniposide (**16**), sweroside (**17**), vomifoliol (**18**), roseoside (**19**), loliolide (**20**). – Abb. 5: Iridoide (**15**–**17**) und Megastigmane (**18**–**20**) aus verschiedenen Arten der *Psychotria*-Verwandtschaft. Asperulosid (**15**), 6α-Hydroxygeniposid (**16**), Swerosid (**17**), Vomifoliol (**18**), Roseosid (**19**), Loliolid (**20**).

noids, many of which are known as important components in wine aroma. Megastigmanes, and especially their glucosides, are widespread and found in many different plant families (Rao 2017). Within several genera of the Psychotrieae and Palicoureeae, megastigmanes such as vomifoliol (**18**), roseoside (**19**) and loliolide (**20**) are found as minor constituents in a few species (Fig. 5). In contrast, these appear to be accumulated in several species of the genus *Notopleura*, which could be of chemosystematic importance (Kostyan 2017). To date, the biological relevance of megastigmane accumulation is unclear, but could likewise be related to defense against herbivores.

Chemical defense against herbivory

Plants have evolved a variety of defense mechanisms to cope with biotic and abiotic threats. Besides phenological and mechanical adaptations such as thorns, trichomes or tough leaves, the production of secondary metabolites is considered one of the most important ways to defend against herbivores and pathogens, thus facilitating survival and reproduction (e.g. Agrawal & Weber 2015). Fieldwork in La Gamba has revealed that species of the *Psychotria* alliance are indeed heavily attacked by a variety of herbivorous insects. Most of these belong to the order Lepidoptera, although leaf-cutter ants occasionally defoliate entire treelets. Two genera of moths constitute the bulk of larvae that were reared or observed feeding on various species in La Gamba: *Desmia* (Crambidae) and *Xylophanes* (Sphingidae). Thus, own observations are in accordance with rearing data from the Guanacaste Conservation Area in northern Costa Rica (Janzen & Hallwachs 2018). Larvae from species of the genus *Desmia* are small, inconspicuous and gregarious leaf-rollers. Their imagines are small black moths with several white spots on the fore and hind wings. In turn, larvae from species of the genus *Xylophanes* are large, often variously coloured, bear conspicuous eye-spots, and feed solitarily on the leaf surface. The large and robust imagines are often green or brown, relatively uniform but with ornamentation such as speckles or streaks on the wings (Fig. 6).

Fig. 6: Late-instar larvae of species of the two singlemost important genera of herbivores feeding on species of the *Psychotria* alliance. A. *Desmia* sp. (Crambidae); B. *Xylophanes chiron* (Sphingidae). Photos: A. Berger. – Abb. 6: Raupen der zwei wichtigsten Herbivorengattungen welche an Arten der *Psychotria*-Verwandtschaft fressen. A. *Desmia* sp. (Crambidae); B. *Xylophanes chiron* (Sphingidae). Fotos: A. Berger.

While studying the effects of plant metabolites in nature is difficult and requires complex experimental setups, laboratory assays such as feeding experiments with model organisms are an easy and well-established method to provide first indications of their effects. In the course of our phytochemical studies, no-choice feeding assays against the cotton leaf worm *Spodoptera littoralis* (Boisduval 1833), Noctuidae – an important agricultural pest insect and model organism – were conducted (Srivastava & Proksch 1991). Briefly summarized, artificial food powder based on beans was spiked with up to 2,500 ppm crude methanolic extracts. After evaporation of the solvent, the spiked food powder was stabilized with Agar-Agar and transferred into a petri dish with ten neonate larvae. After 96 hours of incubation at 26° C and 90 % humidity, average masses and numbers of survivors of the larvae were assessed relative to a control.

The data shows that dopamine-iridoid alkaloid-containing extracts of the genus *Carapichea* are highly toxic to larvae even at very low concentrations (Kornpoitner et al. 2018). By contrast, tryptamine-iridoid alkaloid-containing extracts of *Palicourea* did not exhibit significant effects on the development of caterpillars even at the highest concentrations. In contrast to alkaloids, tannin-containing extracts from *Psychotria* s.str. caused a remarkable growth reduction of approximately 80–90 % in comparison to the control (Berger 2012). These data indicate the relative importance of tannin-content in *Psychotria* when compared to alkaloid glucosides accumulated in *Palicourea* species.

In part, a lack of significant activity of *Palicourea*-alkaloids may be related to their glucosidic nature. Glucosides are often harmless storage forms of otherwise toxic secondary metabolites, as is the case with the famous glucosinolates. These constitute the so-called “mustard oil bomb” defense system and are responsible for insecticidal activity and the characteristic bitter flavor of plants of the mustard family (Brassicaceae). Upon rupture of plant tissue, glucosides come into contact with their dedicated glucosidases and are converted into their active forms, finally exerting toxic activity. According to Guirimand et al. (2010), a similar reaction principle is realized with strictosidine accumulated in *Catharanthus roseus* (L.) G.Don. Upon activation by strictosidine β-glucosidase, a reactive dialdehyde is formed, which is capable of protein cross-linking and precipitation. Extracts used in feeding studies lack glucosidases, and are thus devoid of activity depending on a prior activation.

Conclusion

The obtained phytochemical results indicate a clear differentiation between the studied genera and thus coincide with modern generic concepts: the genus *Palicourea* is characterized by accumulating tryptamine-iridoid alkaloids, which are not found in other genera. Likewise, the genus *Carapichea* appears to be differentiated by the presence of unique dopamine-iridoid alkaloids. By contrast, the genus *Psychotria* is characterized by lacking both of the former alkaloid classes, instead accumulating condensed tannins related to catechin. The genus *Notopleura* appears to lack all of the aforementioned compound classes, instead accumulating megastigmanes, which are otherwise found only irregularly. Finally, flavonoids and iridoids appear to be infrequent, but are found in all of the above-mentioned genera. Altogether, the broad array of compounds and compound classes within different lineages suggests their biosynthetic differentiation such as via the key enzyme strictosidine synthase. We speculate that this may be related to different evolutionary history and selective pressures such as by herbivores acting on the different lineages, which remains open to further studies.

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ABOL meeting 2018 – preface

The 5th ABOL meeting took place from 6th to 7th of December 2018 at the Natural History Museum of Vienna. The meeting was one of our most successful ones, with about 180 participants. The workshops "*Application of DNA based methods in biodiversity monitoring*" and "*Collecting for ABOL – ABOL for collectors*" met with great interest among stakeholders, users and taxonomists. The scientific conference on the second day was stimulated by an excellent keynote talk with the focus on high throughput barcoding approaches in freshwater biology held by Florian Leese from the University of Duisburg Essen, followed by a series of interesting talks on various national and international projects as well as a broad spectrum of different taxonomic groups. Most of the contributions are represented in the following book of abstracts.

Das 5. ABOL-Treffen fand vom 6. bis 7. Dezember 2018 im Naturhistorischen Museum Wien statt. Das Treffen war mit rund 180 Teilnehmern eines unserer erfolgreichsten. Die Workshops „*Anwendung DNA-basierter Methoden im Biodiversitätsmonitoring*" und „*Sammeln für ABOL – ABOL für SammlerInnen*" stießen auf großes Interesse bei Stakeholdern, AnwenderInnen und TaxonomInnen. Die wissenschaftliche Konferenz am zweiten Tag wurde angeregt durch einen exzellenten Keynote-Vortrag mit dem Schwerpunkt auf Hochdurchsatz-Barcoding-Ansätzen in der Süßwasserbiologie von Florian Leese von der Universität Duisburg-Essen, gefolgt von einer Reihe interessanter Vorträge zu verschiedenen nationalen und internationalen Projekten sowie ein breites Spektrum verschiedener taxonomischer Gruppen. Die meisten Beiträge sind im folgenden Abstract-Band enthalten.

Extended abstract

Plant DNA barcoding within the ABOL initiative

Harald Meimberg, Andreas Tribsch, Manuel Curto, Karl Georg Bernhardt, Karin Tremetsberger, Christian Braeuchler, Tobias Grasegger, Anette Wimmer, David Horner, Eva Dornstauder-Schrammel, Leonid Rasran, Barbara Turner, Nora Stoeckl, Monika Kriechbaum, Katharina Schweikl, Matthias Affenzeller, Christoph Mayerhofer & Matthias Kropf

The Austrian Barcode of Life (ABOL) initiative has the goal to sequence a certain region of DNA of all species known from Austria to construct a taxonomically proved DNA reference database for species identification. Within this consortium, we participate in by investigating plant species. Originally, DNA barcoding refers to the use of defined sequences as information about species assignment of a given sample. A number of projects have been undertaken to construct regional or national DNA barcode databases and some shortcomings of the original DNA barcoding concepts are obvious. For plants, these are in particular the difficulties of the scientific community to agree with a certain barcode region, which is complicated by the higher reluctance of botanists to use degenerated primers (Li et al., 2014; Coissac et al., 2016). In addition, the original concepts are difficult to incorporate into the ongoing increase in throughput of sequencing capacities with second and third generation sequencing technologies (Shokralla et al., 2014). Thus, barcoding regions for reference databases are frequently different from regions that are used in metabarcoding applications where these technologies are used for species identification from bulk samples.

Within our approach, we develop a multilocus barcoding system that might overcome some of these constraints. The parallel use of multiple markers results in a higher amount of variation to discriminate species provides clearly defined primer binding sites for unbiased amplification and allows the use of next generation sequencing for data collection. We utilize 22 plastid markers, including 14 published primer pairs that cover insert length of up to 700 base pairs. Twelve primer pairs cover markers shorter than 600 bp that can be sequenced for both directions with the Illumina technology generating overlapping sequences for most samples. A two-step analysis linking sequence length of merged reads to marker information results in a reduction of complexity of amplicon sequence output allowing the *de novo* determination of sequences (Curto et al., 2019). To date, 2,055 plant samples were collected, which belong to about 1,700 different species. 1,042 of these have been processed so far. The application to identify plant species composition in pollen samples using the multilocus marker set was tested. We can show differences in species numbers in collected pollen between poly- and oligolectic wild bees; therefore, verifying the suitability of this method.

Meimberg H., Tribsch A., Curto M., Bernhardt K.G., Tremetsberger K., Braeuchler C., Grasegger T., Wimmer A., Horner D., Dornstauder-Schrammel E., Rasran L., Turner B., Stoeckl N., Kriechbaum M., Schweikl K., Affenzeller M., Mayerhofer C. & Kropf M., 2018: DNA-Barcoding bei Pflanzen im Rahmen der ABOL-Initiative.

Die *Austrian Barcode of Life* Initiative (ABOL) hat zum Ziel, eine bestimmte Region der DNA aller aus Österreich bekannten Arten zu sequenzieren, um eine taxonomisch überprüfte DNA-Referenzdatenbank für die Identifizierung von Arten zu erstellen. Innerhalb dieses Konsortiums beteiligen wir uns an der Untersuchung von Pflanzenarten. DNA-Barcoding bezieht sich ursprünglich auf die Verwendung definierter Sequenzen der Plastiden-DNA als Information über die Artzuordnung einer gegebenen Probe. Eine Reihe von Projekten wurde zum Aufbau regionaler oder nationaler Barcode-Datenbanken durchgeführt, und einige Unzulänglichkeiten der ursprünglichen DNA-Barcode-Konzepte sind dabei erkennbar. Für Pflanzen sind dies insbesondere die Probleme der wissenschaftlichen Gemeinschaft, sich auf eine bestimmte Barcode-Re-

gion zu einigen, was durch die größere Zurückhaltung in der Botanik bei der Verwendung von degenerierten Primern noch verstärkt wird (Li et al., 2014; Coissac et al., 2016). Darüber hinaus ist es schwierig, die ursprünglichen Konzepte mit der fortlaufenden Intensivierung des Durchsatzes in Sequenzierungstechnologien der zweiten und dritten Generation zu integrieren (Shokralla et al., 2014). Barcode-Regionen unterscheiden sich daher häufig von den Regionen, die in Metabarcoding-Anwendungen verwendet werden, die auf diesen Technologien zur Artbestimmung aus Mischproben beruhen.

Im Rahmen unseres Ansatzes entwickeln wir ein Multilocus-Barcode-System, das einige dieser Einschränkungen überwinden könnte. Die parallele Verwendung mehrerer Marker erhöht die Variation, die zur Unterscheidung von Arten zur Verfügung steht, liefert klar definierte Primer-Bindungsstellen für eine gleichmäßige Amplifikation und ermöglicht die Verwendung der Next-Generation-Sequenzierung zur Datenerfassung. Wir verwenden 22 Marker der Plastiden-DNA, darunter 14 veröffentlichte Primerpaare, die eine Insertlänge von bis zu 700 Basenpaaren abdecken. Zwölf Primerpaare amplifizieren Marker von unter 590 bp, die für beide Richtungen sequenziert werden können und die Illumina-Technologie überlappende Sequenzen für die meisten Proben erzeugt. Eine zweistufige Analyse, bei der die Sequenzlänge mit Markierungsinformationen verknüpft wird, führt zu einer Verringerung der Komplexität der Ergebnisse der Amplicon-Sequenzen, was die *de novo* Bestimmung von Sequenzen ermöglicht (Curto et al., 2019). Bis jetzt wurden 2.055 Pflanzenproben gesammelt, die ungefähr 1.700 verschiedenen Arten angehören. Von diesen wurden 1.042 bearbeitet. Die Anwendung zur Identifizierung der Zusammensetzung von Pflanzenarten in Pollenproben mit dem Multilocus-Markerset wurde getestet. Wir können Unterschiede in der Artenzahl in gesammeltem Pollen zwischen poly- und oligolektischen Wildbienen zeigen, was die Eignung dieser Methode unterstreicht.

Keywords: multiclocus DNA barcoding, plants, pollen

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Abstract

Alpine lichens and liverworts – DNA barcoding of Austrian cryptogams

Adriana Atanassova

Lichens are omnipresent cryptogamous organisms. The occurrence of their fungi-like, photosynthetic active thalli range from soil over trees and boulders up to highest alpine risings, where they form comprehensive windswept heaths and defy the most extreme weather conditions. Lichens represent symbiotic communities formed by one fungus and one or more algae, which in suitable microbial environment manage to survive unsteady and hostile conditions and are therefore able to colonize sensible ecological niches. From Austria 2,491 species of lichenized fungi are documented, where the highest biodiversity is found in alpine habitats. Natural and anthropogenic factors, as climate change, pollution, glacial melting and landslides lead to imbalance in alpine eco systems and disappearance of many vulnerable species (*Lobaria pulmonaria, Anaptychia ciliaris, Heterodermia speciosa*).

Lichens often occur in the company of mosses, mostly competing for the same substrates. Numerous representatives of both groups of organisms are suitable as bioindicators and can potentially warn for e.g. air pollution, global warming and migration of the species in higher altitudes.

Bryophytes are green non-vascular plants, which show reptant to erect habitus. Traditionally they divided into mosses (Bryophyta), hornworts (Anthocerotophyta) and liverworts (Marchantiophyta). In our study, we focused mainly on alpine liverworts. With 260 reported species, liverworts form the smaller part of Austrian bryophytes, however many of them are critical biological and ecological indicators growing in humid and shady habitats on similar substrates (soil, wood and rock) as lichens.

For the ABOL project many of the alpine lichen and liverwort species were freshly collected. The ITS (Internal Transcribed Spacer) region of both groups was determined as suggested the most useful gene segment for DNA barcoding lichens and liverworts diverse primers (ITS1, ITS1f, ITS2, ITS4) were tested. Elusive species as well as sterile compounds could be successfully decoded with this method. With ca. 1,800 individuals (about 700 different species, mostly lichens), which were evaluated on sequence level, a substantial part of Austrian common species is already recorded.

With optimization of extraction and sequencing methods, the project realization will proceed with rare and critical species from existing and re-sampled material over the next two years.

Atanassova A., 2018: Alpine Flechten und Lebermoose – DNA-Barcoding österreichischer Kryptogamen.

Flechten sind allgegenwärtige kryptogame Organismen. Ihre pilzartigen, photosynthetisch aktiven Wuchskörper erstrecken sich vom Erdsubstrat auf Bäume über Felsblöcke bis auf die höchsten alpinen Erhebungen, wo sie flächendeckend Windheiden bilden und den extremsten Wetterlagen trotzen. Sie stellen Lebensgemeinschaften aus einem Pilz und einer oder mehreren Algen dar, die im passenden mikrobiellen Umfeld schwankende und lebensfeindliche Bedingungen überstehen und somit sensible ökologische Nischen besiedeln. In Österreich sind 2.491 Arten von lichenisierten Pilzen dokumentiert, wobei die größte Diversität auf alpine Habitate beschränkt ist. Natürliche und anthropogene Faktoren, wie Klimawandel, Luftverschmutzung, Gletscherschmelze und Erdrutschungen führen zu Ungleichgewicht in den alpinen Ökosystemen und zum Verschwinden vieler luft- und schadstoffempfindlicher Arten (*Lobaria pulmonaria, Anaptychia ciliaris, Heterodermia speciosa*).

Flechten treten häufig in Begleitung von Moosen auf, meist in Konkurrenz um dasselbe Substrat. Zahlreiche Vertreter beider Organismengruppen eignen sich als Bioindikatoren und potentielle Warnzeiger für Klimaerwärmung und ein Höherwandern der Arten.

