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Distribution and systematic significance of selected
secondary metabolites within Psychotrieae/Palicoureeae
(Rubiaceae)

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Table of contents

1	Introduction	1
2	Systematics, Taxonomy and Morphology	3
2.1	Gentianales	3
2.2	Rubiaceae	3
2.3	Subfamiliar classification	4
2.4	Rubioideae	5
2.5	Psychotrieae alliance [Supertribe Psychotriidinae]	6
2.6	Psychotrieae sensu lato	6
2.7	Psychotrieae sensu stricto	12
2.7.1	<i>Psychotria</i> subg. <i>Psychotria</i>	12
2.7.2	<i>Psychotria</i> subg. <i>Tetramerae</i>	13
2.8	Palicoureeae	13
2.8.1	<i>Carapichea</i>	14
2.8.2	<i>Chassalia</i>	15
2.8.3	<i>Geophila</i>	15
2.8.4	<i>Margaritopsis</i>	15
2.8.5	<i>Notopleura</i>	16
2.8.6	<i>Palicourea</i>	17
2.8.7	<i>Psychotria</i> subg. <i>Heteropsychotria</i>	18
2.8.8	<i>Rudgea</i>	19
2.9	Lasiantheae	19
2.9.1	<i>Ronabea</i>	19
3	Phytochemistry	21
3.1	Iridoid Glycosides	21
3.1.1	Distribution of iridoids in <i>Psychotria</i> s.l.	22
3.1.2	Plant-Herbivore Interaction	23
3.1.3	Pharmacological Activities	23
3.2	Tryptamine Alkaloids	23
3.3	Monomeric tryptamine alkaloids	23
3.3.1	Distribution in <i>Psychotria</i> s.l.	24
3.3.2	Plant-Herbivore Interaction	24
3.3.3	Pharmacological Activities	24
3.4	Cyclotryptamine alkaloids	25
3.4.1	Biosynthesis	25
3.4.2	Monomeric cyclotryptamine alkaloids	26
3.4.3	Dimeric cyclotryptamine alkaloids	26
3.4.4	Oligomeric cyclotryptamine alkaloids	27
3.4.5	Distribution of cyclotryptamine alkaloids in <i>Psychotria</i> s.l.	27
3.4.6	Plant-Herbivore Interaction	28
3.4.7	Pharmacological Activities	28
3.5	Tryptamine-Iridoid Alkaloids	29
3.5.1	Distribution of tryptamine iridoid alkaloids	30
3.5.2	Plant-Herbivore Interaction	31
3.5.3	Pharmacological Activities	32
3.6	Dopamine-Iridoid Alkaloids	32
3.6.1	Distribution of dopamine iridoid alkaloids	33
3.6.2	Plant-Herbivore Interaction	33
3.6.3	Pharmacological Activities	34
3.7	Tannins	34

3.7.1	Distribution of tannins in <i>Psychotria</i> s.l.....	35
3.7.2	Plant-Herbivore Interaction.....	35
3.7.3	Pharmacological Activities	37
3.8	Other Compounds	37
3.8.1	Coumarins	37
3.8.2	Flavonoids	37
3.8.3	Naphthoquinone derivatives.....	38
3.8.4	Terpenes:	38
4	Material and Methods.....	39
4.1	Plant material.....	39
4.2	Reference compounds	40
4.3	Analytical methods.....	42
4.3.1	Thin layer Chromatography	42
4.3.2	High-Performance Liquid Chromatography	42
4.3.3	Nuclear magnetic resonance spectroscopy.....	43
4.3.4	Mass spectrometry.....	43
4.3.5	Phenolic content, radical scavenging activity and bioassay using <i>Spodoptera littoralis</i>	43
4.4	Preparative methods	44
4.4.1	Liquid-liquid extraction.....	44
4.4.2	Column Chromatography	44
4.4.3	Medium Pressure Liquid Chromatography	45
4.4.4	Preparative Thin Layer Chromatography	45
5	Extraction and Isolation	46
5.1	<i>Palicourea acuminata</i>	46
5.2	<i>Psychotria chiriquiensis</i>	47
5.3	<i>Psychotria cyanococca</i>	47
5.4	<i>Psychotria suerrensii</i>	48
5.5	<i>Notopleura polyphlebia</i>	48
5.6	<i>Psychotria nervosa</i>	49
5.7	<i>Psychotria tenuifolia</i>	49
6	Results	50
6.1	<i>Palicourea acuminata</i> (Palicoureeae)	50
6.2	<i>Psychotria chiriquiensis</i> (Palicoureeae).....	52
6.3	<i>Psychotria cyanococca</i> (Palicoureeae).....	53
6.4	<i>Psychotria suerrensii</i> (Palicoureeae)	54
6.5	<i>Notopleura polyphlebia</i> (Palicoureeae).....	55
6.6	<i>Psychotria</i> subg. <i>Psychotria</i> (Psychotrieae).....	56
6.7	<i>Psychotria nervosa</i> (Psychotrieae).....	58
6.8	<i>Psychotria tenuifolia</i> (Psychotrieae)	58
6.9	Overview of compounds detected.....	60
6.10	Diversification of chemical profiles within the <i>Psychotria</i> alliance	62
7	Chemosystematic Aspects.....	66
7.1	Relevance for infrageneric classification	66
7.1.1	Section <i>Didymocarpos</i>	66
7.1.2	Section <i>Pseudocephaelis</i> , series B. <i>Pseudocephaelis</i>	69
7.1.3	Section <i>Nonatelia</i>	71
7.1.4	Iridoids	73
7.2	Relevance for intergeneric classification	74
7.2.1	<i>Psychotria</i> s.str (Psychotrieae).....	74
7.2.2	<i>Carapichea</i> (Palicoureeae).....	76

7.2.3	<i>Chassalia</i> (Palicoureeae).....	77
7.2.4	<i>Geophila</i> (Palicoureeae).....	77
7.2.5	<i>Margaritopsis</i> (Palicoureeae).....	77
7.2.6	<i>Notopleura</i> (Palicoureeae).....	81
7.2.7	<i>Palicourea</i> s.l. (Palicoureeae)	83
7.2.8	<i>Rudgea</i> (Palicoureeae).....	83
7.2.9	The polyphyletic genus <i>Cephaelis</i>	84
7.3	Relevance for tribal classification	85
7.3.1	<i>Ronabea</i> (Lasiantheae).....	85
7.4	Chemotaxonomic conclusions.....	86
8	Correlation with Plant-Herbivore Interactions	89
9	Correlations with Ethnobotanical Aspects	93
9.1	Ethnopharmacology	93
9.1.1	<i>Psychotria</i> subg. <i>Psychotria</i>	95
9.1.2	<i>Palicourea</i> s.l.	95
9.2	Ayahuasca preparation	96
10	Summary	100
11	Acknowledgements	102
12	Literatur	104
13	Appendix	126
13.1	Corrigenda.....	126
13.2	List of taxa and voucher specimens	126
13.3	Images of voucher specimens	134
13.4	Curriculum Vitae.....	208

1 Introduction

Phytochemical studies traditionally focussed on single species, report their constituents or assess their bioactivity in several model systems. However, most studies fail to put their results in a broad phylogenetic and ecological context. As a consequence, little is known concerning the patterns of diversification and accumulation of secondary metabolites within related lineages and their ecological impact.

