

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

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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.

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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 “Chemo-diversity 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-

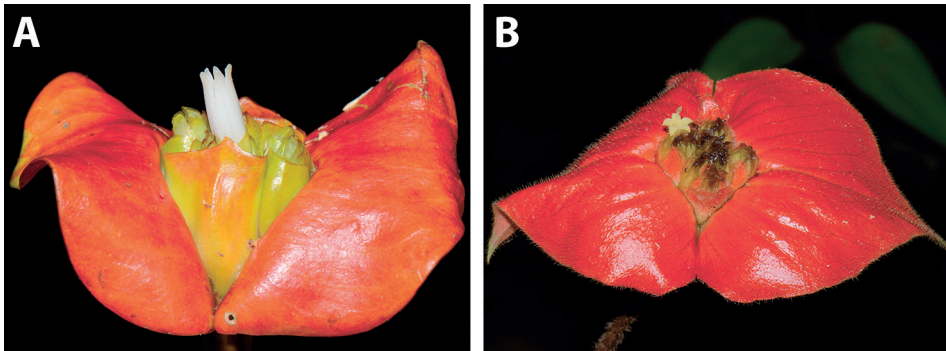


Fig. 1: Inflorescences of *Palicourea elata* (A) and *Palicourea tomentosa* (B, both Palicoureeae), two common shrubs or treelets in the Piedras Blancas National Park, Costa Rica. Both feature showy, red, involucreal bracts advertising flowers to pollinators. Note differences in bract pubescence and 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 involucre 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 ( $2 \times 3$  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 Psychotriaceae occurring in the Piedras Blancas National Park and the adjacent Golfito National Wildlife Refuge, Puntarenas Province, Costa Rica. – Tab. 1: Liste der Palicoureeae und Psychotriaceae, die im Piedras Blancas Nationalpark und dem angrenzenden Golfito National Wildlife Refuge, Provinz Puntarenas, Costa Rica, vorkommen.