Moose sind grüne Landpflanzen, die kein Stützgewebe besitzen und kriechende bis aufrechte Wuchsformen ausbilden. Traditionell werden Moose in folgende drei Gruppen geteilt: Horn- (Anthocerotophyta), Leber- (Marchantiophyta) und Laubmoose (Bryophyta). Das Hauptaugenmerk der vorliegenden Datengenerierung liegt auf alpinen Lebermoosen. Lebermoose bilden mit 260 Arten den kleineren Anteil der Moose Österreichs und sind oftmals kritische biologische und ökologische Zeiger, welche ähnliche Substrate wie Flechten (Erde, Holz und Gestein) in humiden und schattigen Lebensräumen besiedeln.

Für das ABOL-Projekt wurden alpine Flechten und Lebermoose großteils frisch gesammelt. Die ITS-Region wurde für beiden Organismengruppen als den für DNA-Barcoding brauchbaren Genabschnitt determiniert und mit diversen Primern (ITS1, ITS1f, ITS2, ITS4) sequenziert. Schwer bestimmbare Arten oder sterile Wuchskörper konnten anhand von DNA-Barcoding oftmals erfolgreich entschlüsselt werden. Mit ca. 1.800 Individuen (etwa 700 verschiedene Arten, überwiegend Flechten), die auf Sequenzebene ausgewertet wurden, ist bereits ein beträchtlicher Teil der häufig vorkommenden Arten erfasst.

Die Optimierung von Extraktions- und Sequenzierungsmethoden wird anhand seltener und kritischer Arten in den kommenden zwei Jahren aus vorhandenen und erneut gesammelten Proben den Schwerpunkt der Projektverwirklichung darstellen.

Keywords: DNA barcoding, mosses, lichens.

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Abstract

Revealing unrecorded diversity in Austrian rivers – DNA barcoding of *Gyrodactylus*, a genus of hyperdiverse fish ectoparasites

Christoph Hahn, Lukas Zangl, Mario Poglitsch & Stephan Koblmüller

The genus *Gyrodactylus* (Monogenea; Platyhelminthes) contains an estimated number of 20,000 species worldwide, with only little over 400 formally described to date. These fish ectoparasites are characterized by high host specificity and a unique assemblage of reproductive features (hyperviviparity, progenesis). Little is known about the distribution and diversity of *Gyrodactylus* species in Austria. Since their discovery in 1832, taxonomy has been traditionally based on host species ID and the morphology of the hook apparatus, which they use to attach to their hosts. Synonymies, as well as cryptic diversity, may be assumed in this taxon and require further (molecular) investigation, which is currently hampered by the lack of reliable generic barcoding primers for the genus. In the first year of the project, we investigated 19 Austrian fish species, and recorded 17 genetically distinct species of *Gyrodactylus*, 9 of which could (at least tentatively) be assigned to known taxa based on morphological/molecular evidence. We developed new PCR primers, which successfully amplify the Cytochrome Oxidase Subunit I (COI) gene of all *Gyrodactylus* species collected so far in Austria, and are currently being tested on a wide range of additional taxa provided by international collaborators, to further verify their generic nature. We present first data obtained using the portable MinION sequencing platform (Oxford Nanopore) and discuss challenges and future directions.

Hahn C., Zangl L., Poglitsch M. & Koblmüller S., 2018: Unbekannte Vielfalt in österreichischen Flüssen - DNA-Barcoding von *Gyrodactylus*, einer Gattung hyperdiverser Fischektoparasiten.

Die Gattung *Gyrodactylus* (Monogenea; Platyhelminthes) enthält schätzungsweise 20.000 Arten weltweit, von denen bislang nur etwas mehr als 400 formal beschrieben wurden. Diese Fischektoparasiten zeichnen sich durch eine hohe Wirtsspezifität und eine einzigartige Zusammenstellung von Fortpflanzungsmerkmalen (Hyperviviparität, Progenese) aus. Über die Verbreitung und Vielfalt der *Gyrodactylus*-Arten in Österreich ist wenig bekannt. Seit ihrer Entdeckung im Jahr 1832 basiert die Taxonomie traditionell auf der Identifizierung der Wirtsspezies und der Morphologie des Hakenapparats, mit dem sie sich an ihre Wirte anheften. Das Vorhandensein von Synonymien und kryptischer Diversität gilt in dieser Gattung als wahrscheinlich und erfordert weitere (molekulare) Untersuchungen, die derzeit durch das Fehlen zuverlässiger Barcoding-Primer für die Gattung erschwert werden. Im ersten Projektjahr wurden 19 österreichische Fischarten untersucht und 17 genetisch unterschiedliche Arten von *Gyrodactylus* erfasst, von denen 9 (zumindest vorläufig) bekannten Taxa auf Grundlage von morphologischen/molekularen Merkmalen zugeordnet werden konnten. Wir haben neue PCR-Primer entwickelt, die das Cytochrom Oxidase Untereinheit I (COI)-Gen aller bisher in Österreich gesammelten *Gyrodactylus*-Arten erfolgreich amplifizieren. Diese werden derzeit an einer Vielzahl zusätzlicher Taxa (bereitgestellt von internationalen Kooperationspartnern) getestet, um ihre generische Natur weiter zu bestätigen. Wir präsentieren erste Daten, die mit der portablen MinION-Sequenzierungsplattform (Oxford Nanopore) gewonnen wurden, und diskutieren Herausforderungen und zukünftige Vorhaben.

Keywords: DNA barcoding, fish parasites, Platyhelminthes.

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Abstract

DNA barcodes in streptophyte green algae and the problem of morphological species concepts

Andreas Holzinger

Green algae can be divided into the chlorophytic and the streptophytic lineage that separated about 750 million years ago. The streptophytic lineage comprises the phylum Charophyta and all land plants. Within the Charophyta currently 6 different classes can be distinguished, the Mesostinematophyceae, Chlorokybophyceae, Klebsormidiophyceae, Zygnematopyhceae, Coleochaetophyceae and Charophyceae. While some classes contain only a few genera and are rather rare, others occur abundant in freshwater or terrestrial habitats. The classical morphological species concepts are better established in groups where shapes of cells or reproduction organs have a good variety to be distinguished, however still several taxonomic problems have to be resolved; e.g. the Desmids with their peculiar cell shapes are a classical example for morphological species determination, but also reproductive spores of Zygnematopyhceae when available, can be distinguished morphologically.

Klebsormidiophyceae or vegetative Zygnematales are composed of simple filaments, where morphology does not reflect species diversity. There, molecular markers like the ribosomal small subunit (18S rDNA or SSU) are used mostly on genus level. Some conserved regions of SSU (V4 and V9) are used as barcoding markers. Ribosomal spacers ITS1, ITS2 and the secondary structure of ITS2 are useful for species delimitation. Conservative regions of ITS2 detected by secondary structure was suggested as barcoding marker at the species level in green algae. The ribosomal operon starts to be a universal marker for many groups of algae as good comparisons are possible.

Several chloroplast encoded genes could also serve as good barcoding candidates for green algae and have been widely used for phylogenetic analysis. The large subunit of rubisco (*rbc*L), Photosystem II Protein D1 (*psb*A) and the spacer region between these, photosystem II CP43 reaction center (*psb*C) and ATP synthase subunit b (*atp*B) and the plastid encoded protein-coding gene (*mat*K) have been employed to perform species delineation in green algae. Sometimes only a combination of genes (multigene analysis) gives a reasonable resolution; in contrast elongation factor TU1 (*tuf*A) may be particularly useful in chlorophytes. Also the universal plastid amplicon (UPA) was suggested as barcode. In this presentation special examples of e.g. *Zygogonium erictorum*, *Zygnema* sp. and *Klebsormidium* sp. among others will be discussed.

Holzinger A., 2018: DNA-Barcodes in streptophytischen Grünalgen und das Problem des morphologischen Spezies-Konzepts.

Grünalgen können in Chlorophyten und Streptophyten unterteilt werden, die sich vor 750 Millionen Jahren getrennt haben. Die Streptophyten-Linie beinhaltet den Stamm der Charophyta und alle Landpflanzen. Innerhalb der Charophyta werden gegenwärtig 6 Klassen unterschieden: Mesostinematophyceae, Chlorokybophyceae, Klebsormidiophyceae, Zygnematopyhceae, Coleochaetophyceae und Charophyceae. Einige dieser Klassen beinhalten nur wenige Gattungen und sind selten, während andere sehr artenreich und häufig in Süßwasser und terrestrischen Habitaten zu finden sind. Die klassischen morphologischen Spezies-Konzepte sind in Gruppen mit formenreichen Zellen oder Reproduktionsorganen besser etabliert. Trotzdem müssen zahlreiche taxonomische Probleme gelöst werden. Desmidiaceen mit ihren besonderen Zellformen sind ein klassisches Beispiel für morphologische Spezies-Bestimmung, aber auch reproduktive Sporen der Zygnematophyceen können gut morphologisch unterschieden werden.

Klebsormidiophyceae oder vegetative Zygnematales hingegen bestehen aus einfachen Filamenten, bei denen die Morphologie die Artenvielfalt nicht widergibt. Dort sind molekulare Marker wie die ribosomale kleine Untereinheit (18S rDNA or SSU) zur Unterscheidung auf Artniveau im Einsatz. Einige konservierte Regionen der SSU (V4 und V9) werden als Barcoding Marker verwendet. Ribosomale spacer ITS1 und ITS2,

sowie die Sekundärstruktur von ITS2 sind zur Artenauftrennung geeignet. Die konservierten Regionen der ITS2 wurden als Barcoding-Marker auf Artniveau in Grünalgen vorgeschlagen. Auch das ribosomale Operon wird als universeller Marker in vielen Algengruppen verwendet, weil damit gute Vergleichsmöglichkeiten gegeben sind. Auch Chloroplasten codierte Gene können zum Barcoding herangezogen werden, sie sind vielfach für die phylogenetische Analyse im Einsatz. Die große Untereinheit der Rubisco (*rbc*L), das Photosystem II Protein D1 (*psb*A) und die spacer Region zwischen diesen, das Photosystem II CP43 Reaktionszentrum (*psb*C) und die ATP Synthase Untereinheit b (*atp*B) und das Plastiden codierte protein-codierende Gen (*mat*K) wurde in Grünalgen zur Artunterscheidung herangezogen. Manchmal gibt nur eine Kombination von Genen (multigen-Analyse) die nötige Auflösung; der Elongation Faktor TU1 (*tuf*A) ist besonders für Chlorophyten Unterscheidung geeignet. Auch das Universelle Plastid Amplicon (UPA) wurde als barcode Marker vorgeschlagen. Spezielle Beispiele wie *Zygogonium ericetorum*, *Zygnema* sp. und *Klebsormidium* sp. werden diskutiert.

Keywords: DNA barcoding, green algae.

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Abstract

BIO-PLANBAR: Assessing biodiversity in construction project planning by means of DNA barcoding

Stephan Koblmüller, Wolfgang Paill & Werner Holzinger

In Austria, the realization of major construction projects typically requires an environmental impact assessment. A positive evaluation depends on the project's conformity with nature conservation interests. This implies that massive negative impacts on species valuable from a conservation perspective should be avoided or compensated for. To estimate the impact of a construction project on the animals and plants in the affected area, extensive knowledge of the local fauna and flora is required. This means that in the planning phase the project leader has to assess the fauna and flora in the area in which the project should be realized. Typically, this is done by recording the presence/abundance of selected quality-determining key species from certain indicator groups. Very often, traditional species identification by means of classic morphological characters is time and cost intensive. In the framework of BIO-PLANBAR we intend to optimize the process of species identification for environmental impact assessments and make it more cost- and time efficient. Specifically, we aim at a i) faster and ii) more comprehensive species identification. To achieve these goals we want to establish DNA barcoding, or more precisely a comprehensive high quality DNA barcode reference database, for the relevant taxa, which should allow for reliable species identification of morphologically "difficult" developmental stages or (in some taxa) females. Advantages of applying DNA barcoding are i) a larger set of identified species, ii) an extension of the study period, iii) a faster species inventory, and iv) in the long run the potential application of non-invasive methods (e.g. eDNA). Even if, in principle, the application of DNA barcoding (or metabarcoding of mixed samples) is promising, its success depends on the availability of a comprehensive high quality reference data base. Reference data for the relevant taxa should be generated in the framework of the project; i.e. DNA barcodes will be generated and uploaded (together with the corresponding metadata) to the Austrian (ABOL) and international (BOLD) barcoding reference data bases. In addition, we will evaluate i) the limits of DNA barcoding in the relevant taxa, ii) whether in morphological difficult taxa species identification based on DNA data is more straightforward than based on classic morphological data, and iii) whether there are certain taxa for which DNA barcoding does not work. Finally, we intend to establish guidelines for sampling, when data analysis is based at least in part on molecular data.

Koblmüller S., Paill W. & Holzinger W., 2018: BIO-PLANBAR: Biodiversitätserfassung bei Projektplanungen mittels DNA-Barcoding.
In Österreich ist bei großen Bauvorhaben, die außerhalb von gewidmeten Wohn-, Industrie- und Gewerbegebieten umgesetzt werden, ein UVP-Verfahren oder zumindest ein naturschutzrechtliches Bewilligungsverfahren erforderlich. Voraussetzung für einen positiven Bescheid ist eine möglichst naturschutzkonforme Umsetzung des Vorhabens. Dies bedeutet, dass der Projektwerber im Zuge des Planungsprozesses auch die Tier- und Pflanzenwelt des Raumes, in der er sein Projekt umzusetzen gedenkt, erfassen muss. Da es allerdings praktisch unmöglich ist, tatsächlich die gesamte Fauna eines Raumes zu erheben, wird versucht, eine möglichst repräsentative Auswahl an „wertbestimmenden" Arten aus Indikatorgruppen zu erfassen. Diese Artbestimmung anhand klassisch morphologischer Merkmale ist ein in vielen Fällen zeitlich aufwändiger Arbeitsschritt, dessen Optimierung im Fokus des Projekts BIO-PLANBAR liegt. Die spezifischen Ziele dieses Projekts sind i) eine raschere Bearbeitung dieses Arbeitsschritts „Artbestimmung" und ii) eine vollständige Bearbeitbarkeit der Proben zur Steigerung der Effizienz. Um diese Ziele zu erreichen, sollen nun die Voraussetzungen dafür geschaffen werden, zukünftig auch molekulargenetische Methoden (DNA-Barcoding) - ergänzend zu klassischen morphologischen - bei der Probenauswertung und Bestimmung einsetzen zu können, um auch morphologisch unbestimmbare Tiere - das

sind vor allem Jungtiere, Larvenstadien, Eier, aber bei manchen Tiergruppen auch die Weibchen bestimmter Arten – zweifelsfrei bestimmbar zu machen und somit Proben möglichst vollständig auswerten zu können. Vorteile für den Anwender von DNA-Barcoding sind i) eine vollständigere Auswertungsquote, ii) eine Ausdehnung des Bearbeitungszeitraum (da auch diverse Entwicklungsstadien identifiziert werden können), iii) ein raschere Bearbeitbarkeit und iv) mittelfristig auch die Anwendung nichtinvasiver Methoden (Stichwort eDNA). Auch wenn die Anwendung von DNA-Barcoding (bzw. Metabarcoding von Mischproben und die Verwendung von DNA-Barcode-Referenzdatenbanken) prinzipiell vielversprechend ist, steht und fällt der Erfolg mit der Verfügbarkeit einer möglichst vollständigen und qualitativ hochwertigen Referenzdatenbank. Diese soll für einen Großteil der UVP-relevante Tiergruppen im Rahmen dieses Projekts erstellt werden, und es sollen die DNA-Barcodes und dazugehörigen Metadaten in die österreichische (ABOL) und die internationale (BOLD) Referenzdatenbank eingespeist werden. Zusätzlich soll generell geklärt werden i) wo die Grenzen der Artbestimmung anhand von DNA-Barcodes bei den relevanten Tiergruppen liegen, ii) ob Artbestimmung mittels DNA-Barcoding bei morphologisch schwer bestimmbaren Arten einfacher ist/funktioniert, und iii) wo die Methode nicht funktioniert. Nicht zuletzt soll auch ein Leitfaden für die Probennahme ausgearbeitet werden, wenn die Auswertung vorwiegend oder zusätzlich auch mit Hilfe molekularer Methoden erfolgen soll.

Keywords: ABOL, DNA barcoding, environmental impact assessment, indicator species.

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Abstract

DNA barcoding of Austrian molluscs – to BIN or not to BIN

Luise Kruckenhauser, Michael Duda, Julia Schindelar, Oliver Macek,
Susanne Reier & Anita Eschner

Molluscs are the second species-rich animal group, however, up to now DNA barcoding projects, which deal with molluscs in general, and land snails in particular are scarce. Within the Austrian Barcode of Life (ABOL) initiative, we took over the ambitious task to generate DNA barcodes for all Austrian molluscs. Several factors of interest overruled possible difficulties: molluscs are important indicator species, suitable for evaluation of habitat quality. Living in vulnerable habitats leads to a high risk of extinction for many molluscs: about 35 % of the snail and 37 % of the Austrian mussel species are endangered. Also the number of endemics is quite impressive (19.3 %). Approximately 30 % of the 400 native species are divided into subspecies. At the NHM Vienna several projects on snail species in Austria are conducted, hence pre-conditions (collected specimens and experiences) for successful DNA-barcoding are given. However, genetic investigations in land pulmonates showed an extreme high intraspecific diversity. Therefore there is no standard value for genetic distances, which marks taxonomic delimitations. Due to the overlap of intra- and interspecific variation often no barcoding gap can be found, which has to be considered in data analysis.