When studying the question of phytochemical diversification within a given clade, a readily accessible group must be selected, featuring diversity on both, species and chemical level. This is the case in the *Psychotria* alliance, a hyperdiverse group including the genera *Carapichea*, *Chassalia*, *Geophila*, *Margaritopsis*, *Notopleura*, *Palicourea*, *Psychotria* and *Rudgea*. After a series of systematic works concerning mostly neotropical representatives, most species are considerably well known and their generic boundaries and phylogeny are well settled. In the present thesis, it has been selected as a model group in studying metabolic diversity and accumulation patterns using the framework provided by macromolecular phylogenetic and morphological studies.

Psychotria s.l. is known for its species richness (e.g. Davis et al., 2009), phytochemical diversity (e.g. Berger et al., 2012; Lopes et al., 2004; Porto et al., 2009) and ethnobotanical importance (e.g. Rivier & Lindgren, 1972; Sanz-Biset et al., 2009). The pantropical genus *Psychotria*, in its broadest sense, is the largest genus within the Rubiaceae consisting of more than 1800 species (Davis et al., 2009). Its species, mainly herbs, shrubs and occasionally small trees, contribute an important part to rainforest understory species diversity, abundance and biomass (Gentry, 1990). Furthermore, its fruits provide an important food source for frugivore birds (e.g. Poulin et al., 1999; Snow, 1981).

Psychotria s.l. is represented in the Neotropics by at least 600 species (Delprete, 2004), one of the most specious regions is Costa Rica where more than 145 species are reported (Burger & Taylor, 1993). Beside its striking biological diversity, Costa Rica is most suitable for successfully performing scientific studies, because of its research infrastructure, harbouring the La Gamba Field Station, Puntarenas province in the southwestern part of the country.

Following questions are addressed:

1. **Phytochemical diversity:** How diverse are compounds present in selected species of *Psychotria* alliance?
2. **Correlations between botanical and phytochemical diversity:** Does the botanical diversification within the *Psychotria* alliance resulted in biosynthesis of different substance classes?
3. **Search for chemical characters:** Is it possible to find accumulation tendencies and define clade specific chemical markers? Are patterns of secondary metabolite accumulation of phylogenetic importance at infrageneric or intergeneric level? Do exceptions exist?
4. **Ecology and Ethnobotany:** Is the ethnobotanical usage of certain species related to secondary metabolite content? Is the phytochemical diversification of ecological importance for species interactions, especially in defining host plant interactions?

2 Systematics, Taxonomy and Morphology

Due to the fact that the group and especially floral characters are morphologically uniform, systematic treatment and generic delimitations are challenging. Because of the size and pantropical distribution of the group, a comprehensive worldwide treatment is still missing. Nevertheless, on base of morphological and macromolecular phylogenetic data, the genus *Psychotria* as well as the tribe Psychotrieae it was assigned to, have recently experienced considerable rearrangements. Two major clades were recognised on tribal level and several species previously classified as *Psychotria* were segregated at generic level (e.g. Andersson, 2002b; Nepokroeff et al., 1999; Robbrecht & Manen, 2006).

A comprehensive overview of morphology, life history and systematic treatment of species studied in the present work is given in the following chapter and will provide the background for further discussion. Special emphasis will be given to the new tribal classification system and new generic delimitations. Genera referred to in bold letters in the conspectus of the tribes will be discussed in detail regarding their systematic position.

2.1 Gentianales

According to the Angiosperm Phylogeny Group III (2009), the family Rubiaceae belongs to the Lamiids (syn. Euasterids I), a member of core eudicots within the angiosperms. The family is further placed in the order Gentianales Juss. ex Bercht. & J. Presl, and, according to macromolecular phylogenetic data, it represents their basal clade. As currently circumscribed, the Gentianales consists of five families: Apocynaceae, Gelsemiaceae, Gentianaceae, Loganiaceae and Rubiaceae (Angiosperm Phylogeny Group III, 2009; Backlund et al., 2000).

2.2 Rubiaceae

The coffee family (Rubiaceae Juss.) is a monophyletic group (e.g. Bremer, 1996; Robbrecht & Manen, 2006) and consists of about 611 genera and approximately 13.143 species, almost half of whom occur in the Neotropics. It is the largest family of the Gentianales and the fourth largest family of flowering plants (Davis et al., 2009; Govaerts et al., 2011).

The family is recognised by a combination of vegetative and generative characters: opposite or whorled leaves with entire margins, interpetiolar stipules that are entire or divided, bisexual, actinomorphic, tetracyclic 4-5-merous flowers and an inferior ovary with

two carpels (Delprete, 2004). In the tropics, most species of the Rubiaceae are small trees and shrubs, and occasionally large trees or lianas. In temperate climates, annual or perennial herbs predominate. Species of the family have adapted to all habitats, with most species being terrestrial, while epiphytic or aquatic taxa are rarely found. They occur from semiaquatic habitats to arid deserts, from lowland rain forests to high altitude páramo, and from pristine to disturbed habitats (Delprete, 2004). Rubiaceous species have a cosmopolite distribution, but diversity and biomass is predominantly rich in the tropics and decreases towards temperate regions. As an example, outstanding diversity is found in Colombia, where over 1000 species occur (Davis et al., 2009).

Several species of the Rubiaceae are of great economic importance and are used for various purposes. *Coffea arabica* L. is the most prominent example. It is grown for coffee-production and represents one of the worlds major crops. Several genera of the tribe Gardenieae produce large edible fruits (e.g. *Genipa americana* L. or *Borojoa sorbilis* L.), while species of *Ixora* L. or *Mussaenda* L. are grown as ornamentals. Arboreal genera like *Chimarrhis* Jacq. or *Simira* Aubl. are used as source of timber. Many rubiaceous species are sources of important secondary metabolites used as natural medicines, hallucinogens, poisons or as lead compounds for drug development. Some examples include emetine from *Carapichea ipecacuanha*, quinine from *Cinchona pubescens* or tannins from *Uncaria gambir* (Delprete, 2004).

2.3 Subfamilial classification

In the classification of Robbrecht (1988), the Rubiaceae are divided in four subfamilies: Antirheoideae, Cinchonoideae, Rubioideae and Ixoroideae. Later studies using macromolecular data (e.g. Bremer, 1996; Andersson & Rova 1999; Bremer & Manen, 2000) showed the Antirheoideae to be polyphyletic, but supported the recognition of three subfamilies Cinchonoideae, Rubioideae and Ixoroideae. Characterisation of the three subfamilies according to Delprete (2004) is given in Tab. 1. The most comprehensive study, a supertree by Robbrecht & Manen (2006) including 300 genera, recognises only two major clades in Rubiaceae which are proposed as subfamilies Cinchonoideae and Rubioideae. These are characterised by morphology, anatomy, and flower biology.