Species	Selected voucher specimen
<b>Carapichea</b>	
<i>Carapichea affinis</i> (Standl.) L. ANDERSSON	A. BERGER 1838 (WU!)
<b>Geophila</b>	
<i>Geophila repens</i> (L.) I.M. JOHNST. <sup>1</sup>	M. KIEHN et al. MK-920207-1/2 (WU!)
<i>Geophila macropoda</i> (RUIZ & PAV.) DC.	A. BERGER 2247 (WU!)
<b>Notopleura</b>	
<i>Notopleura anomothyrsa</i> (K. SCHUM. & DONN. SM.) C.M. TAYLOR	W. HUBER & A. WEISSENHOFER 1579 (WU!)
<i>Notopleura capacifolia</i> (DWYER) C.M. TAYLOR	M. BERNHARD & H. GREGER HG 2907082 (WU!)
<i>Notopleura epiphytica</i> (K. KRAUSE) C.M. TAYLOR <sup>1</sup>	G. HERRERA CHACON 5046 (MO n.v.)
<i>Notopleura polyphlebia</i> (DONN. SM.) C.M. TAYLOR	A. BERGER 1349 (WU!)
<i>Notopleura uliginosa</i> (SW.) BREMEK.	A. BERGER 1317 (WU!)
<b>Palicourea</b>	
<i>Palicourea acicularis</i> (C.M. TAYLOR) BORHIDI	A. BERGER 1827 (WU!)
<i>Palicourea acuminata</i> (BENTH.) BORHIDI	A. BERGER 1339 (WU!)
<i>Palicourea brachiata</i> (SW.) BORHIDI <sup>2</sup>	W. HUBER & A. WEISSENHOFER 9 (MO!)
<i>Palicourea calidicola</i> (C.M. TAYLOR) C.M. TAYLOR	A. BERGER 1812 (WU!)
<i>Palicourea cyanococca</i> (DOMBRAIN) BORHIDI	A. BERGER 1811 (WU!)
<i>Palicourea deflexa</i> (DC.) BORHIDI	A. BERGER 1338 (WU!)
<i>Palicourea elata</i> (SW.) BORHIDI	A. BERGER 1395 (WU!)
<i>Palicourea glomerulata</i> (DONN. SM.) BORHIDI <sup>2</sup>	G. INDUNI 204 (MO!)
<i>Palicourea grandifructa</i> (C.M. TAYLOR) C.M. TAYLOR	A. BERGER 1863 (WU!)
<i>Palicourea guianensis</i> AUBL.	A. BERGER AB 16021010 (WU!)
<i>Palicourea hoffmannseggiiana</i> (ROEM. & SCHULT.) BORHIDI	A. BERGER 1763 (WU!)
<i>Palicourea hondensis</i> (STANDL.) C.M. TAYLOR	A. BERGER 1849 (WU!)
<i>Palicourea longiinvoluta</i> A.C. BERGER	A. BERGER 1418 (WU!)
<i>Palicourea mortoniana</i> (STANDL.) BORHIDI	M. KIEHN & A. WEISSENHOFER MK 961119-2/1 (WU!)
<i>Palicourea pilosa</i> (RUIZ & PAV.) BORHIDI	A. BERGER 1397 (WU!)
<i>Palicourea pseudaxillaris</i> (WERNHAM) C.M. TAYLOR	A. BERGER 1301 (WU!)
<i>Palicourea racemosa</i> (AUBL.) G. NICHOLSON	A. BERGER 1736 (WU!)
<i>Palicourea solitudinum</i> (STANDL.) BORHIDI	A. BERGER 1392B (WU!)
<i>Palicourea suerrensii</i> (DONN. SM.) BORHIDI	A. BERGER AB 16021005 (WU!)
<i>Palicourea tetragona</i> (DONN. SM.) C.M. TAYLOR & LORENCE	A. BERGER 1852 (WU!)
<i>Palicourea tomentosa</i> (AUBL.) BORHIDI	A. BERGER 1394 (WU!)
<i>Palicourea triphylla</i> DC.	A. BERGER 1738 (WU!)
<i>Palicourea tsakiana</i> (C.M. TAYLOR) C.M. TAYLOR	M. BERNHARD & H. GREGER HG 31070812 (WU!)
<i>Palicourea violacea</i> (AUBL.) A. RICH.	A. BERGER AB 16021009 (WU!)
<i>Palicourea winkleri</i> BORHIDI	A. BERGER 1369 (WU!)
<b>Psychotria</b>	
<i>Psychotria chagensis</i> STANDL.	A. BERGER 1398 (WU!)
<i>Psychotria chitariana</i> DWYER & C.W. HAM.	A. BERGER 1316 (WU!)
<i>Psychotria costivenia</i> GRISEB. <sup>2</sup>	S. WILL 87 (MO n.v.)
<i>Psychotria grandis</i> Sw.	W. HUBER & A. WEISSENHOFER 2447 (WU!)
<i>Psychotria limonensis</i> K. KRAUSE <sup>1</sup>	A. BERGER 1757 (WU!)
<i>Psychotria marginata</i> Sw.	A. BERGER 1410 (WU!)
<i>Psychotria micrantha</i> KUNTH	A. BERGER 1776 (WU!)
<i>Psychotria panamensis</i> STANDL.	A. BERGER 2137 (WU!)
<i>Psychotria psychotriifolia</i> (SEEM.) STANDL.	A. BERGER 2249 (WU!)
<b>Rudgea</b>	
<i>Rudgea cornifolia</i> (KUNTH) STANDL.	A. BERGER 1368 (WU!)
<i>Rudgea raveniana</i> W.C. BURGER	A. BERGER 1420 (WU!)
<i>Rudgea skutchii</i> STANDL.	A. BERGER 1820 (WU!)

<sup>1</sup> 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; <sup>2</sup> 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.