For the ABOL Project Molluscs we used material collected during concerted field trips to different parts of Austria, and from former projects, both specifically preserved for DNA analyses, but also older material from the collections of the Natural History Museum Vienna and the Biology Centre of Linz. DNA from museum material is often fragmented and of low concentration, therefore it is only chosen, when no other material is available. Until now, we generated 569 DNA barcodes with all relevant metadata from 230 different species (about 60 % of the Austrian species) and uploaded them to the BOLD database. From our results, we find that many species are very well placed within a BIN (Barcode Index Number) or at least in one supported clade but we also see high genetic differentiation within one morphological described species, implying the existence of cryptic species. Contrary also different morphologically described species that cluster in one barcoding BIN can be found. We will give an overview of the occurrence of these different patterns within our data.

The project provides DNA barcodes for the challenging group of molluscs. Data are sampled in a relatively small geographic scale, which is essential for taxa with low dispersal capacity. The DNA barcodes will facilitate determination, which is often difficult and vague in molluscs. Therefore, the method can be useful in evaluations of nature conservation issues.

Kruckenhauser L., Duda M., Schindelar J., Macek O., Reier S. & Eschner A., 2018: DNA-Barcoding Österreichischer Mollusken.

Mollusken sind die zweit artenreichste Tiergruppe, dennoch sind DNA-Barcode-Projekte, die sich mit Mollusken im Allgemeinen und Landschnecken im Besonderen befassen, rar. Im Rahmen der Initiative Austrian Barcode of Life (ABOL) haben wir die Aufgabe übernommen, DNA-Barcodes für alle österreichischen Mollusken zu erstellen. Aus verschiedenen Gründen ist die Bearbeitung dieser Gruppe besonders relevant: Weichtiere sind wichtige Indikatorarten, die zur Bewertung der Habitatqualität geeignet sind. Die Gefährdung der Lebensräume führt dazu, dass viele Weichtiere bedroht sind: Etwa 35 % der Schnecken- und 37 % der österreichischen Muschelarten sind vom Aussterben bedroht. 19,3 % der heimischen Mollusken sind Endemiten und ungefähr 30 % der 400 einheimischen Arten sind in Unterarten unterteilt. Genetische Untersuchungen an Landpulmonaten haben oft eine extrem hohe intraspezifische Diversität gezeigt. Daher gibt es keinen Standardwert für die genetische Distanz, der eine taxonomische Abgrenzungen kennzeichnet. Aufgrund der Überlappung von intra- und interspezifischen Distanzen kann häufig keine „barcoding gap“ gefunden werden. Dies muss bei der Datenanalyse berücksichtigt muss.

Für das ABOL-Projekt Mollusken wurde Material verwendet, das speziell für DNA-Analysen während gezielter Exkursionen in verschiedene Teile Österreichs gesammelt wurde, oder aus früheren Projekten stammt. Abgesehen davon wurde auch älteres Material aus den Sammlungen des Naturhistorischen Museums Wien und des Biologiezentrums Linz verwendet. Aufgrund von Fragmentierung und geringeren Konzentrationen der DNA in älterem Museumsmaterial, wurden diese nur ausgewählt, wenn kein anderes Material zur Verfügung stand. Bisher haben wir aus 230 verschiedenen Arten (ca. 60 % der österreichischen Arten) 569 DNA-Barcodes mit allen relevanten Metadaten generiert und in die BOLD-Datenbank hochgeladen. Aus unseren Ergebnissen geht hervor, dass viele Arten innerhalb eines BINs (Barcode Index Number) oder zumindest in einer unterstützten Gruppe positioniert sind. Allerdings haben wir bei manchen Arten auch eine hohe genetische Differenzierung innerhalb einer morphologisch beschriebenen Art festgestellt, was auf die Existenz kryptischer Arten schließen lässt. Im Gegensatz dazu sind auch verschiedene morphologisch beschriebene Arten zu finden, die den gleichen Barcode-BIN angehören. Wir geben einen Überblick über diese unterschiedlichen Muster in unseren Daten.

Das Projekt liefert DNA-Barcodes für die herausfordernde Gruppe der Weichtiere. Die Datenerhebung erfolgt in einem relativ kleinen geografischen Maßstab, was für Taxa mit geringer Ausbreitungskapazität von wesentlicher Bedeutung ist. Die Methode des DNA-Barcodings erleichtert die Bestimmung von Mollusken, die oft schwierig und vage ist und kann daher hilfreich bei der Bewertung von Naturschutzfragen sein.

Keywords: DNA barcoding, BIN congruency, molluscs.

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Abstract

Development and establishment of molecular genetic (eDNA) monitoring methods for waterbodies in the alpine region and comparison to traditional ecological status assessment methods

Hans Rund, Jean Marc Baudoin, Isabelle Domaizon, Tina Eleršek, Georgio Franzini, Claudia Greco, Peter Hufnagl, Aleksandra Krivograd Klemenčič, Rainer Kurmayer, Fabio Lepori, Jochen Schaumburg, Michael Schubert & Nico Salmaso

With the adoption of new methodologies and approaches, the Eco-AlpsWater project sets very ambitious objectives, anticipating the route in the development of the new generation water monitoring systems in Europe. Ecosystem services provided by lakes and rivers are facing serious threats under the pressure of anthropogenic impacts, climate change, biodiversity loss and invasion of alien species. The evaluation of these changes is still carried out by traditional criteria, which include expensive and time-consuming approaches (for example based exclusively on the classical identification of aquatic species with microscopy techniques). The main objective of Eco-AlpsWater is to integrate the traditional monitoring approaches used in the Alpine region and at the European level (Water Framework Directive 2000/60 EC and, in Switzerland, the Water Protection Ordinance - WPO) with advanced and innovative approaches, providing solid and qualified knowledge to further support water resources management plans. The new approach will use Next Generation Sequencing (NGS) technologies to analyze environmental DNA (eDNA) extracted from water samples collected in lakes and rivers. These new techniques, based on the amplification and analysis of millions of DNA sequences and on the use of smart technologies (automation in data processing and storage and information retrieval), allow a rapid and low cost identification of aquatic organisms, from bacteria to fish. Together with the implementation of new monitoring techniques in European regions, the new technologies will provide the census of lake and river biodiversity in the Alpine region at an unprecedented level, based on the analysis of hundreds of samples collected in over 30 bodies of water. The data will in particular identify the areas most at risk due to the presence of toxic cyanobacteria, pathogenic bacteria, and invasive or potentially invasive organisms.

Rund H., Baudoin J.M., Domaizon I., Eleršek T., Franzini G., Greco C., Hufnagl P., Krivograd Klemenčič A., Kurmayer R., Lepori F., Schaumburg J., Schubert M. & Salmaso N., 2018: Entwicklung und Etablierung von molekulargenetischen (eDNA) Monitoring-Methoden für Gewässer im Alpenraum und deren Vergleich mit traditionellen ökologischen Zustandsbewertungs-Methoden.

Mit der Einführung neuer Methoden und Ansätze setzt sich das Projekt Eco-AlpsWater das Ziel, eine neue Generation von Gewässer-Monitoring-Systemen im Alpenraum zu etablieren. Die wertvollen Ökosystemleistungen von Seen und Flüssen sind durch anthropogene Einflüsse, Klimawandel, Verlust der Biodiversität und dem Eindringen invasiver Arten gefährdet. Die Veränderungen der Gewässer werden noch immer anhand von traditionellen Untersuchungen bewertet, was kostenintensive und zeitaufwändige Ansätze umfasst. Das Hauptziel von Eco-AlpsWater besteht darin, die traditionellen Monitoring-Instrumente im Alpenraum und auf europäischer Ebene (Wasserrahmenrichtlinie 2000/60 EG und in der Schweiz die Gewässerschutzverordnung – GSchV) mit fortschrittlichen und innovativen Ansätzen zu verbinden. Der neue Ansatz umfasst Next Generation Sequencing (NGS)-Technologien, um Umwelt-DNA (eDNA) aus verschiedenen Gewässern zu analysieren. Diese neuen Techniken, die auf der Amplifikation und Analyse von Millionen von DNA-Sequenzen und dem Einsatz intelligenter

Technologien (Automatisierung in der Datenverarbeitung, Datenspeicherung und Informationsrückgewinnung) basieren, ermöglichen eine schnelle und kostengünstige Identifizierung von Algen, Bakterien und Fischen. Basierend auf der Analyse von hunderten Proben, die in über 30 Gewässern gesammelt werden, wird dieser Ansatz die Erfassung der Biodiversität von Seen und Flüssen im Alpenraum auf einem bisher beispiellosen Niveau ermöglichen. Mithilfe der gewonnenen Daten wird es auch möglich sein, besonders stark gefährdete Bereiche innerhalb eines Ökosystems zu identifizieren (toxische Cyanobakterien, pathogene Bakterien oder invasive Arten) und Gegenmaßnahmen zu treffen.

Keywords: environmental DNA, Next Generation Sequencing, monitoring.

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Abstract

DNA barcode derived community phylogenies reveal strong phylogenetic clustering of host-plant use in European Lepidoptera

Patrick Strutzenberger & Konrad Fiedler

We used a community phylogeny approach to investigate phylogenetic structuring in European Lepidoptera associated with a set of 200 selected focal plant species. We focused on plant species with a wide distribution in Europe, which can be reliably identified also by lepidopterists, and which represent all major lineages of vascular plants. Host records of Lepidoptera larvae for each of those 200 plant species were extracted from the literature resulting in ~7300 plant-herbivore associations. All available barcodes for the resulting selection of lepidopteran herbivores were extracted from the Barcode of Life Datasystems (BOLD). Eight of the 200 plant species had to be removed from the analysis due to a lack of barcode coverage for their associated herbivores.
This resulted in a sequence alignment containing 2341 species of Lepidoptera from 70 families feeding on 192 different plant species. Only for 5% of lepidopteran species recorded to feed on the focal plants no barcode data were yet available. This comprehensive dataset was used to reconstruct a phylogenetic tree with BEAST. A backbone constraint was used to replicate the currently recognized family level relationships within Lepidoptera and the tree was time-calibrated according to the results of a previously published large-scale phylogenetic study. Measures of phylogenetic diversity of the herbivore assemblages affiliated with each plant species were calculated. The Net Relatedness Index (NRI) revealed that species feeding on herbaceous plants are slightly but significantly more closely related to each other than is the case on graminoid and woody plants. The UniFrac measure was used to gain further insight into phylogenetic clustering. UniFrac measures the fraction of cumulative branch length in a tree that is unique to the assemblage in question, i.e. a measure of shared evolutionary history among herbivores on the same plant species. Obtained UniFrac values were analyzed with ordination analyses (NMDS and canonical analysis of principal coordinates, CAP). Highly significant structuring was revealed between herbivore communities on herbs, graminoids, and woody plants. In a CAP analysis all but 11 plant species clustered with their correct growth-form category. The majority of those 11 misclassified plant species were either highly toxic plants, plants with an unusual phenology, plants with a low number of associated Lepidoptera species, or a combination of more than one of those factors. This case study demonstrates the utility of DNA barcode sequences beyond species discovery, identification, and taxonomy. The amount of DNA sequence data generated through barcoding campaigns is currently unrivaled, rendering those datasets prime sources for large scale macro-ecological studies.

Strutzenberger P. & Fiedler K., 2018: DNA-Barcode basierte Stammbäume zeigen starke phylogenetische Klumpung in mitteleuropäischen Raupengemeinschaften (Lepidoptera).

Die phylogenetische Struktur von mitteleuropäischen Raupengemeinschaften wurden unter Verwendung eines community phylogeny Ansatzes untersucht. Zu diesem Zweck wurden ca. 7300 Fraßbeziehungen zwischen Lepidopteren und Pflanzen aus der Literatur extrahiert. Kriterien zur Auswahl der Pflanzenarten waren eine weite Verbreitung in Mitteleuropa und einfache, auch durch Entomologen durchzuführende Bestimmung. Zur Erstellung einer zeit-kalibrierten Phylogenie wurden DNA-Barcodes aus den Barcode of Life Datasystems (BOLD) zusammen mit einem backbone constraint zur Auflösung tiefer Verzweigungen verwendet. Nach Entfernung der in BOLD nicht mit Sequenzdaten vertretenen Arten ergab sich ein Datensatz von 2341 Lepidopterenarten aus 70 Familien mit Fraßbeziehungen zu 192 Pflanzenarten. Auf Basis des generierten Stammbaums wurden diverse Metriken für phylogenetische Diversität berechnet. Ergebnisse des Net Relatedness Index (NRI) haben gezeigt, dass Lepidopteren auf krautigen Pflanzen signifikant näher miteinander verwandt sind als das für Arten auf graminoiden und holzigen Pflanzen der Fall ist. Zur weiteren Untersuchung der phylogenetischen Struktur der Gemeinschaften wurde das UniFrac Maß verwendet. UniFrac mißt im paarweisen Vergleich der Gemeinschaften die für die jeweilige Artengemeinschaft einzigartige Evolutionsgeschichte. Die errechneten UniFrac Werte wurden mittels Ordinationsverfahren ausgewertet. Eine NMDS Ordination zeigte hochsignifikante Unterschiede in der phylogenetischen Zusammensetzung zwischen Artengemeinschaften auf krautigen, holzigen und graminoiden Pflanzen. Eine CAP Ordination hat gezeigt das 181 von 192 Pflanzenarten aufgrund der phylogenetischen Struktur ihrer Raupengemeinschaften der korrekten Wuchsform zugeordnet wurden. Bei falsch zugeordneten Pflanzen handelte es sich entweder um hochgradig giftige Pflanzen (z. B. Eibe), Pflanzen mit ungewöhnlicher Wuchsform (z. B. Misteln), oder Pflanzen mit sehr wenigen assoziierten Herbivoren. Über die unmittelbaren Ergebnisse hinaus zeigt diese Fallstudie sehr deutlich, dass DNA-Barcodes neben Taxonomie und Biodiversitätsforschung auch für makroökologische Studien eine überaus wertvolle Datenquelle sind.

Keywords: DNA barcoding, food webs, plant-insect interaction.

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Abstracts

eDNA-based fish monitoring in Alpine rivers

Bettina Thalinger, Christian Moritz, Richard Schwarzenberger,
Josef Wanzenböck & Michael Traugott

Environmental DNA (short: "eDNA") segregated into the surrounding water by aquatic organisms is on the verge to revolutionize the monitoring of fish species. The application of this approach is especially beneficial for large scale monitoring efforts as water samples can be taken easily from numerous sampling locations. However, the interpretation of eDNA signals obtained from rivers and streams poses a specific challenge due to downstream transport and high discharge fluctuations. Especially the detection of rare species calls for suitable sampling protocols and highly sensitive molecular analysis. Furthermore, fish abundance estimations based on eDNA signals definitely need to take into account discharge and populations upstream of the sampling location. In the FFG project *„eDNA – AlpFish: Detection und semi-quantitative population estimations of Alpine fish species via eDNA"*, these challenges are addressed with the goal to enable large scale molecular fish monitoring in the future.

In an experimental setup, downstream transport distances of minute eDNA quantities were investigated at different seasonal discharge situations resulting in decreasing signal strength with increasing discharge and distance from the source. Additionally, eDNA signal strengths were directly compared to results of electrofishing in Tyrolean rivers showing a positive correlation between the results obtained by the two techniques, but also highlighting the influence of fish individuals upstream of the sampling location.

The combination of results obtained from different experiments and approaches within the project not only showcases the potential of eDNA-based fish monitoring in the future, but also enables correct and informed choices for sampling strategy, method of analysis, and interpretation of the obtained datasets.

Thalinger B., Moritz C., Schwarzenberger R., Wanzenböck J. & Traugott M., 2018: eDNA basiertes Fischmonitoring in alpinen Fließgewässern.

Umwelt DNA (englisch: „environmental DNA"; kurz: „eDNA"), die von aquatischen Organismen wie z. B. Fischen ins Umgebungswasser abgegeben wird, hat das Potenzial, bereits in naher Zukunft das Gewässermonitoring zu revolutionieren. Da eDNA vergleichsweise einfach durch die Filtration und anschließende Analyse von Wasserproben gewonnen werden kann, ermöglicht die Methode die Untersuchung einer Vielzahl unterschiedlicher Standorte in kurzer zeitlicher Abfolge. Allerdings kann die Interpretation der so gewonnenen Ergebnisse vor allem in Fließgewässern problematisch sein, da hier Abflussschwankungen sowie Individuen, die sich flussaufwärts aufhalten, die Signalstärke wesentlich beeinflussen. Vor allem der Nachweis seltener Arten erfordert eine geeignete Probenahmestrategie sowie hoch sensitive molekulare Analysen. Zusätzlich ist es für semi-quantitative Abschätzungen von Abundanzen einzelner Arten notwendig, die aktuelle Abflusssituation sowie die Transportdistanz mit einzubeziehen.

Im Rahmen des FFG Projekts *„eDNA – AlpFisch: Detektion und semiquantitative Bestandserhebungen alpiner Fischarten mittels eDNA"* wurde in den letzten beiden Jahren der Einfluss von Abfluss und Transport auf die eDNA-Signalstärke verschiedener Fischarten in Flüssen untersucht. Bei einem Käfigversuch führten sowohl gesteigerter Abfluss als auch höhere Distanz zur DNA-Quelle zu geringeren Signalstärken. Zudem wurde die eDNA-Signalstärke direkt mit den Ergebnissen quantitativer Elektrobefischungen in tiroler Flüssen verglichen, wobei einerseits eine positive Korrelation zwischen den Resultaten der beiden Ansätze gezeigt werden konnte und andererseits der Einfluss von Fischpopulationen oberhalb der Beprobungsstelle auf die molekulargenetischen Nachweise deutlich wurde.