Tab. 1: Major diagnostic characters of the three subfamilies of Rubiaceae (Delprete, 2004)*

	Cinchonoideae	Ixoroideae	Rubioideae
habit	trees, shrubs, lianas	trees, shrubs, vines	herbs, shrubs, (trees)
Ca-oxalate crystals	absent	absent	raphides
stipules	entire, (bifid)	entire, (bifid)	entire, bifid, fimbriate
calycophylls	absent	present	absent
flowers	actinomorphic, (zygomorphic)	actinomorphic, (zygomorphic)	actinomorphic

*table based on morphological characters, note that exceptions exist in some cases

Tab. 2: Major diagnostic characters of the two subfamilies of Rubiaceae sensu Robbrecht & Manen (2006).*

	Cinchonoideae	Rubioideae
habit	woody	woody or herbaceous
Ca-oxalate crystals	mostly crystal sand	raphides
corolla aestivation	variable	predominantly valvate
flower biology	secondary pollen presentation frequent	heterostyly frequent
Al-accumulation	only in few species	frequently strong
ploidy levels	predominantly 2x, 4x	2x, 4x and higher

*table based on morphological characters, but supported by DNA phylogenetic data, note that exceptions exist in some cases.

2.4 Rubioideae

The circumscription of the subfamily Rubioideae Verdc. remained stable over decades (e.g. Robbrecht, 1988; Andersson & Rova 1999; Robbrecht & Manen, 2006). According to the latter study (Tab. 2), it is characterised by woody or herbaceous habit, presence of calcium oxalate raphides [$\text{Ca}(\text{COO})_2$] in leaves, predominantly valvate corolla aestivation, frequently heterostylous flowers, higher ploidy levels (Kiehn, 1995, 2010) and aluminium accumulation (Jansen et al., 2000a, 2002b).

2.5 Psychotrieae alliance [Supertribe Psychotriidinae]

The subfamily Rubioideae consists of two major clades classified as supertribes, the predominantly herbaceous Rubioidae and the predominantly woody **Psychotriidinae**, the latter is a well supported monophyletic group that currently includes 8 tribes: Cratispermeae, Gaertnereae, Mitchelleae, Morindeae, Palicoureeae, Pristomerideae Psychotrieae and Schradereae (Razafimandimbison et al., 2008; Robbrecht & Manen, 2006 but see Bremer & Eriksson, 2009). Table 3 lists changes in tribal classifications of the Psychotriidinae [also known as Psychotrieae s.l. sensu Andersson & Rova (1999) or *Psychotrieae* alliance sensu Bremer & Manen (2000)].

Tab. 3: Different tribal circumscriptions of the Psychotrieae alliance [Psychotriidinae].¹

Andersson & Rova (1999)	Bremer & Manen (2000)	Robbrecht & Manen (2006)	Razafimandimbison et al. (2008)
	Cratispermeae	Cratispermeae	Cratispermeae
Gaertnereae	Gaertnereae	Gaertnereae	Gaertnereae Mitchelleae
Morindeae	Morindeae	Morindeae	Morindeae
		Palicoureeae	Palicoureeae Pristomerideae
Psychotrieae s.l.²	Psychotrieae s.l.²	Psychotrieae s.str.	Psychotrieae s.str.
Schradereae	Schradereae	Schradereae	Schradereae

¹ based on macromolecular phylogenetic studies (modified from Razafimandimbison et al., 2008).

² including Palicoureeae sensu Robbrecht & Manen (2006).

The tribes Palicoureeae and Psychotrieae (previously treated together as Psychotrieae s.l.) are the focus of the present work and will be discussed in detail.

2.6 Psychotrieae sensu lato

The tribe Psychotrieae Cham. & Schltdl. s.l. [Psychotrieae sensu Taylor, 1996 and Bremer, 1996; Psychotrieae s.str. sensu Bremer & Manen, 2000; Psychotriidinae sensu Bremer & Manen, 2006] is the most speciose tribe of the Rubioideae and the Rubiaceae in general. In a broader sense, the tribe includes 36 genera and well over 2000 species (Taylor, 1996, Andersson, 2002b). By taking into account several recent surveys, it may well consist of more than 3000 species (Govaerts et al., 2011; Davis et al., 2009; Razafimandimbison et al., 2008; Taylor, 2003; Zappi, 2003). The tribe is characterised by fleshy drupaceous fruits with one single basal ovule per locule and seeds with non-oily (often horny) endosperm and small embryos (Andersson, 2002b; Bremer & Manen, 2000; Taylor, 1996).

Several genera of the Psychotrieae s.l. like *Chassalia*, *Hymenocoleus* and the Hydnophytinae (myrmecophilous epiphytes *Anthorrhiza*, *Hydnophytum*, *Myrmecodia*, *Squamellaria* and related genera, Huxley & Jebb, 1990, 1991) are paleotropic, the genus *Margaritopsis* is pantropical in distribution (Andersson, 2001). Based upon the generic circumscription of Steyermark (1972), Taylor (1996) lists the following genera of the Psychotrieae s.l. for the Neotropics: *Aphanocarpus*, *Coccochondra*, *Coryphothamnus*, *Declieuxia*, *Geophila*, *Pagamea*, *Pagameopsis*, *Palicourea*, *Psychotria*, *Rudgea* and *Stachyococcus*. Several more genera have subsequently been recognized. In the context of the present work, and in order to avoid confusion with the Psychotrieae s.str., all genera of the tribe Psychotrieae s.l. are informally called the *Psychotria* alliance.

History of Classification within the *Psychotria* alliance

The pantropic genus *Psychotria* s.l. is the largest genus within the Psychotrieae s.l. and the Rubiaceae, consisting of more than 1800 species (Davis et al., 2009; Govaerts et al., 2011). It is represented in the Neotropics by at least 600 species (Delprete, 2004). These are mainly shrubs and occasionally small trees, forming an important part of understory rainforest species diversity and abundance (Gentry, 1990). Costa Rica is one of the most speciose regions, from which over 115 species of *Psychotria* s.l. are reported (Burger & Taylor, 1993). Species of *Psychotria* s.l. do not possess any striking similarities beyond widespread characters defining the whole tribe Psychotrieae s.l.: distinctive interpetiolar stipules which are often bilobed and sometimes bare an appendage, small and usually white flowers and drupaceous fruits with mostly 2 pyrenes [a pyrene denotes a stone of a drupe consisting of endocarp and enclosed seed].