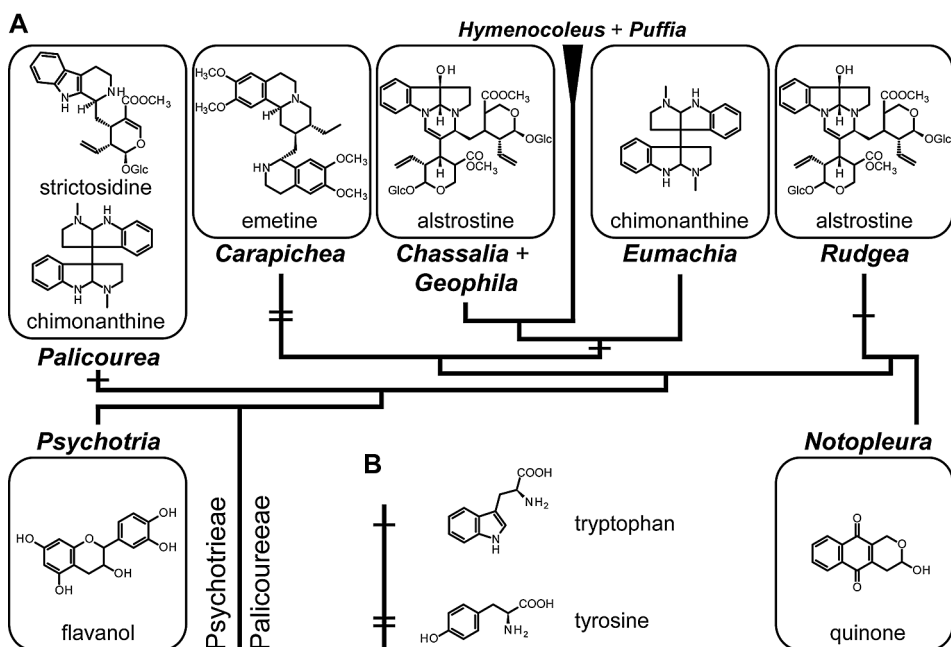


Fig. 2: Major groups of secondary metabolites mapped on a phylogeny of the *Psychotria* alliance (Palicoureeae + Psychotrieae). A: Boxes with representative chemical formulae illustrate the compound classes here understood to characterize each genus. Branches and boxes of lineages showing alkaloid accumulation 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-beinhaltenen 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/Psychotriaceae. 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 Psychotriaceae. 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-iridoid 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 deglycosylation 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 deoxostriatosamide (**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,  $\beta$ -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 5 $\alpha$ -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

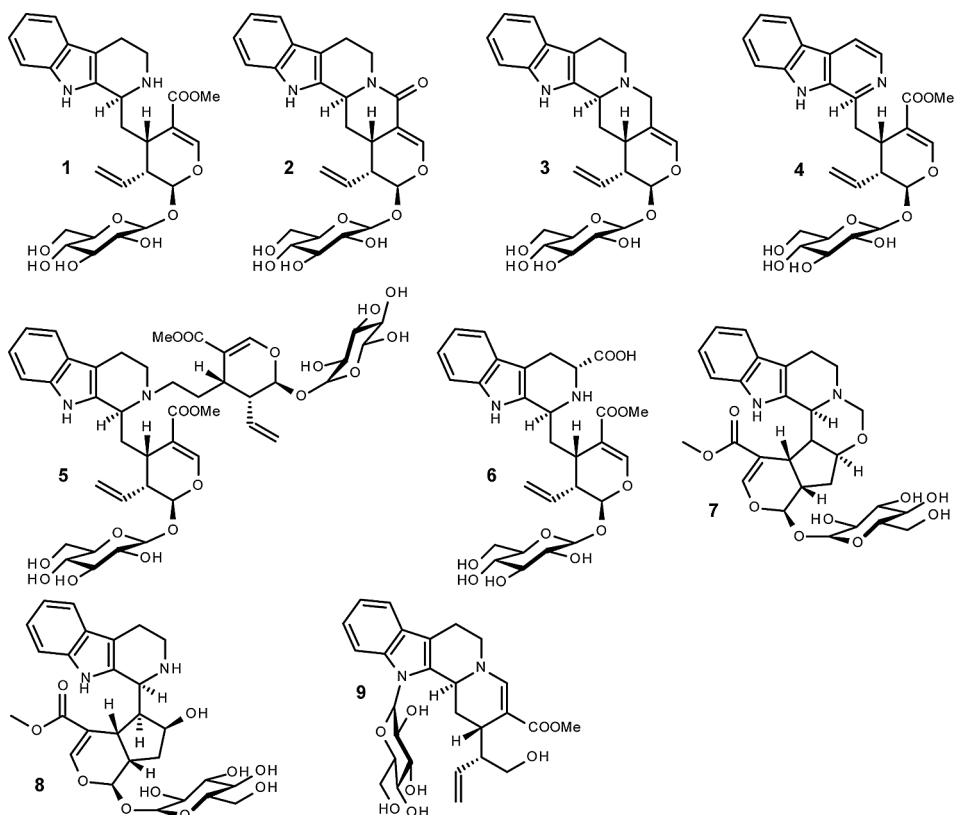


Fig. 3: Examples of structural diversity of tryptamine-derived alkaloids isolated from *Palicourea* species. Strictosidine (1), strictosamide (2), deoxostrictosamide (3), lyaloside (4), bahienoside B (5), 5 $\alpha$ -carboxystrictosidine (6), palicroceain (7), brachycerin (8), lagamboside (9). – Abb. 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), 5 $\alpha$ -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 deglycosylation of strictosidine (**1**) by a dedicated strictosidine  $\beta$ -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 *Rauwolfia 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).