Die innerhalb des Projekts mit unterschiedlichen DNA-basierten Methoden erzielten Ergebnisse zeigen nicht nur beispielhaft die vielfältige Anwendbarkeit von eDNA zum Nachweis von Fischarten in alpinen Flüssen, sondern erlauben auch die gezielte Auswahl von Beprobungsstrategien für zukünftige Monitorings sowie eine korrekte Interpretation der dabei gewonnenen Daten.

Keywords: DNA barcoding, vertebrata, DNA transport, lotisch.

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Abstract

Power of ecological status assessment tools based on molecular data

Simon Vitecek, Wolfram Graf, Patrick Leitner,
Christian Moritz & Astrid Schmidt-Kloiber

Molecular approaches have become standard tools in ecological research. Yet, in context of monitoring and assessment schemes, e.g. of biodiversity or ecological status of waterbodies, they are rarely used in Europe. Ongoing initiatives aim at implementing molecular tools such as meta-barcoding in European standard assessment protocols, in particular for use in aquatic ecosystems. These ecosystems comprise a great variety of waters and are regulated by the Water Framework Directive (WFD) where compliant assessment tools and protocols based on identification and quantification of ecological communities have been put to use in all EU member states. Molecular data could enhance WFD-compliant ecological status assessment, as it holds the potential to increase temporal and spatial resolution by affording higher sample throughput. However, best practice protocols for using molecular approaches in standard assessment still need to be identified. In principal, there are two options: (a) calibrate new ecological status assessment tools based on molecular data; (b) make use of molecular tools by adapting existing protocols and ecological status assessment tools. Here we present results from a computational exercise aimed at clarifying potential of the second option for the detailed Austrian macrozoobenthos assessment method as a test case. We discuss assessment outcomes when using pseudo-molecular data, and identify potential pitfalls to the way to the implementation of molecular tools in ecological status assessment. Our results indicate that this is not a trivial feat, and will require substantial effort due to the comprehensiveness of the Austrian methodology.

Vitecek S., Graf W., Leitner P., Moritz C. & Schmidt-Kloiber A., 2018: Potenzial von Gewässerzustandsbewertungsverfahren auf Basis von molekular-genetischen Daten.

Die Anwendung molekularer Methoden ist mittlerweile Standard in der ökologischen Forschung. Dennoch wird dieser Ansatz im Rahmen der Überwachung und Einschätzung von z. B. Biodiversität oder des ökologischen Status von Wasserkörpern auf europäischer Ebene selten angewandt. Laufende Initiativen befassen sich mit der Einbindung molekularer Methoden wie dem Meta-Barcoding in standardisierte europäische Umweltüberwachungsprotokolle, insbesondere zur Bearbeitung aquatischer Ökosysteme. Aquatische Ökosysteme umfassen eine Vielzahl an Gewässern, die der Wasser-Rahmenrichtlinie (WRRL) unterliegen. Zu deren Einhaltung werden in allen EU-Mitgliedsstaaten adäquate Bewertungsverfahren angewandt, die sämtlich auf die Identifikation und Quantifizierung ökologischer Gemeinschaften abzielen. Molekulare Methoden könnten die WRRL-gemäße Erhebung des ökologischen Status erweitern, da durch sie die zeitliche und räumliche Auflösung durch einen schnelleren Probendurchsatz erhöht werden könnte. Jedoch muss ein optimaler methodischer Ansatz zur Nutzung molekularer Werkzeuge in standardisierten Einstufungsverfahren erst identifiziert werden. Grundlegend müssen hier zwei Herangehensweisen unterschieden werden: (a) Entwicklung und Kalibrierung neuer Methoden zur Erhebung des ökologischen Status auf Basis molekularer Daten; (b) Abwandlung existierender Bewertungsprotokolle, sodass molekulare Methoden einfach eingebunden werden können. Wir diskutieren Ergebnisse eines rechnerischen Experiments zur Abschätzung des Potenzials des zweiten Ansatzes für die *Detaillierte Makrozoobenthos Methode* zur Bewertung österreichischer Fließgewässer. Insbesondere stellen wir Bewertungsergebnisse, die auf Basis pseudo-molekularer Daten ermittelt wurden vor und identifizieren absehbare Fallstricke auf dem Weg zur Einbindung molekularer Werkzeuge. Unsere Ergebnisse weisen auf die Komplexität dieses Unterfangens hin, welches auf Grund der Genauigkeit der österreichischen Methode außerordentlichen Aufwand erfordern wird.

Keywords: DNA metabarcoding, EU Water Framework Directive, aquatic ecosystem assessment, benthic invertebrate fauna, biomonitoring, detailed macrozoobenthos method.

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Abstract

DNA barcoding in Tropical East Asia

John-James Wilson

Over the past 50 years, Tropical East Asia has lost more biodiversity than any other tropical region. Tropical East Asia is a megadiverse region with an acute taxonomic impediment. DNA barcodes (or DNA metabarcodes from high-throughput sequencers) may be especially useful for characterizing and connecting species-level biodiversity units in inventories encompassing taxa lacking formal description (particularly arthropods) and in large-scale, minimal-impact approaches to vertebrate monitoring and population assessments through secondary sources of DNA (invertebrate derived DNA and environmental DNA). DNA barcodes also illuminate interaction networks and provision of ecosystems services. Interest and capacity for DNA barcoding are slowly growing in Tropical East Asia, particularly among the younger generation of researchers who can connect with the barcoding analogy and understand the need for new approaches to the conservation challenges being faced.

Wilson J.-J., 2018: DNA-Barcoding in Ostasien.
In den letzten 50 Jahren hat das tropische Ostasien mehr Artenvielfalt eingebüßt als jede andere tropische Region. Das tropische Ostasien ist eine megadiverse Region, in der unser taxonomisches Wissen noch sehr beschränkt ist. DNA-Barcodes können besonders nützlich sein, um Arten zu charakterisieren und Taxa ohne formale Beschreibung (insbesondere Arthropoden) zuzuordnen und für Monitoring und Populationsbewertungen von Wirbeltieren mit minimalem Eingriff (DNA aus Parasiten und Umwelt-DNA). Mittels DNA-Barcodes können auch Interaktionsnetzwerke und die Bereitstellung von Ökosystemdienstleistungen untersucht werden. Das Interesse an und die Kapazität für DNA-Barcoding nimmt in Ostasien langsam zu, insbesondere bei der jüngeren Generation von Forschern. Diese sehen die Notwendigkeit neuer Ansätze, um den Herausforderungen im Biodiversitätsschutz gerecht zu werden.

Keywords: DNA barcoding, eDNA, metabarcoding

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Poster Abstract

First steps of systematic DNA barcoding in Croatia – example of caddisfly fauna (Trichoptera)

Anđela Ćukušić, Martina Podnar & Mladen Kučinić

Today DNA barcoding is a widely used method and many research organizations and national governments invest in DNA barcoding initiatives to reach the ultimate goal of a DNA barcode reference library for all life on Earth. In Croatia, one of the first steps was made with building up a comprehensive mtDNA *COI* barcode reference library for the caddisfly fauna within the Croatian barcode of life initiative – CroBOL. A major step forward in the construction of a genetic national library of biodiversity was facilitated by the ongoing project DNA barcoding of Croatian faunal biodiversity (CroBar Fauna). We not only continued to generate DNA barcodes of caddisflies, but likewise included other taxa.

The Trichoptera inventory was conducted on more than 100 localities in Croatia in various aquatic habitats starting from 2014 till today. We used the standard DNA barcoding approach with specimens vouchered in the Trichoptera DNA Barcode collection of the Natural History Museum in Zagreb. Full-length *COI*-5P DNA barcodes for most of the specimens were amplified using a standard set of primers: LCO1490/HCO2198. In several cases modified Folmer primers had to be used for successful amplification. So far we successfully amplified mt*COI* gene fragment for more than 250 individuals representing about 150 species or 70 % of the known Croatian caddisfly fauna. DNA barcoding confirmed our morphological identifications, helped to identify dubious species, enabled the recognition of morphologically cryptic species and connected sexes and life stages.

Ćukušić A., Podnar M. & Kučinić M., 2018: Erste Schritte zum systematischen DNA-Barcoding in Kroatien – am Beispiel der Köcherfliegenfauna (Trichoptera). Heutzutage ist DNA-Barcoding eine weit verbreitete Methode und viele Forschungsorganisationen und nationale Regierungen investieren in DNA-Barcode-Initiativen, um das ultimative Ziel einer DNA-Barcode-Referenzbibliothek für alles Leben auf der Erde zu erreichen. In Kroatien wurde mit dem Aufbau einer umfassenden mtDNA-*COI*-Barcode-Referenzbibliothek für die Köcherfliegenfauna im Rahmen der kroatischen Barcode-of-Life-Initiative CroBOL ein erster Schritt unternommen. Ein wesentlicher Fortschritt beim Aufbau einer genetischen Biodiversitätsdatenbank wurde durch das laufende Projekt DNA-Barcoding der kroatischen Artenvielfalt (CroBar Fauna) ermöglicht. Wir haben nicht weitere DNA-Barcodes von Köcherfliegen generiert, sondern auch andere Taxa inkludiert.

Die Bestandsaufnahme von Trichopteren wurde seit 2014 an mehr als 100 Orten in Kroatien in verschiedenen aquatischen Lebensräumen durchgeführt. Wir verwendeten den Standard-DNA-Barcoding-Ansatz, die Belegexemplare befinden sich im Naturhistorischen Museums in Zagreb. DNA-Barcodes wurden für die meisten Proben mittels Standardprimern amplifiziert (LCO1490 / HCO2198). In einigen Fällen mussten für eine erfolgreiche Amplifikation modifizierte Folmer-Primer verwendet werden. Bisher haben wir erfolgreich mt*COI*-Genfragmente für mehr als 250 Individuen amplifiziert, die etwa 150 Arten oder 70 % der bekannten kroatischen Köcherfliegenfauna repräsentieren. Die DNA-Barcodes bestätigten unsere morphologischen Bestimmungen, halfen bei der Identifizierung zweifelhafter Arten, ermöglichten die Erkennung kryptischer Arten sowie dazugehörender Geschlechter und Lebensstadien.

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Poster Abstract

Genetic species delimitation of selected arctic-alpine and boreo-montane animals in South Tyrol – a first view beyond the horizon

Andreas Eckelt, Paul D.N. Hebert, Peter Huemer, Petra Kranebitter,
Marko Mutanen & Alexander Rief

Due to long isolation and interrupted gene flow arctic-alpine and boreo-montane animals are among the outstanding examples of possible allopatric speciation processes. Generally, the comparison between central and north European populations is limited to a few taxa and has hitherto been focused on morphology. In the frame of a project supported by the South Tyrol Research Fund, representatives of species rich arthropod orders including Araneae, Orthoptera, Coleoptera, and Lepidoptera are surveyed for the first time in South Tyrol using DNA barcoding and, in the case of Lepidoptera, Next-Generation Sequencing analysis. The taxa are then examined and evaluated in view of genetic consistency with the Nordic populations. Throughout the groups all kind of different genetic variation was observed. In some cases the hypotheses could be confirmed and allopatric speciation as well cryptic diversity was found. On the other hand there were highly disjunct species which show no or very low genetic differences.

Eckelt A., Hebert P.D.N., Huemer P., Kranebitter P., Mutanen M. & Rief A., 2018: Genetische Artabgrenzung ausgewählter arktoalpiner und boreomontaner Tiere Südtirols – ein erster Blick über den Tellerrand.
Arktoalpine und boreomontane Tiere zählen auf Grund von Isolationsvorgängen und dadurch unterbrochenem Genfluss zu den Musterbeispielen möglicher allopatrischer Speziationsprozesse. Vergleiche von mittel- und nordeuropäischer Populationen beschränken sich jedoch üblicherweise auf wenige Taxa bzw. waren bislang morphologisch geprägt. Im Rahmen eines vom Südtiroler Forschungsfond geförderten Projektes konnten erstmals in Südtirol Vertreter artenreicher Tierordnungen, Schmetterlinge (Lepidoptera), Käfer (Coleoptera), Spinnen (Araneae) und Heuschrecken (Orthoptera) genetisch mittels DNA-Barcoding bzw. im Falle der Lepidopteren zusätzlich durch Next Generation-Sequencing-Methoden untersucht und in Hinblick auf ihre genetische Kongruenz mit arktischen Pendants geprüft und bewertet werden.

Im Focus der Untersuchungen standen 80 Arten mit vorwiegend stark disjunkter Verbreitung (300–3000 km lineare Distanz zwischen den einzelnen Populationen). Ein Großteil dieser Arten konnte erfolgreich gesammelt und sequenziert werden. Von ca. 20 Schmetterlingsarten liegen zusätzlich auch ddRADseq Daten vor. Erste Detailergebnisse weisen bei einigen Arten auf allopatrische Speziationsprozesse hin und belegen kryptische Diversität innerhalb dieser. Es zeigt sich aber auch, dass die genetische Diversität bei mehreren der disjunkt verbreiteten Arten sehr gering ist und die Populationstrennung noch zu keiner nachweisbaren Speziation geführt hat.

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Poster Abstract

Differentiation between blueberry cultivars and bilberry by high-resolution melt based DNA barcoding

Doris Feurle, Iva Nikolikj, Katja Zappe, Milena Stojkovska,
Jasmina Petreska Stanoeva, Marina Stefova & Margit Cichna-Markl

Bilberries (*Vaccinium myrtillus*) are considered to have higher health benefits than blueberries (*Vaccinium corymbosum*). As the cultivation of *V. myrtillus* is also much more challenging and cost intensive, this species is often adulterated on the market. In addition to the detection of food adulterations, the preservation of biodiversity plays a crucial role.

The authenticity of plants is often verified with high-performance liquid chromatography – mass spectrometry (HPLC – MS) or HPLC-UV/VIS methods, determining anthocyanin profiles. The advantage of DNA based methods is that in contrast to the anthocyanin profiles, the DNA sequence is independent from variations by environmental conditions, storage and treatment of the plants. Consequently, DNA based methods are more suitable for the identification of different varieties.

The aim of this study was to develop an appropriate DNA barcoding assay to differentiate between *V. myrtillus* and *V. corymbosum* and to identify different *V. corymbosum* varieties. A region of the internal transcribed spacer 1 (ITS1), which is located between the genes of ribosomal RNA, was amplified from DNA extracts from fruits and leafs by real-time polymerase chain reaction (PCR). Subsequently, the DNA strands were melted in high resolution. *V. myrtillus* and *V. corymbosum* could be distinguished by their different melt temperatures and different groups of *V. corymbosum* varieties by the different melt profiles of the ITS1 barcode region.

Feurle D., Nikolikj I., Zappe K., Stojkovska M., Stanoeva J.P., Stefova M. & Cichna-Markl M., 2018: Unterscheidung von Kulturheidelbeersorten und wilder Heidelbeere mittels einer auf hochauflösenden Schmelzkurven basierenden DNA-Barcoding Methode.

Der Wildheidelbeere (*Vaccinium myrtillus*) wird eine stärkere gesundheitsfördernde Wirkung als der Kulturheidelbeere (*Vaccinium corymbosum*) zugeschrieben. Da zudem der Anbau von *V. myrtillus* deutlich schwieriger und teurer ist, wird diese Spezies im Verkauf häufig verfälscht. Neben der Aufdeckung von Lebensmittelverfälschungen spielt auch der Erhalt der Biodiversität, z. B. die Re-Identifizierung von Sorten eine zentrale Rolle.

Zur Authentizitätsbestimmung von Pflanzen werden häufig Anthocyanprofile mittels Hochleistungsflüssigkeitschromatographie – Massenspektrometrie (HPLC – MS) oder HPLC-UV/VIS bestimmt. DNA-basierte Methoden haben den großen Vorteil, dass DNA-Sequenzen im Gegensatz zu Anthocyanprofilen nicht je nach Umweltbedingungen, Lagerung und Behandlung der Pflanzen variieren. Daher sind DNA-basierte Methoden besser zur Identifizierung von verschiedenen Sorten geeignet.

Das Ziel dieser Studie war es, einen geeigneten DNA-Barcoding Assay zu entwickeln, um *V. myrtillus* von *V. corymbosum* zu unterscheiden, sowie verschiedene *V. corymbosum* Sorten zu identifizieren. Ein Abschnitt des Internal transcribed spacer 1 (ITS1), der zwischen den Genen der ribosomalen RNA liegt, wurde aus DNA-Extrakten von Früchten sowie Blättern mittels Real-Time Polymerasekettenreaktion (PCR) vervielfältigt. Anschließend wurden die DNA-Stränge hochauflösend aufgeschmolzen. Aufgrund der verschiedenen Schmelztemperaturen konnten *V. myrtillus* von *V. corymbosum*, sowie verschiedene *V. corymbosum* Sortengruppen anhand ihrer unterschiedlichen Schmelzprofile des ITS1 Barcodes voneinander unterschieden werden.

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Poster Abstract

Barcoding Austria's damsel- and dragonflies

Iris Fischer, Lukas Zangl, Marcia Sittenthaler, Andreas Chovanec,
Kristina M. Sefc, Gernot Kunz, Stephan Koblmüller & Elisabeth Haring

The order Odonata, consisting of the suborders Anisoptera (dragonflies) and Zygoptera (damselflies), is one of the oldest groups of winged insects and originated more than 300 Million years ago. Even though the number of recognized species is fairly small compared to other groups of insects (Austria: 78 species, Europe: 143 species), they are important bioindicators and flagship species, as they have high species specific habitat claims and they are well known in the non-scientific community. In the framework of ABOL and associated projects we aim at cataloguing and DNA barcoding all Austrian species of dragonflies and damselflies, covering their geographic distribution in Austria to finally provide a complete DNA barcode reference data base for these taxa and to roughly asses their intraspecific variation. For this task, new sets of Odonata-specific primers have been designed. So far, 78 % of the Austrian dragonfly species have been collected and from 64 % DNA barcodes were obtained. The data obtained for Austrian samples will (1) complete the European data set by providing species and sequences hitherto not included in international barcode reference libraries and (2) contribute to the assessment of intraspecific variation of species with a wider distribution in Europe.