Several attempts were made towards a more natural grouping of *Psychotria* resulting in a subdivision in subgenera (Petit, 1964; Steyermark, 1972) which were finally placed in two tribes (Bremer & Manen, 2006). Petit (1964), in his treatment of African Psychotrieae, made the first modern attempt and restricted the genus *Psychotria* to comprise only plants having pyrenes with ethanol-soluble red seed coat pigment (RSCP) and lacking preformed germination slits (PGS), whereas he included species with opposite characters in *Chassalia* or *Chazaliella*. Steyermark (1972), in his revision of neotropical Psychotrieae, defined *Psychotria* in a broader sense and included most neotropical species of the Psychotrieae s.l. He concluded that species placed in *Cephaelis*, *Grumilea*, *Mapouria*, *Psychotria*, *Uragoga* or several other segregate genera must be grouped under one comprehensive genus, *Psychotria*. Similarly, Petit (1964, 1966) synonymised African taxa of *Cephaelis*, *Grumilea*, *Mapouria* and *Uragoga* under *Psychotria*. Steyermark (1972)

subdivided neotropical representatives of the genus in two subgenera: subg. *Psychotria* (corresponding to *Psychotria* sensu Petit, 1964, 1966) and subg. *Heteropsychotria*. Steyermark used different characters than Petit to define subg. *Psychotria*: deciduous stipules, ferruginous stipule colleters (i.e. secretory hairs at the base of the stipule scar), and dried leaves of reddish-brown, greyish-black, or blackish colour, and the commissural face of the seeds usually plane, and with the dorsal surface of the seeds 4-5 lobed, whereas he defined subg. *Heteropsychotria* by the absence of the former characters.

It has recently been shown by macromolecular phylogenetic data that the genus *Psychotria* is highly paraphyletic, comprising several distinct lineages. It was therefore split into several smaller monophyletic groups separated at generic level (e.g. Andersson and Rova, 1999; Nepokroeff, 1999; Robbrecht & Manen, 2006). Segregation of species added three further genera to the Psychotrieae s.l.: *Carapichea* (Andersson, 2002a), *Margaritopsis* (Andersson, 2001) and *Notopleura* (Taylor, 2001) whereas *Pagameopsis* was excluded due to unclear tribal classification (Piesschaert et al., 2001a). Two genera were transferred to other tribes: *Pagamea* is now included in Gaertnereae (Andersson and Rova, 1999; Bremer & Manen, 2000; Robbrecht & Manen, 2006) and *Declieuxia* is included in Coussareeae (Nepokroeff et al., 1999; Bremer & Manen, 2000; Robbrecht & Manen, 2006). Some species of *Psychotria* were transferred to the genus *Ronabea* which belongs to the tribe Lasiantheae (Taylor, 2004). The former genera *Cephaelis*, *Mapouria*, *Montamans* and *Uragoga* were treated as synonyms (Taylor, 1996). Therefore, the total number of neotropical genera in Psychotrieae s.l. is 11.

The tribe Psychotrieae s.l., also classified as Palicoureeae-Psychotrieae s.str. clade, can be subdivided in two major groups i.e. tribes: Psychotrieae s.str. (*Psychotria* complex sensu Andersson, 2001, 2002a, 2002b) and Palicoureeae sensu Robbrecht & Manen (2006) (*Palicourea* complex sensu Andersson, 2001, 2002a, 2002b) (Robbrecht & Manen, 2006 but see Bremer & Eriksson, 2009). Table 4 lists all genera and infrageneric groups treated in the present work.

Tab. 4: Selected genera and infrageneric groups traditionally assigned to the tribe Psychotrieae s.l., and their current tribal placement in either Psychotrieae s.str. or Palicoureeae.¹

	Psychotrieae s.l	Psychotrieae s.str.	Palicoureeae
<i>Psychotria</i> subg. <i>Psychotria</i>	x	x	
<i>Psychotria</i> subg. <i>Tetramerae</i>	x	x	
<i>Psychotria</i> subg. <i>Heteropsychotria</i>	x		x
<i>Carapichea</i>	x		x
<i>Chassalia</i>	x		x
<i>Geophila</i>	x		x
<i>Margaritopsis</i> ²	x		x
<i>Notopleura</i>	x		x
<i>Palicourea</i>	x		x
<i>Ronabea</i>	x	now included in Lasiantheae	
<i>Rudgea</i>	x		x

¹ according to Robbrecht & Manen, 2006.

² including *Chazaliella*, *Chytropsia* and *Readea*.

Fig. 1 illustrates part of the morphological diversity of species within *Psychotria* subg. *Psychotria* (Psychotrieae), *Psychotria* subg. *Heteropsychotria*, *Notopleura* and *Palicourea*. Note differences in inflorescence structure: bracteous and congested vs. ebracteose and branched, and corolla structure: small and white vs. large and conspicuously coloured. Fig. 2 shows a molecular phylogenetic tree of the Psychotrieae s.l. indicating differentiations at tribal and generic level. The tree has been modified from Nepokroeff et al. (1999) in order to reflect modern generic classification of species according to literature treated in the following chapters.



Fig. 1: Morphological diversity among species of the *Psychotria* alliance. Clockwise: whole plant and inflorescence of *Psychotria chiriquiensis* (Palicoureeae), inflorescence of *Psychotria nervosa* (Psychotrieae), whole plant and inflorescence of *Notopleura polyphlebia* (Palicoureeae), infructescence of *Psychotria marginata* (Psychotrieae), inflorescence and flower of *Palicourea padifolia* (Palicoureeae).