Lagambose (**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  $\beta$ -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.

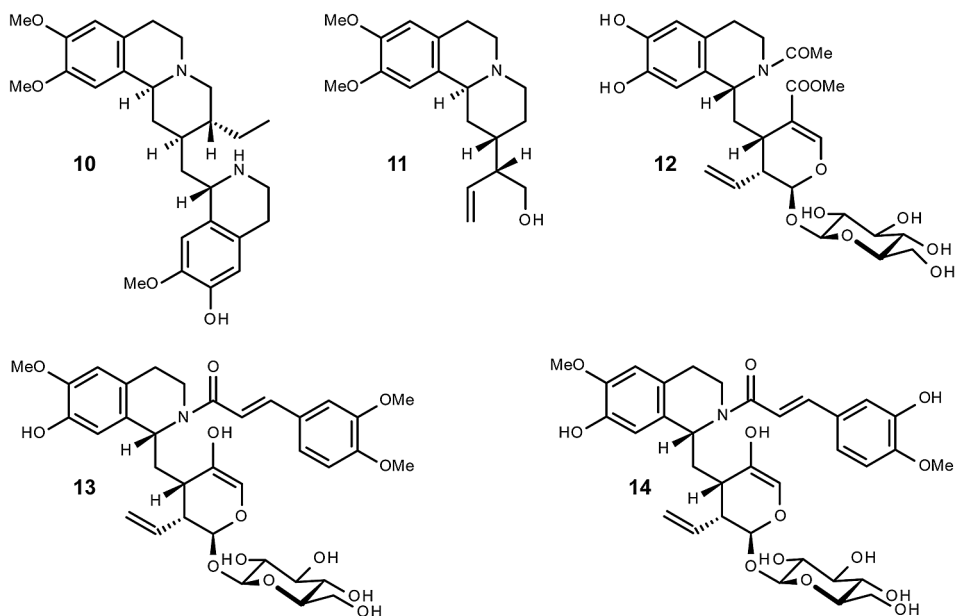


Fig. 4: Structural variation of dopamine-derived alkaloids isolated from *Carapichea affinis*. Cephaeline (10), 7-*O*-methyl-alangine (11), ipecoside (12), borucoside (13), 3'''-*O*-demethyl-borucoside (14). – Abb. 4: Diversität der von Dopamin abgeleiteten Alkaloide der Art *Carapichea affinis*. Cephaelin (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 Psychotriaceae and placed in the Lasiantheae, was shown to be characterized by iridoids including asperuloside (**15**), 6 $\alpha$ -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-

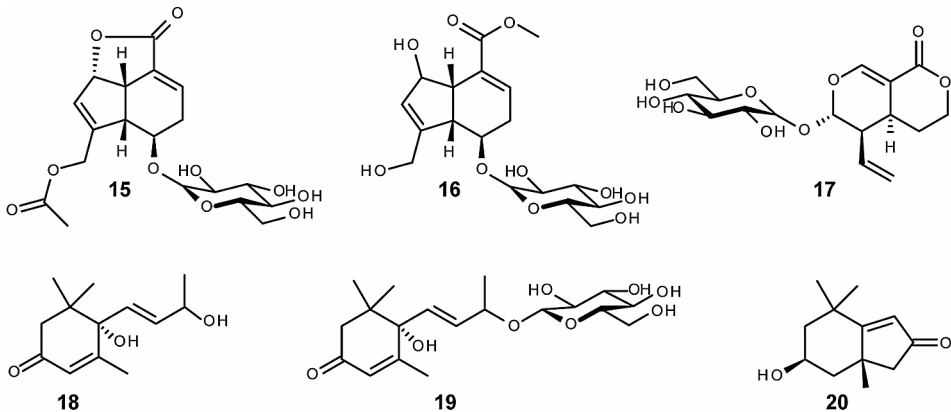


Fig. 5: Iridoids (**15–17**) and megastigmanes (**18–20**) isolated from various species of the *Psychotria* alliance. Asperuloside (**15**), 6 $\alpha$ -hydroxygeniposide (**16**), sweroside (**17**), vomifoliol (**18**), roseoside (**19**), loliolid (**20**). – Abb. 5: Iridoide (**15–17**) und Megastigmane (**18–20**) aus verschiedenen Arten der *Psychotria*-Verwandtschaft. Asperulosid (**15**), 6 $\alpha$ -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 Psychotriaceae 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 single-most 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  $\beta$ -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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