Fischer I., Zangl L., Sittenthaler W., Chovanec A., Sefc K.M., Kunz G., Koblmüller S. & Haring E., 2018: Barcoding der österreichischen Libellenfauna.
Mit einem Alter von mehr als 300 Millionen Jahren, zählt die Gruppe der Odonata zu einer der ältesten Gruppen geflügelter Insekten. Sie umfasst, verglichen mit anderen Insektengruppen, eine überschaubare Artenzahl: Das österreichische Arteninventar besteht aus 78 Libellenspezies, das europäische umfasst 143 Arten. Ihre hohen artspezifischen Ansprüche an ihre Brutgewässer und ihre Popularität in der Öffentlichkeit machen sie zu wichtigen Bioindikatoren und Flaggschiffarten. Im Rahmen der ABOL-Initiative und assoziierten Projekten werden DNA-Barcodes von allen österreichischen Arten generiert, mit dem Ziel eine vollständige DNA-Datenbank für diese Artengruppe zu erstellen. Dabei soll die geographische Verbreitung jeder Art Berücksichtigung finden, um die intraspezifische Variation einschätzen zu können. Hierfür wurden neue libellenspezifische Primer konstruiert. Bisher wurden 78 % der österreichischen Libellenspezies gesammelt und von 64 % DNA-Barcodes generiert. Mit dem österreichischen Datenset werden einerseits internationale Referenzdatenbanken um Arten ergänzt, für die bisher noch keine DNA-Barcodes verfügbar waren, andererseits dient es dazu, intraspezifische Variation von Arten mit einer weiten Verbreitung besser beurteilen zu können.

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Poster Abstract

Who am I? – DNA analyses of root stalagmites

Tobias Grasegger, Petra Cech, Matthias Kropf & Andreas Tribsch

Plant species identification can be challenging. Standard identification keys for plants are mainly based on individuals with flowers and other morphological characters. The Austrian Barcode of Life initiative tries to create DNA barcodes for every Austrian plant species to be able to easily identify specimens by DNA barcoding analyses. This could help for identifying plant individuals where "traditional" plant identification is difficult like plant roots.

Root stalagmites (Pavuza & Cech, 2013) are a rather poorly studied phenomenon, first mentioned in 1975 by speleologists, and at the moment known from caves in ten countries. These root structures develop near to cave entrances, where water is dripping from the ceiling onto roots and growth against gravity as well as the formation of tiny root hairs is induced. Which tree species are involved is largely unknown.

This study aims for identifying the plant species of the eight root stalagmites collected in caves in Upper and Lower Austria studied by Pavuza & Cech (2013) by DNA analyses.

To do so, a rough vegetation survey of trees and shrubs was done for the caves. Three different chloroplast DNA regions (*matK*, *trnL-trnF*, *atpI-atpH*) were amplified and sequenced, and these obtained sequences were compared to reference sequences of contemplable plant species and thus identified at the genus and species level.

Grasegger T., Cech P., Kropf M. & Tribsch A., 2018: Wer bin ich? – DNA-Analysen von Wurzelstalagmiten.

Die Bestimmung von Pflanzen kann einen vor Herausforderungen stellen. Übliche Bestimmungswerke basieren auf Blüten der Pflanzen und anderen morphologischen Merkmalen. Die „Austrian Barcode of Life"-Initiative versucht für jede Pflanzenart in Österreich DNA-Barcodes zu erstellen, um einzelne Individuen leicht identifizieren zu können. Das könnte helfen, Pflanzen zu bestimmen, wenn eine „traditionelle" Bestimmung nicht möglich ist – wie zum Beispiel bei Wurzeln.

Wurzelstalagmiten (Pavuza & Cech, 2013) sind ein bisher noch relativ wenig untersuchtes Phänomen. Die erste Erwähnung war im Jahr 1975 von Speläologen, und im Moment kennt man Vorkommen in zehn Ländern. Diese Wurzelgebilde entstehen in Höhlen nahe dem Eingang, wo kontinuierlich Wasser auf Wurzeln tropft. Dadurch wird Wachstum gegen die Schwerkraft und die Bildung von Wurzelhärchen gefördert. Welche Baumarten hinter den Wurzelstrukturen stecken ist größtenteils noch unbekannt.

Diese Studie versucht, die Pflanzenarten von acht Wurzelstalagmiten mittels DNA-Analysen zu identifizieren. Die Proben stammen aus Höhlen in Ober- und Niederösterreich, die von Pavuza & Cech (2013) untersucht wurden.

Dafür wurde eine grobe Vegetationsaufnahme von Bäumen und Sträuchern rund um den Höhleneingang durchgeführt und drei verschiedene Chloroplastenregionen (*matK*, *trnL-trnF*, *atpI-atpH*) amplifiziert und sequenziert. Die erhaltenen Sequenzen wurden mit vorhandenen Referenzsequenzen von in Frage kommenden Pflanzenarten verglichen und dadurch auf Gattungs- und Artniveau identifiziert.

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Poster Abstract

Analyzing historical plant-pollinator interactions by conducting pollen metabarcoding on natural history collections of German bumblebee species

Andreas Kolter, Michael Ohl, Markus Ankenbrand, Alexander Keller,
Ingolf Steffan-Dewenter & Birgit Gemeinholzer

Bumblebees are considered keystone species in ecosystems and declines in their abundance and diversity negatively affects other wildlife. 19 of the 38 bumblebee species that occur in Germany are classified as rare or extremely rare. Very few studies have addressed the question of whether bumblebee declines are associated with loss of floral resources due to contemporary anthropogenic changes in landscapes. The advent of metabarcoding has provided new tools with the potential to investigate dependencies between floral resources and pollinator diversity in greater detail than was possible in the past.

In-silico evaluation of newly generated primers demonstrates a reduction of mismatches across the European flora compared to currently available primers. Sequences generated from biomass (pollen, plant tissue, fungal spores and other unknown components) adhering to the bumblebee's body, provided information on environmental interactions that were not observed in the analysis of the pollen provisioned for larval nutrition.

Our effort to metabarcode pollen found on the bodies of bumblebee collections from three time periods of important agricultural shifts (before 1950, 1950–1980, after 1980) has the potential to provide valuable insight of interactions between bumblebees and the environment, possibly explaining bumblebee declines in Germany. Our sampling effort will be comprehensive, comprising 20 specimens per species and time period (~2250 specimen in total).

Kolter A., Ohl M., Ankenbrand M., Keller A., Steffan-Dewenter I. & Gemeinholzer B., 2018: Pollen Metabarcoding von naturkundlichen Sammlungen ermöglicht historische Betrachtungen der Bestäubernetzwerke deutscher Hummelarten.

Hummeln werden oftmals als Schlüsselarten angesehen und der Rückgang ihrer Häufigkeit und Verbreitung hat weitreichende Folgen auf das betroffene Ökosystem. Von den 38 deutschen Arten werden offiziell 19 als mindestens selten eingestuft. Relativ wenige Studien haben sich bisher mit der Frage beschäftigt welcher Zusammenhang zwischen einem Rückgang floraler Ressourcen durch Landreformen und Veränderungen in der Artenvielfalt der Hummeln in einem betroffenen Areal besteht. Die Standardisierung der mit dem Metabarcoding verbundenen Methoden stellt neue Werkzeuge zur Verfügung, die einen tieferen Einblick in das Wechselspiel zwischen Bestäuber und Umwelt erlauben als dies bisher möglich war.

Bisherige Ergebnisse umfassen beispielsweise in silico Evaluationen von neu erstellten ITS Primern, welche eine bessere Übereinstimmung zu Zielsequenzen der europäischen Flora aufweisen als bisher publizierte Primer, währenddessen aber gleichzeitig ihre Präferenz zum Pflanzenreich erhalten bleibt. Körperpollenproben erschließen vielfältigere Wechselwirkungen mit Pflanzen, als dies aus den Beinpollen, die der Nahrung der Larven dienen, ersichtlich wäre.

Unsere Anstrengungen Metabarcoding auf Hummelpollen anzuwenden hat das Potential wertvolle Einblicke in vergangene ökologische Zusammenhänge zu eröffnen und letztendlich Erklärungsansätze für rückläufige Hummelausbreitungen in Deutschland zu liefern. Die Proben, gesammelt aus 3 Zeitabschnitten (<1950, 1950–1980, >1980), umfassen etwa 20 Individuen pro Art und Zeiteinheit werden (insgesamt 2250 Individuen) aus allen Bundesländern umfassen.

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Poster Abstract

Biodiversity Austria – A science-policy-society interface for biodiversity and ecosystem services in Austria

Tanja LUMETSBERGER & Andrea HÖLTL

Over the last years a series of national and international biodiversity networks were established, like IPBES (INT), the Forum Biodiversität Schweiz (CH), nefo (DE), and the Belgian Biodiversity Platform (B). With September 2017 a coordination centre for an Austrian-wide network on biodiversity and ecosystem services was installed at the Danube University Krems – the Biodiversity-Hub – with the objective to fully establish a similar national platform in Austria – the Biodiversity Network Austria. The two core areas of the Biodiversity-Hub, a project funded by the state government of Lower Austria in the course of the FTI programme, are (1) to connect the various stakeholders in the field of biodiversity and ecosystem services and (2) to develop and install a central data repository for biodiversity data – the Biodiversity Atlas Austria. By providing the infrastructure in form of a science-policy-society interface, the Biodiversity-Hub aims to strengthen biodiversity in Austria and to highlight the value of ecosystems and their services for society. The goals are to (i) stimulate communication and knowledge transfer between the different stakeholders (e.g. researchers, policy-makers, practicioners, general public), (ii) support stakeholders in their research and decision-making processes, (iii) improve the understanding of Austria's biodiversity, (iv) increase the knowledge of Austria's biodiversity in society, but also (v) identify scientific gaps and the needs for research in the field of biodiversity and ecosystem services.

LUMETSBERGER T. & HÖLTL A., 2018: Biodiversität Österreich - Eine Schnittstelle zwischen Wissenschaft, Politik und Gesellschaft für Biodiversität und Ökosystemleistungen in Österreich.

In den letzten Jahren sind eine Reihe von nationalen und internationalen Biodiversitätsnetzwerken entstanden, wie IPBES (INT), das Forum Biodiversität Schweiz (CH), nefo (DE) oder die Belgian Biodiversity Platform (B). Mit September 2017 wurde eine Koordinationsstelle für ein österreichweites Netzwerk zu Biodiversität und Ökosystemleistungen an der Donau-Universität Krems eingerichtet – der Biodiversitäts-Hub – mit dem Ziel eine ähnliche nationale Plattform in Österreich aufzubauen – das Netzwerk Biodiversität Österreich. Die zwei Schwerpunkte des Biodiversitäts-Hubs, einem Projekt gefördert durch das Land Niederösterreich im Zuge des FTI-Programms, sind (1) die Vernetzung von unterschiedlichen Stakeholdern im Bereich Biodiversität und Ökosystemleistungen und (2) der Aufbau eines zentralen Datenbankmanagements für Biodiversitätsdaten – dem Biodiversitäts-Atlas Österreich. Durch die Bereitstellung der Infrastruktur in Form eines Science-Policy-Society Interface, zielt der Biodiversitäts-Hub darauf ab, die Biodiversität in Österreich zu stärken und den Wert von Ökosystemen und ihrer Leistungen für die Gesellschaft hervorzuheben. Die Ziele bestehen darin (i) die Kommunikation und den Wissenstransfer zwischen den verschiedenenen Stakeholdern (u. a. ForscherInnen, EntscheidungsträgerInnen, PraktikerInnen, Bevölkerung) zu fördern, (ii) sie in ihrer Forschung und in ihren Entscheidungsprozessen zu unterstützen, (iii) das Wissen über Österreichs Biodiversität zu verbessern, (iv) das Verständnis von Biodiversität in der Gesellschaft zu erhöhen, aber auch (v) Forschungslücken und Forschungsbedarf im Bereich Biodiversität und Ökosystemleistungen zu identifizieren.

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Poster Abstract

Game of Clones – DNA barcoding as a tool to discriminate *Fallopia* hybrids

Christina Pichler-Koban, Anneliese Fuchs,
Michael Jungmeier & Roland Eberwein

Fallopia japonica as an invasive alien species to Europe and North America presents a significant problem to the existing flora and is extremely difficult to get rid of. Besides the ecological implications, the plant is also of economic significance since it poses a threat to infrastructures, agricultural land and humus depots. This issue is subject to numerous research projects, "Game of Clones" is one of them and approaches the topic in a playful manner. The results lead to the development of a board game and a computer simulation that teach about the behavior of knotweed and the methods to control the plant.

The difference between *Fallopia japonica* and *Fallopia sachalinensis* is clear when comparing size and hairiness of the leaves. Due to the hybridization of both species and its product *Fallopia x bohemica*, a solid phenological identification has become considerably harder and sometimes impossible. For a serious discussion about the plant and the development of a management concept to control its distribution, it is important to know what we are dealing with. That is where DNA barcoding becomes a powerful tool to contribute to the identification of an invasive species. 95 leaf samples from *Fallopia* species were collected in July 2018 in Carinthia and Styria in Austria to be dried and sent to the Canadian Centre for DNA Barcoding (CCDB). Ten of the samples are from botanical gardens and herbaria that provide competently identified specimens of *F. sachalinensis* and *F. japonica* and will serve as references. The procedure requires the analysis of two DNA compartments (chloroplast and nuclear) – The chloroplast marker is inherited from the maternal organism, so by using it, it will be clear what species was maternal. The nuclear marker will indicate if the plant is homo- or heterozygote, therefore a hybrid.

The expected outcome will be a clear picture of the occurrence and distribution of the *Fallopia* species and hybrids in Carinthia and Styria. Genetic differences in the specimens could have implications for the recommendation of measures and actions in order to combat the invasive species.

Pichler-Koban C., Fuchs A., Jungmeier M. & Eberwein R., 2018: Game of Clones – DNA-Barcoding als Werkzeug zur Unterscheidung von *Fallopia*-Hybriden.
Fallopia japonica ist eine nach Europa und Nordamerika eingewanderte invasive Pflanze. Sie bereitet ob ihrer Konkurrenzkraft der bestehenden Flora erhebliche Probleme und ist nur schwer unter Kontrolle zu bringen. Neben den ökologischen Auswirkungen ist die Verbreitung dieser Pflanze auch ökonomisch von Bedeutung. Sie kann Schäden an baulichen Infrastrukturen, auf landwirtschaftlichen Flächen und auf Humusdeponien bewirken. Dieses Problem ist Gegenstand zahlreicher Forschungsprojekte. Das im Sparkling Science-Programm laufende „Game of Clones" ist eines davon, es nähert sich dem Thema auf spielerische Art und Weise. Die Erkenntnisse aus dem Projekts fließen in ein Brettspiel und eine Computersimulation, die das Verhalten der Pflanze und die Methoden, sie in den Griff zu bekommen, vermitteln sollen.

Die Unterscheidung von *Fallopia japonica* und *Fallopia sachalinensis* fällt auf den ersten Blick leicht, die Größe und Behaarung der Blätter sind sehr verschieden. Aufgrund fortlaufender Hybridisierung der beiden Arten und deren Produkt *Fallopia x bohemica*, ist eine solide phänologische Bestimmung erheblich schwieriger geworden, teilweise sogar unmöglich. Um eine sachliche Diskussion über die Pflanze zu ermöglichen und ein geeignetes Managementkonzept zu entwickeln, ist es wichtig zu wissen, welche Arten im Forschungsgebiet tatsächlich vorkommen. DNA Barcoding kann hier einen wichtigen Beitrag zur Bestimmung und Erforschung einer invasiven Art leisten.

95 Blattproben von *Fallopia*-Arten wurden im Juli 2018 in Kärnten und in der Steiermark gesammelt, getrocknet und an das *Canadian Centre for DNA Barcoding* (CCDB) übermittelt. Zehn der Proben stammen aus Botanischen Gärten und Herbarien, die im Besitz von verlässlich bestimmten Exemplaren der Arten *F. sachalinensis* und *F. japonica* sind, diese Proben fungieren als Referenzen. Die Prozedur erfordert die Analyse von zwei DNA-Abschnitten (Chloroplast und Kernmarker). Der Chloroplastenmarker wird von der mütterlichen Pflanze vererbt, dadurch wird klar, von welcher Art der Samen stammt. Der Kernmarker gibt an, ob die Pflanze homozygot ist oder heterozygot und folglich ein Hybrid.

Das erwartete Ergebnis soll ein klares Bild des Vorkommens und der Verbreitung von *Fallopia*-Arten und -Hybriden in Kärnten und der Steiermark darstellen. Genetische Unterschiede zwischen den Proben können Auswirkungen auf die Maßnahmenempfehlung nach sich ziehen, um diese invasive Art zu bekämpfen.