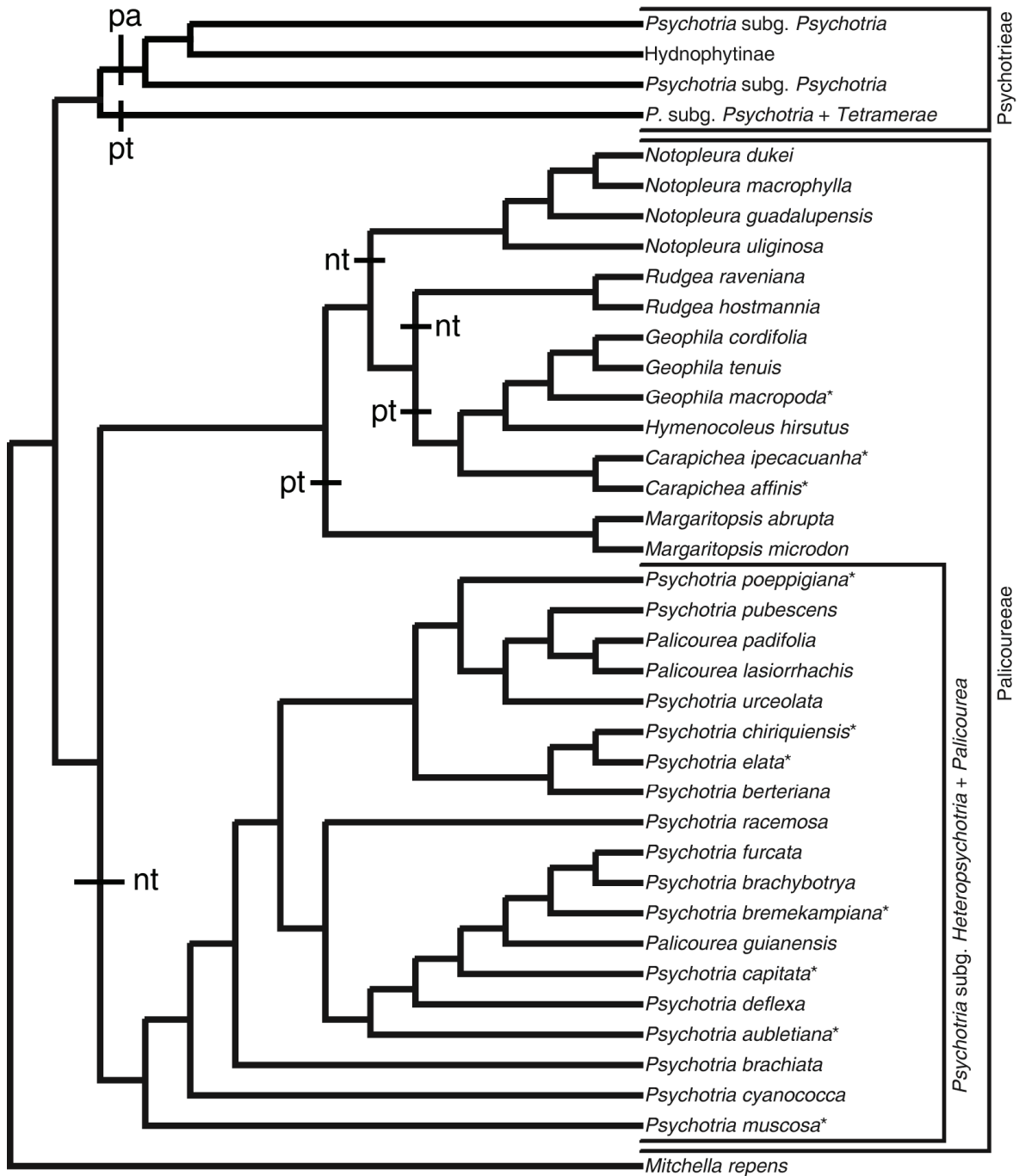


Fig. 2: One of 48 trees resulting from maximum parsimony analysis of ITS sequence data . Modified from Nepokroeff et al. (1999), updated with currently accepted names, tribal placement according to Bremer & Manen (2006). Clades of species of *Psychotria* subg. *Psychotria* are combined to single lines, whereas relationships within the Palicoureae are fully shown. Distribution of lineages is indicated by neotropical (nt), pacific (pa) and pantropical clades (pt). Species traditionally classified as *Cephaelis* are additionally marked with an asterisk.

2.7 Psychotrieae sensu stricto

The tribe Psychotrieae s.str. [clade subg. *Psychotria*, subg. *Tetramerae* and Hydnophytinae of Nepokroeff et al., 1999; *Psychotria* complex sensu Andersson, 2001, 2002a, 2002b] has long been treated in a broader sense as Psychotrieae s.l., including species now placed in the Palicoureeae. According to both morphological characters and macromolecular phylogenetic data (Razafimandimbison et al., 2008; Robbrecht & Manen, 2006 but see Bremer & Eriksson, 2009), the tribe was restricted to comprise the pantropical *Psychotria* subg. *Psychotria*, the African subg. *Tetramerae*, the paleotropical subtribe Hydnophytinae (myrmecophilous tuberous epiphytes *Anthorrhiza*, *Hydnophytum*, *Myrmecodia*, *Myrmephytum* and *Squamellaria*, Huxley & Jebb, 1990, 1991) and several small and little known Paleotropic genera like *Straussia* or *Streblosa*. The Hydnophytinae are nested within the Psychotrieae clade and their recognition creates the paraphyletic autonym Psychotriinae for the remaining species (Nepokroeff et al., 1999; Robbrecht & Manen, 2006).

The tribe Psychotrieae is largely characterized by caducous stipules shed by an abscission layer (except for myrmecophilous Hydnophytinae and the nodule bearing African subg. *Tetramerae*), absence of PGS (except for Pacific representatives of *Psychotria* and Hydnophytinae) and the presence of RSCP (e.g. Andersson, 2002b; Robbrecht & Manen, 2006).

2.7.1 *Psychotria* subg. *Psychotria*

Species of *Psychotria* subg. *Psychotria* [*Psychotria* s.str. sensu Nepokroeff et al. 1999] are currently placed in the tribe Psychotrieae s.str. (Razafimandimbison et al., 2008; Robbrecht & Manen, 2006). According to Andersson (2002b), subg. *Psychotria* can be subdivided in two major clades: *Psychotria* s.str. and the Pacific clade including the Hydnophytinae and species of *Psychotria* sometimes referred to as *Calycosia* for Polynesian and *Straussia* for Hawaiian species.

Species of *Psychotria* subg. *Psychotria* are characterised by caducous stipules leaving a ring of reddish colleters (secretory hairy structures), leaves drying gray or rusty brown, mature fruits usually red, pyrenes with soluble RSCP and lacking PGS (Hamilton, 1989a, 1989b, 1989c; Steyermark, 1972; Taylor, 1996; Robbrecht & Manen, 2006). They are pantropic in distribution and usually found in relatively drier habitats below 1000 m

elevation (Taylor, 1996). The Pacific clade differs in pyrenes having marginal PGS, the Hydnophytinae additionally through non caducous stipules (Andersson, 2002b).

Species of *Psychotria* subg. *Psychotria* have small, possibly melittophilous and psychophilous flowers, but no detailed studies on pollination have been undertaken (Csekits, 2008). The fruits are red and fleshy drupes that are dispersed by birds (Poulin et al., 1999; Snow, 1981).

2.7.2 *Psychotria* subg. *Tetramerae*

The group was initially established as *Psychotria* sect. *Tetramerae* Hiern (Hiern, 1877). It was later elevated to subg. *Tetramerae* (Hiern) E. M. A. Petit and includes all African species possessing bacterial leaf galls whilst all species lacking these features were included in subg. *Psychotria* (Petit, 1964). Subg. *Tetramerae* is additionally characterised by persisting stipules and its African distribution.

Whilst Steyermark (1972) included *P. microdon*, a neotropical species lacking bacterial nodules, in subg. *Psychotria*, Hamilton (1989a) suggested its placement in subg. *Tetramerae*. Molecular studies showed this species to be misplaced in either *Psychotria* subg. *Psychotria* or in the Psychotrieae s.str. and it was transferred to the recently expanded genus *Margaritopsis* (Andersson, 2001). Although subg. *Tetramerae* was essentially supported as a monophyletic clade, it was shown to be deeply nested within subg. *Psychotria*. Recognising it at the subgeneric level would leave the remainder of subg. *Psychotria* paraphyletic. Therefore it seems favourable to follow Hiern (1877) and re-classify subg. *Tetramerae* as a section within *Psychotria* s.str. (Andersson, 2002b; Nepokroeff et al., 1999).