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Poster Abstract

DNA barcoding of harvestmen (Arachnida, Opiliones)

Miriam Schaider, Sylvia Schäffer, Tone Novak,
Michaela Bodner & Günther Raspotnig

Many harvestmen species are small, not easily collectable and can only be determined by specialists. Moreover, a particular issue refers to cryptic speciation as well as unresolved species-complexes that, specifically in soil-dwelling taxa, make taxonomical work difficult. These issues affect genetic databases: published barcodes on harvestmen have remained fragmentary, currently covering conspicuous and frequently occurring taxa only. We here exemplify these problems on the soil-dwelling harvestmen genus *Nemastoma*. 1) Poor database: In GenBank/BOLD, 25 and 37 records, respectively, on species of *Nemastoma* are compiled for sequences of subunit 1 of CO1, 10 (20, resp.) of which refer to *N. lugubre*, 6(7) to *N. triste*, 6(6) to *N. dentigerum*, 1(2) to *N. bimaculatum*, and 1(1) to *N. bidentatum*. In addition, *N. hankiewiczii*, actually a synonym for *Centetostoma hankiewiczii*, is mentioned. There is no data on the remaining species, *N. schuelleri*, *N. transsylvanicum* nor on sub-species of *N. bidentatum*: *N. b. bidentatum*, *N. b. pluridentatum*, *N. b. relictum, N. b. sparsum.* 2) Misidentification, undescribed species: A serious problem in barcoding of *Nemastoma* refers to the difficult determination of species/subspecies and to an unknown number of undescribed species/subspecies. For instance, one record refers to *N. bidentatum* from Croatia, but it is impossible to assign this data to a particular subspecies. Similar to the situation in Slovenia, a large number of currently undescribed subspecies in the *N. bidentatum*-complex is expected for Austria too. 3) Neither common barcoding primer in Opiliones (LCO1490/HCOoutout) nor designed primer work for particular taxa in *Nemastoma*. Alternative marker on subunit 2 though work, but not a single sequence for subunit 2 is currently available in genetic databases. Conclusion: Barcoding, only in combination with a thorough revision of particular harvestmen-groups, will lead to first general insights into true opilionid diversity.

Schaider M., Schäffer S., Novak T., Bodner M. & Raspotnig G., 2018: DNA-Barcoding von Weberknechten (Arachnida, Opiliones).
Viele Weberknecht-Arten sind klein, schwer zu sammeln und nur vom Spezialisten bestimmbar. Zusätzlich erschweren kryptische Arten/ nicht aufgelöste Artenkomplexe die taxonomische Arbeit. Diese Probleme spiegeln sich in genetischen Datenbanken wider: es sind bislang nur wenige Barcodes verfügbar, die hauptsächlich auffällige und häufige Arten abdecken. Am Beispiel der Gattung *Nemastoma* weisen wir auf Probleme beim Barcoding hin. 1) Unvollständige Datenlage: Für *Nemastom*a gibt es in Genbank/BOLD 25 bzw. 37 Einträge für die Region 1 von CO1, 10(20) beziehen sich auf *N. lugubre*, 6(7) auf *N. triste*, 6(6) auf *N. dentigerum*, 1(2) auf *N. bimaculatum*, und 1(1) auf *N. bidentatum*. Außerdem gibt es einen Eintrag für „*N. hankiewiczii*“, eigentlich ein Synonym für *Centetostoma hankiewiczii*. Für *N. schuelleri*, *N. transsylvanicum* bzw. die Unterarten von *N. bidentatum* (*N. b. bidentatum, N. b. pluridentatum, N. b. relictum, N. b. sparsum)* fehlen Daten. 2) Fehlbestimmungen/unbeschriebene Arten: Ein großes Problem beim Barcoding von *Nemastoma* ist einerseits die Bestimmung bekannter Arten/Unterarten, andererseits noch unbeschriebene Arten/Unterarten. Beispielsweise kann ein Eintrag von *N. bidentatum* aus Kroatien unmöglich einer der Unterarten zugeordnet werden. Da neue Unterarten für Slowenien beschrieben werden, sind auch für Österreich weitere Unterarten des *N. bidentatum*-Komplexes zu erwarten. 3) Primer: Die üblichen Primer für die Barcoding-Region (LCO1490/HCOoutout) funktionieren bei einigen *Nemastoma*-Arten nicht, dasselbe gilt für zurzeit neu entwickelte Primer. Die Region 2 von CO1 konnte bei den problematischen Taxa zwar amplifiziert werden, aber in den Datenbanken sind dazu keine entsprechenden Sequenzen enthalten. Conclusio: Nur in Kombination mit einer ausführlichen Revision problematischer Weber-

knecht-Taxa kann das Barcoding erste Einblicke in die tatsächliche Diversität der heimischen Weberknechte liefern.

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Poster Abstract

Austrian Biodiversity Monitoring "ÖBM-Kulturlandschaft": concept and first results of the baseline survey

Stefan Schindler, Gebhard Banko, Dietmar Moser, Roland Grillmayer, Klaus Peter Zulka, Wolfgang Rabitsch, Franz Essl, David Paternoster, Markus Staudinger, Thomas Zuna-Kratky, Nina Gallmetzer, Martin Neuwirth, Stefan Lackner, Elia Guariento,
Kathrin Pascher & Maria Stejskal-Tiefenbach

The Austrian biodiversity monitoring program "ÖBM-Kulturlandschaft" is focussed on habitat and species diversity in cultural landscapes, including alpine pastures. A stratified random selection of sampling sites was conducted based on the 1 km² grid of Statistics Austria with the condition that at least 50 % is open cultural landscape. The 100 selected nested sampling plots are arranged hierarchically by (i) remote sensing based landscape surveys: 3 × 3 km² - landscape plots, (ii) habitat mapping: 625 m x 625 m test areas, and (iii) 10 test circles (20 m radius) for surveys of vascular plants, grasshoppers and butterflies per sampling area. The baseline survey (funded by the Rural Development Programm) took place in 2017/18, with 50 locations sampled per year. The repetition of surveys should take place every three to five years. Remote sensing data are applied on three levels: phenological characterizations of the habitat types (within the 625 m x 625 m sampling plots); detection of changes in ecosystem functions (e.g. NDVI) and ecosystem structure (e.g. land cover of the landscape plots of 3 × 3 km²) and nation-wide analysis of land cover change (with the COPERNICUS products available for the entire EU).

The recording of habitat types is based on the red lists published by the Environment Agency Austria. Vascular plants, grasshoppers and butterflies were chosen mainly for being optimal surrogates for overall biodiversity, suppliers of ecosystem services, and due to practical advantages in surveying.

Preliminary results from 2017 are that approximately 230 habitat types, 1250 species of vascular plants (42 % of Austrian species; n = 49 test areas), 69 species of grasshoppers (49 % of Austrian species; n = 48 test areas), and 103 species of butterflies (48 %, n=49) were detected. Average species richness of test areas was 10.6±4.6 for grasshoppers (3.9±2.9 per test circle) and 10.5±4.7 for butterflies (2.8±2.2 per test circle).

Schindler S., Banko G., Moser D., Grillmayer R., Zulka K.P., Rabitsch W., Essl F., Paternoster F., Staudinger M., Zuna-Kratky T., Gallmetzer N., Neuwirth M., Lackner S., Guariento E., Pascher K. & Stejskal-Tiefenbach M., 2018: Österreichisches Biodiversitätsmonitoring „ÖBM-Kulturlandschaft": Konzept und erste Ergebnisse der Basiserhebung.

Das Österreichische Biodiversitätsmonitoring-Programm "ÖBM-Kulturlandschaft" beschäftigt sich mit der Lebensraum- und Artenvielfalt in der offenen Kulturlandschaft, inklusive der Almen. Die stratifizierte Zufallsauswahl der Erhebungsstandorte basiert auf dem 1 km² Raster der Statistik Austria, mit einem Mindestanteil an landwirtschaftlicher Nutzfläche von 50 % als Bedingung. Die 100 ausgewählten Stichprobenflächen sind hierarchisch angeordnet nach (i) Fernerkundungsbasierte Landschaftserhebung: 3 × 3 km² - Landschaftsausschnitte, (ii) Lebensraumtypenerhebung: 625 m x 625 m Aufnahmeflächen, und (iii) 10 Aufnahmepunkte (20 m Radius) für die Erhebung von Gefäßpflanzen, Heuschrecken und Tagfaltern pro Aufnahmefläche. Die Basiserhebung (gefördert durch das Programm für ländliche Entwicklung) wurde 2017/18 durchgeführt, wobei 50 Standorte pro Jahr erhoben wurden. Die Erhebungen sollten alle drei bis fünf Jahre wiederholt werden. Fernerkundungsdaten werden auf drei Ebenen genutzt: phänologische Charakterisierungen der Lebensraumtypen (innerhalb der 625 m x 625 m Aufnahmeflächen); Erfassung von Änderungen von

Ökosystemfunktionen (z. B. NDVI) und Ökosystemstrukturen (z. B. Landbedeckung auf den 3 × 3 km^2 Landschaftsausschnitten), und österreichweite Analysen der Landbedeckungsveränderung (anhand der EU-weit vorliegenden COPERNICUS-Produkte). Die Erfassung der Lebensraumtypen basiert auf den vom Umweltbundesamt veröffentlichten Lebensraumtypenlisten. Die Wahl auf Gefäßpflanzen, Heuschrecken und Tagfalter fiel aufgrund ihrer Eigenschaften als geeignete Surrogat-Taxa für die Gesamtbiodiversität, als Bereitsteller von Ökosystemleistungen, und ihren praktischen Vorteilen bei der Erhebung.

Laut vorläufigen Ergebnissen aus 2017 wurden ca. 230 Lebensraumtypen, 1250 Gefäßpflanzenarten (42 % der Österreichischen Arten; n = 49 Aufnahmeflächen), 69 Heuschreckenarten (49 % der Österreichischen Arten; n = 48), und 103 Tagfalterarten (48 %, n = 49) erfasst. Die durchschnittliche Artenvielfalt der Aufnahmeflächen betrug für die Heuschrecken 10.6±4.6 (3.9±2.9 pro Aufnahmepunkt) und 10.5±4.7 für die Tagfalter (2.8±2.2 pro Aufnahmepunkt).

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Poster Abstract

Differentiation of American cranberry, common cranberry and lingonberry by DNA barcoding and high resolution melting (HRM) analysis

Alexandra Wanka, Doris Feurle, Iva Nikolikj, Milena Stojkovska, Jasmina Petreska Stanoeva, Marina Stefova & Margit Cichna-Markl

Red berries and products thereof have become increasingly popular due to their high content of bioactive and acclaimed health promoting ingredients. Foodstuffs – including berry products – have to be safe as well as authentic so there is a demand for analytical methods capable of authentication.

The *Vaccinium* genus covers – among other berries – the species American cranberry (*Vaccinium macrocarpon* Ait.), common cranberry (*Vaccinium oxycoccos* L.) and lingonberry (*Vaccinium vitis-idaea* L.). Translational errors or false declaration, as well as the replacement of one berry species for another, show the basic need for a method to detect such adulterations. DNA barcoding as an analytical tool gained attention in the analysis of foodstuff. Within the scope of this work two barcoding regions (MatK and ITS) were investigated via polymerase chain reaction (PCR) and subsequently analyzed by high resolution melting (HRM) analysis. Different primer sets for both barcoding regions were designed and different food groups tested (juices, jams, supplements, chocolate, and teas).

The developed method using the ITS region turned out to be capable of differentiating between the three *Vaccinium* species and was also applicable to highly processed foodstuffs, such as juices or jams, and even food supplements.

Wanka A., Feurle D., Nikolikj I., Stojkovska M., Stanoeva J.P., Stefova M. & Cichna-Markl M., 2018: DNA-Barcoding-Regionen zur Unterscheidung von Preiselbeere, Moosbeere und Cranberry.

Rote Beeren erfreuen sich auf Grund der positiven Wirkung ihrer Inhaltsstoffe auf die Gesundheit steigender Beliebtheit. Auch für diese Lebensmittel gilt, dass sie nicht nur sicher, sondern auch authentisch sein müssen. Dazu werden Analysenmethoden benötigt, die diese Authentifizierung ermöglichen.

Neben diversen anderen Beeren umfasst die Gattung *Vaccinium* (Heidelbeeren) auch die häufig in Lebensmitteln vorkommenden Spezies Preiselbeere (*Vaccinium vitis-idaea* L.), die europäische Cranberry oder Moosbeere (*Vaccinium oxycoccos* L.) und die amerikanische Cranberry (*Vaccinium macrocarpon* Ait.). Diese Namen werden allerdings fälschlicherweise oft synonym verwendet, weshalb Bedarf an einer Methode zur Speziesidentifizierung (in Lebensmitteln) besteht. DNA-Barcoding spielt im Bereich der Lebensmittelanalytik eine große Rolle. Im Rahmen dieser Arbeit wurden die Barcodingregionen MatK und ITS zur Unterscheidung herangezogen und als Analysenmethode die Polymerasekettenreaktion (PCR) mit anschließender hochauflösender Schmelzkurvenanalyse (HRM) angewandt. Neben Positivkontrollen wurden kommerziell erhältliche Säfte, Fruchtzubereitungen, Nahrungsergänzungsmittel, Tees und Schokolade analysiert. Für beide Barcodingregionen wurden Primerpaare entworfen. In der ITS-Region konnten so *V. macrocarpon*, *V. oxycoccos* und *V. vitis-idaea* anhand der Schmelztemperatur und des Kurvenverlaufes der Schmelzkurve unterschieden werden, was in der MatK Region nicht (oder nur zum Teil) möglich war.

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Poster Abstract

Testing methods of species delimitation with tiger moth samples from a mega-diverse Neotropical rainforest

Martina Wieser, Patrick Strutzenberger,
Brigitte Gottsberger & Konrad Fiedler

Lepidoptera are one of the most species-rich insect taxa worldwide, and their diversity is especially high in the tropics. However, a large proportion of extant species are not yet described in taxonomic terms or are still entirely unknown. This study focused on the genus *Eucereon* (Erebidae, Arctiinae) found in Central and South America, with currently more than 150 described species. Species of *Eucereon* are notoriously hard to distinguish due to their very similar external morphology. Thus far, this genus never underwent any proper taxonomic revision. On the Barcode of Life Data Systems (BOLD) there are currently 800 COI barcode sequences available for *Eucereon*, classified into 70 BINs. Over 70 % of those samples originate from Costa Rica. In this work, novel COI barcode sequences of 107 *Eucereon* specimens, collected around the Tropical Research Station La Gamba in the south-west of Costa Rica, were generated and compared to sequences available on BOLD. The aim of this study is to apply several state-of-the-art methods of species delimitation to *Eucereon* and compare their performance and resulting hypotheses about species boundaries. Several DNA-barcode based methods of species delimitation (e.g.: GMYC, ABDG, and pairwise distance thresholds) were applied to this dataset. Initial results indicate a high degree of concordance between morphology-based species delimitations and threshold-based clustering. The results will later be interpreted in a taxonomic and evolutionary context and used for improved regional biodiversity estimates.

Wieser M., Strutzenberger P., Gottsberger B. & Fiedler K., 2018: Vergleich verschiedener Methoden der Artabgrenzung von Bärenspinner-Proben aus einem megadiversen neotropischen Regenwald.
Lepidoptera sind eine der artenreichsten Insektenordnungen weltweit und ihre Diversität ist in den Tropen besonders hoch. Allerdings ist ein großer Anteil der bestehenden Arten bisher nicht taxonomisch beschrieben oder sind gänzlich unbekannt. Diese Studie konzentrierte sich auf die Gattung *Eucereon* (Erebidae, Arctiinae), welche mit mehr als 150 beschriebenen Arten in Zentral- und Südamerika vorkommt. Aufgrund ihrer sehr ähnlichen äußerlichen Morphologie sind *Eucereon*-Arten besonders schwierig zu unterscheiden. Bisher wurde diese Gattung nie formell taxonomisch überarbeitet. In der Datenbank Barcode of Life Data Systems (BOLD) sind gegenwärtig 800 COI Barcode-Sequenzen für *Eucereon* verfügbar, welche in 70 BINs eingeordnet werden. Mehr als 70 % dieser Proben stammen aus Costa Rica. In dieser Arbeit wurden die COI Barcode-Sequenzen von 107 *Eucereon*-Exemplaren bestimmt, welche in der Nähe der Tropenstation La Gamba im Südwesten von Costa Rica gesammelt wurden, und mit auf BOLD verfügbaren Sequenzen verglichen. Ziel dieser Studie ist es, verschiedene moderne Methoden der Artabgrenzung auf *Eucereon* anzuwenden und deren Leistung und daraus resultierenden Hypothesen über Artgrenzen zu vergleichen. Verschiedene DNA-Barcode-basierte Methoden der Artabgrenzung (z. B. GMYC, ABDG und paarweise Distanzschwellen) wurden bei diesem Datensatz angewendet. Erste Ergebnisse deuten auf eine sehr hohe Übereinstimmung zwischen morphologischer Artabgrenzung und Schwellenwert-basiertem Clustering hin. Die Ergebnisse werden später in taxonomischem und evolutionärem Kontext interpretiert und für verbesserte Abschätzungen der regionalen Biodiversität genützt.

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Poster Abstract

Barcoding Austria's scorpionflies: unexpected cryptic diversity

Lukas Zangl, Angelina Hubmann & Stephan Koblmüller

With only several hundred known species worldwide, scorpionflies (Mecoptera, Panorpidae) are a rather species-poor family of winged insects. Five species, *Panorpa alpina*, *P. cognata*, *P. communis*, *P. germanica* and *P. vulgaris* are reported for Austria. First DNA barcodes of these species, generated in the framework of the ABOL (Austrian Barcode of Life; www.abol.ac.at) initiative, however, cluster into seven distinct mitochondrial lineages. *Panorpa communis* samples fall in two distinct lineages that are resolved as paraphyletic with respect to *P. vulgaris*. Similar results were found in *P. alpina*, which also revealed a second mitochondrial lineage. Whether these two *P. communis* and *P. alpina* lineages indeed represent distinct species shall be clarified by means of morphological/morphometric and nuclear multi-locus data.