2.8 Palicoureeae

The tribe Palicoureeae Robbr. & Manen [clade subg. *Heteropsychotria* + *Palicourea* of Nepokroeff et al., 1999; *Palicourea* complex sensu Andersson, 2001, 2002a, 2002b] was previously treated as a part of the Psychotrieae s.l. Contrary to a broad circumscription of the Psychotrieae as Psychotrieae s.l., morphological characters and macromolecular phylogenetic data favoured the recognition of a second tribe, the Palicoureeae (Razafimandimbison et al., 2008; Robbrecht & Manen, 2006 but see Bremer & Eriksson, 2009). The tribe is recognised by persisting (sometimes marcescent) stipules, pyrenes with distinct PGS, the absence of RSCP and non ruminant endosperm (Andersson, 2002b; Razafimandimbison et al., 2008; Robbrecht & Manen, 2006).

Due to the lack of morphological characters, generic delimitation in the Palicoureeae is challenging. Based upon macromolecular phylogenetic data (e.g. Nepokroeff et al., 1999; Andersson, 2001, 2002a, 2002b), several rearrangements were proposed, separating species formerly included in *Psychotria* subg. *Heteropsychotria* on generic level as *Carapichea* (Andersson, 2002a), *Margaritopsis* (Andersson, 2001, 2002c; Taylor, 2005), *Notopleura* (Taylor, 2001, 2003) and *Ronabea* (Taylor, 2004). Together with previously recognised genera such as *Geophila* and *Rudgea*, these genera are closely related to each other showing a sister group relationship to the subg. *Heteropsychotria* and *Palicourea* clade. Based upon a combination of morphological and macromolecular phylogenetic data, species of the genus *Ronabea* were excluded from the tribe and are currently classified in the Lasiantheae (Taylor, 2004)

The tribe Palicoureeae is currently circumscribed to contain the neotropical genera *Carapichea*, *Margaritopsis*, *Notopleura*, *Palicourea*, *Psychotria* subg. *Heteropsychotria* and *Rudgea*, the pantropical *Geophila* and the palaeotropical *Hymenocoleus* and *Chassalia*. Based upon morphological data, other genera belong to the Palicoureeae as well, but those were insufficiently studied, and not yet included (Robbrecht & Manen, 2006).

2.8.1 *Carapichea*

The genus *Carapichea* Aubl. includes at least 6 neotropical species occurring from Nicaragua to Bolivia (Andersson, 2002a; de Boer & Thulin, 2005; Jardim & Zappi, 2008; Taylor & Zappi, 2006). *Carapichea ipecacuanha* (Brot.) L. Andersson [syn. *Callicocca ipecacuanha* Brot. and *Psychotria ipecacuanha* (Brot.) Stokes] is well known as source of the emetic drug Ipecac, and still of some economic importance.

Carapichea was first recognised by Aublet (1775), but subsequently included in *Cephaelis* and *Psychotria* subg. *Heteropsychotria* (e.g. by Steyermark, 1972). Macromolecular phylogenetic data has shown that species of *Carapichea* comprise a distinct lineage and should be treated as a separate genus (Andersson 2002a; Nepokroeff et al., 1999). *Carapichea* was therefore re-established by Andersson (2002a) to include *C. affinis* (Standl.) L. Andersson, *C. guianensis* Aubl. and *C. ipecacuanha*. Three species were added on base of morphological data: *C. dolichophylla* (Standl.) C. M. Taylor, *C. ligularis* (Rudge) Delprete and *C. lucida* J. G. Jardim & Zappi (Delprete, 2003, Jardim & Zappi, 2008). Species of this genus show great morphological diversity, thus recognition of generic characters is challenging. However, species can be recognised by a combination of characters: marcescent stipules with margins fragmenting with age, mostly green

glomerulate and involucrate inflorescences, fruit colour ranging from red, deep purple to black and pyrenes with one basal-ventral PGS (Andersson 2002a; Jardim & Zappi, 2008; Taylor & Zappi, 2006). The genus is sister to *Margaritopsis*, the resulting clade in turn is sister to *Chassalia*, *Geophila* and *Hymenocoleus* (see Fig. 2; Andersson, 2002a).

2.8.2 *Chassalia*

Chassalia Comm. ex J. L. M. Poiret includes at least 42 species with paleotropical distribution (Piesschaert et al., 2001). According to macromolecular phylogenetic data, *Chassalia* is closely related to *Geophila* and *Hymenocoleus*, but also related to *Carapichea*, *Margaritopsis* and *Rudgea* (Bremer, 1996; Andersson & Rova, 1999; Nepokroeff et al., 1999; Piesschaert et al., 2000; Andersson, 2001, 2002a, 2002b). Nepokroeff et al. (1999, ITS and rbcL data) found *Chassalia* and *Geophila* belonging to a well supported clade, which is sister to *Carapichea*. Although with low support, their clade is further related to *Hymenocoleus*, *Margaritopsis*, *Notopleura* and *Rudgea*. According to Andersson (2001, 2002a, 2002b, rps16 intron data), *Chassalia*, *Geophila* and *Hymenocoleus* belong to the same clade, which is in turn sister to a group consisting of *Carapichea* and *Margaritopsis*. *Chassalia* also shares similarities with *Geophila* and *Hymenocoleus* in pyrene anatomy confirming their close relationship (Piesschaert et al., 2001).

The genus is recognised by persisting corky stipules, terminal, fleshy-succulent and often coloured inflorescence axes, winged floral buds, long and at least slightly curved corolla tubes, pyrenes with a ventral excavation as well as a single dorsal PGS and large porate or colpate pollen grains with reticulate exine (Piesschaert et al., 2001).

2.8.3 *Geophila*

The pantropical genus *Geophila* D. Don comprises about 20 species of creeping herbs or shrubs, of which approximately 10 occur in the Neotropics. Members of the genus are easily recognised by their creeping herbaceous habit, long petiolate, cordate leaves and dorsally ribbed pyrenes with ribs frequently twisted (Taylor, 1996; Piesschaert et al., 1999). As already discussed under *Chassalia*, *Geophila* is closely related to that genus.

2.8.4 *Margaritopsis*

The genus *Margaritopsis* C. Wright was previously regarded as being endemic to the Greater Antilles, but it was subsequently shown by molecular data to form a strongly supported pantropical clade with several other rubiaceaceous taxa (Andersson, 2001). It was

therefore suggested to include species of the genera *Chazaliella*, *Chytropsia* and *Readea*, but as most of them are poorly known, new combinations were only provided for the type species of these genera (Andersson, 2001, 2002c). Andersson (2001) found these species forming a well supported clade [*Margaritopsis* s.l.] with four neotropical species of *P.* subg. *Heteropsychotria* and of subg. *Tetramerae* as well as eight Asian and Australasian species of *Psychotria* subg. *Psychotria*. Taylor (2005) recognised 27 species of *Margaritopsis* in the Neotropics. All these are shrubs found in wet to seasonally dry forests in the Greater Antilles and from Mexico southwards to Paraguay. In total, *Margaritopsis* might comprise more than 60 species (Andersson, 2001; Taylor, 2005). In macromolecular phylogenetic studies, the genus is sister to *Carapichea*, the resulting clade is sister to a clade consisting of *Chassalia*, *Geophila* and *Hymenocoleus* (Andersson, 2002a).

The genus is difficult to characterise and its species have long been included in *Psychotria*, but these can be distinguished by their persisting stipules, absence of bacterial nodules, planoconvex pyrenes with two distinct marginal PGS and seeds lacking RSCP. Species of *Margaritopsis* are further recognised by their shrubby habit, usually flattened and longitudinally ridged stems, their raphidate leaves drying greenish or greyish, marcescent stipules, sometimes forming a circumnodal sheath which is becoming corky and fragmented with age. The flowers are arranged in a terminal inflorescence, fruits are small red to orange drupes, and their pyrenes are dorsally ridged or nearly smooth and ventrally smooth or with a short medial longitudinal crest. They differ from other genera of the Palicoureeae by the absence of characters defining these groups, succulent habit, pseudoaxillary inflorescence and fleshy stipules in *Notopleura*, glandular stipules in *Rudgea* or coloured inflorescence axes and corolla in *Palicourea* (Andersson, 2001; Taylor, 2005; Taylor & Zappi, 2006).

2.8.5 *Notopleura*

The genus *Notopleura* (Benth. & Hook. f.) Bremek. is a neotropical genus with species found in wet tropical forests from Mexico and the Antilles south to Bolivia and Brazil. It was formerly included in *Psychotria* subg. *Heteropsychotria* and comprises of about 100 species of terrestrial and epiphytic herbs and subshrubs placed in two subgenera. Species of subg. *Notopleura* are terrestrial, whereas species of subg. *Viscagoga* (Baill.) C. M. Taylor are epiphytic. An overview of species of *Notopleura* including detailed descriptions and keys is given by Taylor (2001), with further species recognised by Taylor (2003).

The group was first described as *Psychotria* sect. *Notopleura* Benth. & Hook. f. (Bentham & Hooker, 1874) and erected to generic level by Bremekamp (1934), an opinion rejected by subsequent authors e.g. Steyermark (1972). Recent morphological (Taylor, 2001) and macromolecular phylogenetic data (Nepokroeff et al., 1999) led to the segregation of *Notopleura* from *Psychotria* (Taylor, 2001). Species of *Notopleura* can easily be distinguished by their usually less than 2 m tall and little branched, fleshy-succulent habit, distinctive fleshy stipules united around the stem to form a sheath bearing a central succulent appendage at each side, inflorescences that are usually pseudoaxillary, small white flowers and 2-4(-6) pyrenes per fruit opening through two small basal adaxial and marginal PGS (Taylor, 2001, 2003; Taylor & Zappi, 2006).

Species of *Notopleura* have small, possibly melittophilous and psychophilous flowers, but no pollination studies have been undertaken (Csekits, 2008). The fruits are succulent or somewhat spongy drupes, which are assumed to be dispersed by birds. Their colour is species specific and varies from white to red or black (Taylor, 2001).

2.8.6 *Palicourea*

The genus *Palicourea* Aubl. is neotropical in distribution, reaching from central Mexico and the Antilles southwards to northern Argentina (Taylor, 1997). It is the sixth largest genus of the Rubiaceae and includes more than 310 species of shrubs or small trees, a number which is currently expanded (Borhidi, 2011; Davis et al., 2009; Taylor et al., 2010). Macromolecular phylogenetic data (Andersson & Rova, 1999; Nepokroeff et al., 1999; Robbrecht & Manen, 2006) indicate that the genus is polyphyletic and nested within *Psychotria* subg. *Heteropsychotria*, from which it differs only in a suite of floral traits associated with hummingbird pollination. Hummingbird pollinated flowers have repeatedly evolved in several groups of bee-pollinated ancestors (e.g. Castellanos et al., 2004; Fenster et al., 2004). Similarly, *Palicourea* spp. could be considered as derived lineages of *Psychotria* subg. *Heteropsychotria* or vice versa and it was hypothesised, that species of *Palicourea* have evolved multiple times out of subg. *Heteropsychotria* in adaption to pollinator shift (Taylor, 1996, 1997). In order to render *Psychotria* a monophyletic group, species of subg. *Heteropsychotria* have to be transferred to *Palicourea*. The process of providing the necessary combinations was recently started with the initial publications of Taylor et al. (2010) and Borhidi (2011). Furthermore most species are quite similar in characters and therefore thought to be closely related (Taylor, 1997).

Species of *Palicourea* are typically found in understory or subcanopy of wet rainforests and are especially frequent in high elevation habitats above 1500 m, where other groups are less speciose (Taylor, 1996, 1997). Species of *Palicourea* have persisting stipules united around the stem having a bilobed part interpetiolarly. They frequently have coloured inflorescence axes together with brightly coloured corollas. Corollas are usually 5-merous, have a well developed tube, a gibbous swelling at their base and an internal ring of protective hairs situated above the nectar accumulating basal swelling.

Palicourea spp. show brightly coloured, odourless flowers with long tubes and are consequently pollinated by hummingbirds. Nectar accumulates at the enlarged base of the corolla and is protected by a ring of stiff hairs, which may be overcome only by birds while blocking insects from reaching the nectar. Their fruits are fleshy blue or purple-black fruits and are dispersed by birds (Taylor, 1997; Ornelas et al., 2004a, 2004b).

2.8.7 *Psychotria* subg. *Heteropsychotria*

Species of *Psychotria* subg. *Heteropsychotria* Steyerm. are currently placed in the tribe Palicoureeae (Razafimandimbison et al., 2008; Robbrecht & Manen, 2006, but see Bremer & Eriksson, 2009). In its traditional circumscription, subg. *Heteropsychotria* is characterised by persisting, triangular to bilobed stipules, leaves generally drying green or gray, flowers usually white, mature fruits usually blue, purple or black, pyrenes with PGS mostly present and RSCP absent (Taylor, 1996; Robbrecht & Manen, 2006). Its members are exclusively neotropical in distribution and are usually found in wet habitats below 1500 m elevation (Taylor, 1996).

In macromolecular phylogenetic studies, species of subg. *Heteropsychotria* were shown to be more closely related to *Palicourea* than to subg. *Psychotria* (Andersson & Rova, 1999; Nepokroeff et al., 1999; Robbrecht & Manen, 2006). Furthermore, subg. *Heteropsychotria* itself turned out to be paraphyletic and interspersed with species of *Palicourea*. It was therefore hypothesised that the corolla structure characterising *Palicourea* has evolved several times out of subg. *Heteropsychotria*. In order to render *Psychotria* a monophyletic group, the genus needs to be restricted to subg. *Psychotria*. In an ongoing effort, species of subg. *Heteropsychotria* are therefore continuously transferred to *Palicourea* (Borhidi, 2011; Taylor et al., 2010)

Species of *Psychotria* subg. *Heteropsychotria* have small, melittophilous, psychophilous or rarely ornithophilous flowers. A comprehensive summary for pollination

syndromes of Costa Rican species is given in Csekits (2008). Its fruits are blue, purple or black fleshy drupes, and are dispersed by birds (Poulin et al., 1999; Snow, 1981).