Zangl L., Hubmann A. & Koblmüller S., 2018: Barcoding österreichischer Skorpionsfliegen: unerwartete kryptische Diversität.
Skorpionsfliegen (Mecoptera, Panorpidae) stellen mit nur einigen wenigen hundert Arten weltweit eine der artenärmeren Insektenfamilien dar. In Österreich wurden mit *Panorpa alpina, P. cognata, P. communis, P. germanica* und *P. vulgaris* bisher fünf Arten nachgewiesen. Anhand der für die „Austrian Barcode of Life“ Biodiversitätsinitiative (ABOL, www.abol.ac.at) generierten DNA-Barcodes zeigten sich jedoch sieben distinkte mitochondrielle Linien. Ob diese zwei zusätzlichen Linien neue, selbstständige Arten in Bezug auf *P. communis* und *P. alpina* darstellen, soll in Zukunft anhand morphologisch-morphometrischer sowie nuklearer Multi-Lokus-Analysen ermittelt werden.

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Buchbesprechungen

Helen Bynum & William Bynum, 2017: Das Wesen der Pflanze. Botanische Skizzenbücher aus 6 Jahrhunderten. Haupt Verlag in Zusammenarbeit mit den Royal Botanic Gardens Kew. ISBN 978-3-258-08078-9.

In diesem Buch wird vom „Bedürfnis zu dokumentieren" geschrieben – gemeint ist: als *Bild* zu dokumentieren. Warum wohl machen wir in allen möglichen und unmöglichen Situationen und an vielen aufgesuchten Orten *selfies*? Wir wollen im Bild festhalten, was wir sehen und erleben. Ein Bild kann als Grundlage für sprachliche Kommunikation dienen, es liefert gewissermaßen Informationen auf einem optischen Kanal – nicht umsonst heißt es *„Ein Bild sagt mehr als 1000 Worte"*. Dabei werden an botanische Illustrationen, also bildliche Quellen über Pflanzen, besondere Anforderungen gestellt. Es gilt geradezu einen Kodex an Regeln zu erfüllen, die wichtigste ist: die differentialdiagnostischen Merkmale müssen erkannt und so wiedergegeben werden, dass die Abbildung dem lebenden Vorbild entspricht. Das Bild muss die Pflanze ersetzen können. Dazu sind botanisches Wissen und maltechnische Erfahrung Voraussetzung.

Im deutschen Sprachraum hat sich für ein spontanes dynamisches Herangehen an die Darstellung der Begriff *Skizze* etabliert, also für eine Methode, visuelle Information zu sammeln und aufzubewahren. Erst Ausarbeitung und Komposition machen daraus die botanische Illustration. Die Skizze ist ein wichtiger Schritt bei der Erarbeitung des Objekts und genau genommen viel interessanter als das fertige Bild – so wertet es auch die Autorin der Rezension, je länger sie sich mit diesem Thema beschäftigt. Die Skizze ist eine Gedächtnisstütze auf dem Weg, Vergängliches festzuhalten, ein Probierraum. Sie bietet mehr Spielraum für Experimente und persönlichen Ausdruck als die botanische Zeichnung, die bereits strengen künstlerischen und botanischen Vorgaben folgen muss. Die Skizze gewinnt im Lauf ihrer Entstehung durch die Erarbeitung der Farbschattierungen und Strukturen an Komplexität. Eine Ergänzung durch Notizen ist möglich und sogar typisch – dadurch wird das Bild mit dem Wort verbunden. Man bedenke jedoch, dass sie meist nicht für die Öffentlichkeit bestimmt war!

Vom Skizzieren geht es im Ablauf der Entstehung weiter zu *Studien*, in denen die Komposition des Bildes ausgearbeitet wird, also die Darstellung sich so entwickelt, dass Klarheit im Aufbau der dargestellten Pflanze hergestellt ist, und dann zur ausgearbeiteten botanischen Illustration.

Warum gewinnen plötzlich die lange vernachlässigten Skizzenbücher an Interesse? Ein möglicher Grund dafür ist der vermehrte Nachdruck von „Prachtbänden". Man hat dadurch die Skizzen als deren Quellen „entdeckt" und auch publiziert; dies ermöglicht einen Einblick in die Entstehung der Abbildungen.

Skizzen werden und wurden sehr oft im Freien angefertigt, z. B. von Margaret Mee (p. 36; die angegebenen Seitenzahlen beziehen sich jeweils auf das besprochene Buch), die bis ins hohe Alter im Amazonasgebiet mit dem Boot unterwegs war, um anders unerreichbare Orte aufzusuchen und - z. T. bis ins 20. Jahrhundert unbekannte - Pflanzen in ihrem Lebensraum zu porträtieren. Auch Albrecht Dürer nutzte seine vielen Reisen zu Naturstudien. Er schreibt: *„Denn wahrhaft steckt die Kunst in der Natur; wer sie heraus kann reißen, der hat sie"*. Wer kennt nicht sein „Großes Rasenstück" (p. 197), in dem er ein perfektes

Abbild der Natur schaffen wollte? Hier ist eine kleine Pflanzengesellschaft abgebildet, mit den „richtigen“, zusammengehörenden Arten; Friedrich EHRENDORFER analysierte 1985 aus Anlass der damaligen Ausstellung in der Albertina in Wien DÜRERS Pflanzenbilder auf ihre botanische Korrektheit.

Auf Grund der von Europa ausgehenden Kolonisierung der Welt strömten immer neue Pflanzen in die botanischen Gärten. Hier wurden sie dann wissenschaftlich untersucht, wobei häufig Herbarbelege und botanische Illustrationen die lebenden Pflanzen ersetzen mussten. Beispielsweise gewann man in Europa erst durch die Skizzen von Joseph Dalton HOOKER (auf seiner Reise 1847–1851) Eindrücke von den Himalajalandschaften (vgl. p. 173). Informationen aus Australien und Norfolk Island (vgl. p. 247) verdanken wir Ferdinand BAUER, der allein im Oktober 1805 die schier unglaubliche Zahl von 1750 Pflanzenskizzen anfertigte; dabei beschränkte er sich auf Bleistift und gab aus seinem eigenen System Farbcodes an, nach denen er später die richtige Farbe in die ausgearbeiteten Zeichnungen einfügen konnte. Seine Farbtabellen umfassten dabei 999 (!) Farbtöne. Um diese Form der Erforschung der Pflanzenwelt noch mit einem Detail abzurunden sei erwähnt, dass Conrad MARTENS als offizieller Zeichner des Forschungsschiffes HMS Beagle (p. 250–251) 1833 auch auf jener Reise dabei war, die Charles DARWIN 1831–1836 um die Welt führte und zu vielen seiner später publizierten Ideen inspirierte.

Das vorliegende Buch ist in folgende Kapitel gegliedert: „Im Feld“, „Wissenschaftler“, „Künstler“ und „Ein angenehmer Zeitvertreib“. Auf insgesamt 288 Seiten werden Skizzen und erklärende Texte geboten. Anhand der einzelnen vorgestellten Personen wird man episodenhaft mit der Geschichte der botanischen Illustration, der Entwicklung der Botanik als Wissenschaft und der Erforschung der Pflanzenwelt aus einem europazentristischen Gesichtspunkt bekannt gemacht. So sieht man Skizzen von berühmten Botanikern und Künstlern vereint mit solchen, die namentlich nicht so bekannt sind – eine sehr interessante Mischung! Besonders hervorzuheben ist die ungeheure Vielfalt, auf welche Weise Pflanzen dargestellt werden können, und die hohe Qualität der Abbildungen. Der persönliche Stil zeigt sich sehr individuell in den vielen, vielen Beispielen.

Die Literaturhinweise sind anstelle einer Bibliographie s.str. in Form einer Liste mit Büchern und online-Quellen (deren Abfragetermine, wie eigentlich üblich, nicht angegeben sind) zusammengestellt, die weitere Informationen über die vorgestellten Zeichner liefern. Hier sei angemerkt, dass im Kapitel „Ein angenehmer Zeitvertreib“ sehr viele zeichnerisch aktive Frauen aufscheinen, deren Werke also als nicht beruflich motiviert, nichts desto weniger aber sehr professionell das Vorkommen der „heimischen“ Floren in ihren Umgebungen dokumentieren, seien sie in England bzw. in der Kolonialzeit in Indien, Birma oder China zu Hause. Ein Register der Personen und Orte sowie der Pflanzennamen – wobei extra darauf hingewiesen wird, dass nach Möglichkeit die Pflanzen auf dem Bild mindestens bis zur Gattung bestimmt wurden; unbenannte Pflanzen oder solche, deren heutiger Name in der Bildlegende nicht genannt ist, werden extra angegeben – bietet weitere Möglichkeiten, Wissenswertes zu verknüpfen.

Ganz wichtig ist natürlich der Bildnachweis! Prominente Institutionen wie das British Museum London, Royal Botanic Gardens Kew, die Universitätsbibliothek Erlangen-Nürnberg und das Hunt Institute for Botanical Documentation in Pittsburgh haben Abbildungen aus ihren Archiven zur Verfügung gestellt, die hier zum Teil erstmals veröffentlicht wurden.

Die Autoren Helen Bynum (Biologin) und William Bynum (Mediziner) waren und sind vielfach wissenschaftsgeschichtlich tätig. Den Zeichnerinnen und Zeichnern mit ihren Mühen, Talenten, Abenteuern und Beweggründen sowie den Pflanzen, die deren Fantasie anregten, ist dieses Buch gewidmet.

Das Buch ist allen zu empfehlen, die sich für botanische Illustrationen und für die Geschichte der Botanik interessieren – und solchen, die Freude an wunderschönen Pflanzenbildern haben. Es steht in der Bibliothek der Zoologisch-Botanischen Gesellschaft zur Verfügung.

Petra Hudler und Wolfgang Punz

Leander Khil, 2018: Vögel Österreichs. 368 Seiten. Franckh-Kosmos Verlags-GmbH & Co. KG, Stuttgart. ISBN 978-3-440-15915-6.

Unter Ornithologen geht es in Gesprächen über Vogelbestimmungsbücher zunehmend um eine Art „Glaubensfrage“ Illustrationen versus Fotos. Das europäische Standardwerk – *Der Kosmos Vogelführer* von Lars Svensson – basiert auf Illustrationen von Killian Mullarney und Dan Zetterström, die in ihrer Qualität neue Maßstäbe in diesem Feld gesetzt haben. Für gezeichnete Illustrationen spricht unter anderem die Möglichkeit, Bestimmungsmerkmale etwas klarer herausarbeiten zu können als sie im Feld (oder auf einem Foto) tatsächlich zu sehen wären – also eigentlich im Sinne des besseren Verständnisses etwas zu „schummeln“. Als Beispiel soll eine Merkmalskombination an Bauch und Rücken eines Vogels dienen, die in der Realität nur bei idealer Körperhaltung des Vogels gleichzeitig zu sehen wäre, und selbst dann wohl meist unter unausgewogenen Lichtverhältnissen. Solche Schwierigkeiten können beim Zeichnen einer Illustration leicht überwunden werden.

Gerade für Neulinge im Bereich der Vogelbeobachtung kann es wiederum schwierig sein, die etwas abstrahierte Zeichnung in einem Bestimmungsbuch wieder auf den realen Vogel vor den eigenen Augen zu „übersetzen“. Dies gilt im Übrigen auch für erfahrenere Beobachter, wenn es um die Einarbeitung in ein neues Spezialgebiet in der Vogelbeobachtung geht – z. B. die Altersbestimmung von Greifvögeln. Hier bietet ein gutes Foto klarerweise eine unmittelbarere Entsprechung und oftmals einen leichteren Einstieg. Und in der Tat hat sich die Qualität solcher Fotos in den letzten Jahrzehnten derart gesteigert, dass viele der genannten Gegenargumente bereits weitgehend entkräftet sind – wenngleich es trotzdem mehrere Fotos brauchen kann, um den gleichen Informationsgehalt einer einzigen Illustration wiederzugeben.

Gerade für „Birdwatching“-Anfänger bietet *Vögel Österreichs* von Leander Khil deshalb wohl die größten Vorteile. Abgesehen von den 1157 hervorragenden Fotos (fast zur Hälfte vom Autor selbst) ist hier die namensgebende Beschränkung auf die heimische Vogelwelt an sich ein wesentlicher Faktor. Die hunderten zusätzlichen Arten, die selbstverständlich im *Kosmos Vogelführer* abgebildet sind aber in Österreich nie vorkommen, können hiesige Vogelbeobachtungs-Einsteiger förmlich erschlagen – und nebenbei zu spektakulären Fehlbestimmungen verleiten. Da hilft es enorm, sich auf eine tatsächlich in Frage kommende Auswahl an Arten (in diesem Buch 390 an der Zahl) beschränkt zu wissen; von den Verbreitungskarten ganz zu schweigen, die die regionale Verbreitung in Österreich (nach Jahreszeiten aufgeschlüsselt) klarerweise viel deutlicher zeigen als es eine Europakarte jemals könnte.

Auf jeder Doppelseite werden meist zwei, maximal drei Arten behandelt – Text und Karten auf einer Seite, Fotos auf der anderen. Je nach Art sind beide Geschlechter, unterschiedliche Kleider bzw. Altersstufen abgebildet. In der Aufteilung dieser Fotos zeigt sich einer der wenigen Schwachpunkte dieses Buches: werden mehrere Arten auf einer Seite präsentiert, ist die Aufteilung nämlich nicht immer einheitlich. Mal zeigen die vier Fotos in der linken Spalte die eine Art und die in der rechten Spalte die andere, mal sind es die oberen und unteren vier Fotos. Bei drei Arten auf einer Seite ist die Aufteilung natürlich wieder anders, das gleiche gilt aber auch, wenn Fotos in unterschiedlichen Formaten verwendet wurden. Die Konsequenz dessen ist, dass auch erfahrenere Beobachter fast immer einen Blick auf die Beschriftungsnummern werfen müssen, um zu verstehen welche Fotos zu welchen Arten gehören. Man fragt sich als Benutzer des Buches ob hier wirklich keine visuelle Hilfe, sei es in Form von dicker gezogenen Linien zwischen den Arten oder farblich unterschiedlichen Hintergrundflächen, möglich gewesen wäre.

Die „Illustrationen versus Fotos“ Frage ist bei diesem Buch aus weiterem Grund eigentlich hinfällig: Es verbindet nämlich das Beste aus zwei Welten und beinhalten sehr wohl auch 263 hervorragende Illustrationen, größtenteils vom Ungarischen Künstler Szabolcs Kókay (man fragt sich als Leser direkt wieso er nicht an prominenterer Stelle genannt wird als in den Danksagungen). Gerade auf den Doppelseiten mit vergleichenden Illustrationen ähnlicher Arten (z. B. die Watvögel auf S. 134/135 oder die Adler auf S. 204/205) bietet dieses Buch eine Besonderheit, die man sogar im *Kosmos Vogelführer* vergeblich sucht.

Bei Bestimmungsliteratur sticht die Bebilderung zuerst ins Auge, mindestens genauso bedeutend ist aber der Text. Auch hier besticht dieses Buch mit einer gelungenen Kombination aus verständlichen Beschreibungen der Merkmale und des Vorkommens (mit Österreich-spezifischen Detailangaben), und ergänzenden „wissenswerten“ Informationen. Da in der Vogelbeobachtung sehr vieles über die Akustik läuft, werden die Vogelstimmen nicht nur in den Arttexten lautmalerisch umschrieben, es steht auch eine kostenlose Smartphone-App zum Download zur Verfügung, über die die Stimmen abgespielt werden können. Das ist ein nicht zu unterschätzendes Extra, da kurz nach dem Kauf des ersten Vogelbuchs oftmals die Suche nach einer geeigneten Vogelstimmenressource beginnt.

Zusammenfassend sei *Vögel Österreichs* von Leander Khil nicht nur beginnenden Vogelbeobachtern wärmstens empfohlen, auch erfahrene Beobachter können von diesem Buch als Ergänzung in der eigenen Bibliothek profitieren – etwa bei der Bestimmung mancher Arten anhand der Fotos gegenüber den Illustrationen, oder durch die Österreich-spezifischen Verbreitungskarten und Informationen im Text.

Benjamin Seaman

Christian Schönwiese, 2017: Klimawandel kompakt. Ein globales Problem wissenschaftlich erklärt. 132 Seiten, 30 Abbildungen, 11 Tabellen, 15 × 21cm, 320 g. Borntraeger, Stuttgart; ISBN 978-3-443-01104-8.

Der Autor des vorliegenden Buches, ein international renommierter Klimatologe und Gutachter des IPCC, der sich seit Jahrzehnten in Forschung und Lehre intensiv mit dem Klimawandel befasst, hat sich viel vorgenommen. Es soll *„aktuell und streng wissenschaftlich, aber trotzdem leicht verständlich“* der Klimawandel mit seinen vielfältigen Ursachen beschrieben werden, wobei sich das Buch *„an alle Leser, die an der Klimawandel-Problematik interessiert sind und sich fachlich korrekt, aber kompakt informieren wollen“* wendet. Gegliedert ist es in 14 Kapitel *(Klimaforschung; Atmosphäre und Wetter; Von der Wetterstatistik zum Klima; Klimainformationen; Klimasystem; Klimaphysik; Klimamodelle; Paläoklima; Klima im Holozän; Neoklima; Ursachendiskussion und Zukunftsperspektiven; Extremereignisse; Auswirkungen des Klimawandels; Klimaschutz und Klimapolitik)* und enthält darüber hinaus umfangreiche Literaturangaben, nationale sowie internationale Internet-Links und ein ausführliches Stichwortverzeichnis.

Angesichts der Tatsache, dass aus dem immer mehr zunehmenden Einfluss der Menschen auf das Klima uns eine besondere Verantwortung für die Welt, welche wir künftigen Generationen hinterlassen, erwächst, und angesichts des Umstands, dass gleichwohl in der Diskussion Sachlichkeit und solide Information erforderlich sind, kann ein derartiges Vorhaben nur begrüßt werden. *„Jeder, der sich für die Klimawandel-Problematik interessiert, ob mit wissenschaftlichem Hintergrund oder nicht, ist angesprochen."* Zu diesem Zweck hat der Autor auch weitestgehend auf Formeln verzichtet, sondern verlässt sich auf die (allerdings von zahlreichen Tabellen und Abbildungen unterstützte) verbale Darstellung.

Kompakt ist die Darstellung tatsächlich: der rezente Klimawandel im „Anthropozän" (also etwa in den letzten 200 Jahren) wird, genau genommen, auf lediglich 50 Seiten abgehandelt, denn der Autor hält es zum Verständnis der Thematik für notwendig, zuvor eingehend meteorologische, physikalische und statistische Grundbegriffe zu erläutern und auch noch auf Klimaänderungen in vorhistorischer Zeit einzugehen. So gerüstet folgt dann die Auseinandersetzung mit den „heutigen" Problemen: welche Szenarien zum Temperaturanstieg erscheinen realistisch? mit welchen Konsequenzen bzw. Auswirkungen ist zu rechnen? in welchen Bereichen kann es zu Beeinträchtigungen kommen? Das letzte Kapitel ist dem *„Was tun?"* gewidmet, also den zwei Aspekten des notwendigen Handelns, nämlich der Anpassung an die (als unvermeidlich angesehene) Temperaturerhöhung einerseits, der Vorsorge (um den Klimawandel in erträglichen Grenzen zu halten) andererseits. Auch hier ist der Autor möglichst auf Vollständigkeit bedacht, scheut sich jedoch nicht, problematisch erscheinende Lösungsvorschläge, wie etwa das Abfangen und Speichern von Kohlendioxid (CCS) oder das „Impfen" der Stratosphäre mit Partikeln zwecks Erzielen von Abkühlungseffekten kritisch zu besprechen. Nach der Lektüre – so jedenfalls die Hoffnung des Verfassers – sollte *„jeder in der Lage sein, die Fehler und Schwächen der Klimadebatte zu erkennen und sich ein realistisches und fundiertes Urteil zu bilden"*.

Ist diese optimistische Annahme tatsächlich angebracht? Kompakte Darstellung ja – ist es aber wirklich möglich, bloß „Interessierten" an der Klimawandel-Problematik auf so knappem Raum ausreichendes Verständnis zu vermitteln? Der Umstand, dass der Autor in einem Hochschultaschenbuch („Klimatologie") die im Wesentlichen gleiche Thematik (hier mit Schwerpunkt auf den „Grundlagen") auf fast 500 Seiten abhandelt, muss diesen Zweifel notgedrungen nähren.

Nehmen wir als Beispiel die wichtige globale Strahlungsbilanz („Solare und terrestrische Strahlungsflüsse in der Atmosphäre usw.; Abb. 8") auf den Seiten 36ff. Was normalerweise in eine Fachvorlesung langsam in Teilschritten aufgebaut wird, ist hier in einer notabene winzigen Abbildung zusammengepfercht. Der Autor bemüht sich allerdings redlich, die nicht unmittelbar einsichtigen Terme zu erläutern. Wenn aber dann in der Abbildung die beiden Begriffe „latenter" und „sensibler" Wärmefluss angeführt sind, letzterer im Text (p. 42) aber nicht vorkommt und überdies ein Querverweis auf die falsche Abbildung (10 statt 8) erfolgt, dürfte dies einem Leser, der erstmals mit der Materie zu tun hat, doch gehörige Schwierigkeiten bereiten.

Es wären noch etliche andere problematische Stellen anzuführen (die vermutlich der Kürze der Darstellung geschuldet sind). So werden etwa bei der Einleitung, die historisch konzipiert ist und langsam zum Thema hinführen soll, weder „Klima“ noch „Wetter“ begrifflich definiert. Ein didaktischer Kunstgriff, gewiss: aber hätte man nicht ganz kurz darauf hinweisen können, dass eine solche Definition alsbald nachfolgt, oder zumindest formulieren, dass von diesen Begriffen ja ohnedies jeder eine gewisse Vorstellung habe. (Wenn danach gleich von Witterungstagebüchern gesprochen wird, in welchen das Wetter zu bestimmten historischen Zeiten zusammenfassend beschrieben wurde, möchte man doch gerne wissen, was genau in diesen Aufzeichnungen denn tatsächlich notiert wurde.)

Begrifflich nicht ganz sauber ist die scharfe Gegenüberstellung von *einerseits* toxischen, *andererseits* klimarelevanten Spurengasen (p. 10). *„Hot spots“* ist mittlerweile ein allgemeiner Begriff, der nicht auf vulkanische Erscheinungen eingeschränkt ist (p. 28). Dass die Atmosphäre anfänglich wirklich zu 98 % aus Kohlendioxid bestanden habe, mag sein (es finden sich allerdings anderswo auch andere Prozentangaben); die Formulierung, diese habe sich in eine Stickstoff-Sauerstoffatmosphäre *umgewandelt*, erscheint jedoch ganz unglücklich und für Nichtfachleute ausgesprochen missverständlich (p. 51). Inwieweit die Verwitterung von Karbonaten der Atmosphäre wirklich dauerhaft bedeutende Mengen von Kohlendioxid entzieht, wirkt nicht plausibel (p. 56). Und bei den anschließend folgenden Darstellungen von Kalt- und Warmzeiten ist es selten klar, ob sich die Angaben auf die ganze Erde oder nur auf die Nordhemisphäre (oder womöglich gar nur auf Teile davon?) beziehen.

Es sei noch einmal betont, dass weder die Kompetenz des Autors noch die begrüßenswerte Zielsetzung des Buches mit diesen Beispielen in Zweifel gezogen werden sollen. Es mag ja sogar sein, dass der Leser durch die knappe Darstellung die Lücken in seinem Wissen erkennt und dadurch motiviert wird, diese in geeigneter Weise zu schließen. Sinnvoll ist der große Bogen, den der Verfasser von den Grundlagen bis zur eingehenden Besprechung der aktuellen Probleme schlägt, auf jeden Fall. Ob er aber wirklich *„leicht verständlich“* ist? Der Rezensent hofft inständig - auch im Sinne des wirklich wichtigen Anliegens dieses Buches - dass die LeserInnen seine diesbezüglichen Zweifel ad absurdum führen mögen.

Wolfgang Punz

Matthias Stolze, Rainer Weisshaidinger, Andreas Bartel, Othmar Schwank, Adrian Müller & Roger Biedermann (Red.), 2018: Chancen der Landwirtschaft in den Alpenländern. Wege zu einer raufutterbasierten Milch- und Fleischproduktion in Österreich und der Schweiz. Haupt Verlag. Bristol-Schriftenreihe Band 58. ISBN 978-3-258-08099-4.

Martin Stuber & Matthias Bürgi, 2018: Vom „eroberten Land“ zum Renaturierungsprojekt. Geschichte der Feuchtgebiete in der Schweiz seit 1700. Haupt Verlag. Bristol- Schriftenreihe Band 59. ISBN 978-3-258-08115-1.

Wieder können wir uns freuen, dass der Haupt Verlag zwei seiner in der Bristol-Schriftenreihe erschienenen Bücher für eine Buchbesprechung zur Verfügung gestellt hat.

Band 58 beschäftigt sich mit einem Thema, das Ökologie, Biodiversität und Landschaftsentwicklung im Zusammenhang mit der Landwirtschaft, vorrangig Rinderhaltung im alpinen Raum, darstellt. Für die „Grasländer“ Schweiz und Österreich stellt sich die Frage, welche Lösungen für die Milch- und Rindfleischproduktion zu einer ökologisch nachhaltigen Landnutzung beitragen können. Eine umfassende Bestandsaufnahme hinsichtlich agrarpolitischer Entwicklungen und deren Folgen für die Umwelt bilden die Grundlage des Berichtes, wobei der Stickstoff der zentrale Faktor der Untersuchung ist. Eine wichtige wie auch globale Leitfrage war dabei, wie sich eine sichere Ernährung der Bevölkerung erreichen lässt, ohne die Tragfähigkeit des Ökosystems zu gefährden. Mit der „regenerativen Milch- und Rindfleischproduktion" stellen die Autoren einen neuen und innovativen Lösungsweg für eine ökologisch nachhaltige Landwirtschaft im Alpenraum vor, der sich von der momentanen Hochleistungsstrategie unterscheidet; denn zurzeit verursacht die Landwirtschaft durch die gängigen Methoden von Bodenbearbeitung und Tierfütterung 9 bzw. 13 % (Schweiz bzw. Österreich) des Ausstoßes von Treibhausgasen.

Verschiedene Modellberechnungen bieten die Möglichkeit, die Machbarkeit einzuschätzen, die von Praxisbeiträgen veranschaulicht werden. Dies ist ein Ansatz, der artenreiche Wiesen fördert, also Ökosysteme stabilisiert, und durch regionales Futter sehr nachhaltig ist. Das hochkarätige Autorenteam stellt dieses Beziehungsgeflecht vielseitig und verständlich dar.

Im Band 59 zeichnet ein Autorenteam mit Schwerpunkt seiner Tätigkeit an der Eidgenössischen Forschungsanstalt für Wald, Schnee und Landschaft WSL (Birmensdorf) mit Akribie den Schwund und die Nutzungsgeschichte der Feuchtgebiete im Alpenrheintal nach. Sie sind im Lauf der letzten zwei Jahrhunderte durch geänderte, vor allem auch technisierte, Nutzung auf 10 % ihrer ursprünglichen Ausdehnung geschrumpft. Das Bestreben der Menschen, fruchtbares Ackerland durch Trockenlegung zu gewinnen, verlief zulasten der Biodiversität, wie am Beispiel ausgewählter Artengruppen gezeigt wird. Durch eine quantitative Untersuchung erhalten wir einen Überblick über die gegebene Situation, nicht zuletzt durch die innovative Einbindung und Verknüpfung von geisteswissenschaftlichen und naturwissenschaftlichen Aspekten. Damit erfolgt eine ganzheitliche Darstellung mit vertiefenden Betrachtungen mittels vieler Beispiele.

Die historische Entwicklung der Feuchtgebiete in der Schweiz wird weder als Fortschritts- noch als Verlustgeschichte beschrieben. Im Vordergrund stehen vielmehr die unterschiedlichen historischen Akteure, ihre Ziele und Praktiken im zeitgenössischen Kontext sowie die Folgen ihres Tuns für Landschaft und Ökologie.

Die Sensibilisierung für naturnahe Lebensräume mit ihren bedeutenden ökologischen Vernetzungen im 21. Jahrhundert könnte durch die Lektüre dieses Buches verbessert werden. Die Vernichtung von Mooren z. B., diesen wichtigen Kohlenstoffspeichern, setzt beträchtliche Mengen an Treibhausgasen frei; nimmt man Klimaschutz ernst, müssten sie großteils wieder vernäßt werden.

Der zeitliche Bogen spannt sich von der Mitte des 18. Jahrhunderts bis zur Gegenwart, beschreibt großflächige Meliorationen und Renaturierungsprojekte.

Das Ehepaar Herbert und Ruth Uhl, die Stiftungsgeber, war besonders tierliebend und wollte mit dieser ihrer Bristol-Stiftung für die Natur etwas Gutes tun. Unterstützt werden Projekte die ebendies zum Ziel haben. Unter https://www.bristol-stiftung.ch/ sind alle unterstützten Projekte und die daraus resultierenden Publikationen zu finden.

Beide Bände sind in der Bibliothek der ZooBot verfügbar.

Petra Hudler

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Aus der Gesellschaft

Bericht über das Vereinsjahr 2018/19

Mi. 3.10.2018
Marieluise Weidinger (CIUS Universität Wien)
„Den Elementen auf der Spur: Möglichkeiten der Visualisierung mit dem Rasterelektronenmikroskop“

Do. 18.10.2018
Christian Pichler (WWF)
„The WWF BIG 5 – Seeadler, Biber, Wolf, Luchs, Fischotter“
Eine Veranstaltung aus der Reihe „Wissenschaft trifft Schule – Schulbrücke“

Sa. 20.10.2018
Christian Gilli (Universität Wien)
„Flora und Vegetation im Herbstaspekt. Im Speziellen Gräser“ (Exkursion)

Sa. 3.11.2018
„Pflegeeinsatz im Natura 2000 Schutzgebiet Zeiserlberg“
[teilweise im Eigentum der Zoologisch-Botanischen Gesellschaft]
Mit Schutzgebietsbetreuer Manuel Denner
In Kooperation mit ENU und der Gemeinde Ottenthal

Mi. 14.11.2018
Werner Gamerith
„Naturjuwel Kamptal erneut bedroht“

Mi. 21.11.2018
Michael Wagreich (Universität Wien)
„Das Anthropozän“
Generalversammlung des Vereins zur Verbreitung naturwissenschaftlicher Kenntnisse in Wien

Di. 22.01.2019
Silke Schwaiger (NHM Wien)
„Die Herpetofaunistische Datenbank: 36 Jahre Langzeitmonitoring der Amphibien und Reptilien Österreichs“

Mi. 13.03.2019
Anthony D. Sheridan (Stiftung Artenschutz Universität Wien)
„Artenschutzprojekt für den Gelbwangen-Schopfgibbon in Vietnam“

Mi. 20.03.2019
Monika Bright (Universität Wien)
„Mit dem Tauchboot zu den Heissen Quellen (Hot Vents)“ – Eine Wissenschafterin erkundet den extremsten Lebensraum des Meeres
Veranstaltet von der Sektion proMare der Zoologisch-Botanischen Gesellschaft

Mi. 3.04.2019
Leonida Fusani (Universität Wien)
„Food, fat and Zugunruhe“
In Kooperation mit dem Konrad Lorenz für Vergleichende Verhaltensforschung

Generalversammlung der Zoologisch-Botanischen Gesellschaft

Sa. 18.05.2019
Matthias Kropf (BOKU) & Margareta Pertl (Wien und Dublin)
„Orchideen in der Lobau“ (Exkursion mit Möglichkeit zum Zeichnen)

Mi. 12.06.2019
„Die wilden Flüsse des Balkan – Handlungsbedarf für Politik und Wissenschaft“

Vortragsabend in Kooperation mit den Freunden des NHM

Ulli Eichelmann (Riverwatch)
Die Schönen und das Biest – Europas beste Flüsse und deren Bedrohung

Fritz Schiemer (Zoobot)
Die Vjosa – ein Fluss-System von internationaler Bedeutung

Christoph Hauer (BOKU)
Ungezähmte Dynamik am Balkan –
Hydromorphologie und Sedimenttransport der Vjosa

Wolfram Graf (BOKU)
Letzte Refugien – Wirbellose Wasserorganismen der Vjosa

Univ. Prof. Thomas Frank (BOKU)
Einzigartige Landtiere an den Ufern der Vjosa

Moderation: Elisabeth Haring (NHMW)

Sa. 07.09.2019
Herbert Marth & Michael Götzinger (Wien)
„Interdisziplinäre Exkursion zum Csaterberg“

Veranstaltungsort (wenn nicht anders angegeben): Karl-Burian-Hörsaal (HS 2), UZA1, Biozentrum der Universität Wien, 1090 Wien, Althanstrasse14

Botanische Illustration im Botanischen Garten (2019)

30./31. Mai 2019
Kurs „Botanische Malerei im Botanischen Garten“ (Botanische Illustration im HBV)
Margareta Pertl

1./2. Juni 2019
Kurs „Botanische Malerei im Botanischen Garten“ (Botanische Illustration im HBV)
Margareta Pertl

21./22. September 2019
Kurs „Botanische Malerei im Botanischen Garten“ (Botanische Illustration im HBV)
Margareta Pertl

BioTaxSyst Summerschools (2019)

Gräserkurs – 2. Teil: Cyperaceae

1.–5. Juli 2019
Kursleitung: Andreas Berger (Wien)
Kursort: Wien und Wiener Umland

Heimische Zikaden

15. – 19. Juli 2019
Kursleitung: Werner Holzinger (Ökoteam), Gernot Kunz (Institut für Biologie der Universität Graz), finanziert von der ÖH Graz.
Kursort: Graz

Hinweis: Unser Archiv bietet noch weitere Informationen zu den stattgehabten Veranstaltungen: <http://www.univie.ac.at/zoobot/wordpress/?page_id=55>

Zoologisch-Botanische Gesellschaft in Österreich

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Dr. Harald Schillhammer

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"Acta ZooBot Austria" (formerly "Verhandlungen der Zoologisch Botanischen Gesellschaft in Österreich") is published in annual volumes. The journal accepts original scientific work dealing with ecological or systematic issues. As stipulated in the society's statutes, preference is given to work related to Austria or its neighbouring countries. All submitted manuscripts are subject to critical review by two experts in the relevant subject matter.

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Structure and form of the manuscript should follow these norms:

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All authors will receive the PDF file of their article per e-mail. Reprints of the manuscript can be ordered at cost price. The PDF files will be uploaded to the literature platform of the Biologiezentrum Linz after two years.

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For the editorial board
Rudolf Maier

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