2.8.8 *Rudgea*

The genus *Rudgea* Salisb. includes at least 120 neotropical species found from Mexico to Argentina (Taylor & Zappi, 2006; Zappi, 2003). Species can usually be recognised by their shrubby habit, their usually domatia bearing leaves and their persisting stipules with distinct fimbriate to pectinate-glandular appendages. Corollas are usually white to cream and larger than in other genera of the *Psychotria* alliance, reaching up to 7 cm in length. Fruits are usually white, spongy and crowned by a persisting calyx. The (1-)2 pyrenes per fruit open through 2 long basal marginal and 1-3(-5) abaxial PGS, containing seeds that are deeply furrowed adaxially (Taylor, 1996; Zappi, 2003; Taylor & Zappi, 2006).

Species of *Rudgea* have white flowers that are either melittophilous or psychophilous. The fruits are fleshy, white drupes, which are assumed to be dispersed by birds, but no detailed information is available in this respect (Csekits, 2008; Zappi, 2003).

2.9 Lasiantheae

Based upon the rps12 intron region, Piesschaert et al. (2000) found the genera *Lasianthus*, *Ronabea* (as *Psychotria emetica*), *Saldinia* and *Trichostachys* being closely related, but showing no affinities with the *Psychotria* alliance as previously expected. Bremer & Manen (2000) confirmed *Lasianthus* and *Trichostachys* as being distantly related to the Psychotrieae and assigned them to their newly delimited tribe Lasiantheae B. Bremer & Manen. The genus *Ronabea* also belongs here (Taylor, 2004).

2.9.1 *Ronabea*

The genus *Ronabea*, initially established by Aublet (1775), was subsequently included in *Psychotria* (e.g. by Steyermark, 1972) but later resurrected, its species segregated from *Psychotria* and assigned in the newly delimited tribe Lasiantheae (Taylor, 2004). Its species are distributed from Belize south to Bolivia and comprises three shrubby species formerly included in *Psychotria* subg. *Heteropsychotria*: *Ronabea emetica* (L. f.) A. Rich. [syn. *Psychotria emetica* L. f.], *R. insanae* (J. H. Kirkbr.) C. M. Taylor, and *R. latifolia* Aubl. [syn. *Psychotria erecta* (Aubl.) Standl. & Steyerm.].

Ronabea was first recognised by Aublet (1775), but has later been included in *Psychotria* sect. *Oppositiflorae* Benth. & Hook. (Bentham & Hooker, 1873). Its species

were found to be quite distantly related to *Psychotria* by morphological (Piesschaert, 2001) and macromolecular phylogenetic data (Piesschaert et al., 2000). They were shown to belong to a genus of their own, which is now placed in the tribe Lasiantheae (Bremer & Manen, 2000; Piesschaert et al., 2000; Taylor, 2004; Taylor & Zappi, 2006). *Ronabea* may be recognized by a combination of widespread characters within the Rubiaceae: simple, persisting triangular stipules, axillary inflorescences with small white flowers and blue-black drupaceous fruits with two pyrenes being smooth on the adaxial surface. The pyrenes bare oily endosperm and open through ventral PGS (Taylor, 2004; Taylor & Zappi, 2006).

Species of *Ronabea* have small white flowers that are possibly insect pollinated, but no information is known in this respect (Csekits, 2008). Their fruits are fleshy blue to black drupes, which are dispersed by birds (Poulin et al., 1999).

3 Phytochemistry

Several species of *Psychotria* and related genera are used as medicinal plants by indigenous tribes, making use of their bioactive compounds, e.g. the San Martin Quechuas of the Amazon basin (Sanz-Biset et al., 2009). Such species with traditional ethnomedical usage have often been the starting point of phytochemical studies within *Psychotria*. Together with numerous other surveys, a broad variety of secondary metabolites of different biosynthetic pathways was discovered (e.g. Lopes et al., 2004; Porto et al., 2009). Nevertheless, when compared to the total number of species, only a small portion has yet been studied.

Compounds already known from *Psychotria* s.l. as well as those found in the present study are grouped according to their biosynthetic origin in iridoids, tryptamine alkaloids (i.e. simple indole alkaloids), cyclotryptamine alkaloids (i.e. pyrrolidinoindoline alkaloids), tryptamine-iridoid alkaloids (i.e. monoterpene indole alkaloids), dopamine-iridoid alkaloids (i.e. isoquinoline alkaloids) flavonoids and tannins, coumarins, naphthoquinones and terpenoids.

3.1 Iridoid Glycosides

Iridoids are monoterpenes bearing a cyclopentanopyran core. They are usually oxygenized and glycosylated, in most cases with β -D-glucose. Cleavage of the cyclopentane ring results in seco-iridoids, cleavage of the pyran ring leads to various other derivatives (Dinda et al., 2007a). One of the most important iridoids is secologanin, being a key progenitor in monoterpene indole alkaloid (MIA) biosynthesis (O'Connor & Maresh, 2006; El-Sayed & Verpoorte, 2007).

Iridoids are derived from condensation of two C₅-moieties (isopentenyl diphosphate, IPP and dimethylallyl pyrophosphate, DMAPP). Two independent pathways are involved in the biosynthesis of these C₅ units, the precursors of all terpenoids: the mevalonate (MVA) and the alternative 2-C-methyl-D-erythritol 4-phosphate or methyl-erythritol phosphate (MEP) pathway. The MVA pathway is situated in the cytoplasm, whereas the MEP pathway is located in the plastids (e.g. Lichtenthaler et al., 1997; El-Sayed & Verpoorte, 2007). Radiotracer studies in *Catharanthus roseus* (L.) G. Don (Apocynaceae) and *Ophiorrhiza pumila* Champ. ex Benth. (Rubiaceae) showed that the iridoid secologanin, the terpene precursors in MIA biosynthesis, is derived via the MEP-pathway (Contin et al., 1998; Yamazaki et al., 2004). Fig. 3 shows the biosynthesis of secologanin.

Important steps in secologanin biosynthesis are the condensation of two C₅-moieties to geraniol, which is hydroxylated by the enzyme geraniol-10-hydroxylase (G10H) and further processed to iridodial and iridotrial intermediates. Subsequently, loganin is formed and finally transferred to secologanin by the enzyme secologanin synthase (SLS). The conversion to secologanin is hypothesized as rate limiting step in monoterpene indole alkaloid biosynthesis (O'Connor & Maresh, 2006; El-Sayed & Verpoorte, 2007).

Iridoids are sensitive to artifact formation, as shown for asperuloside during prolonged boiling in H₂O and MeOH, where asperulosidic acid, daphylloside, deacetylasperuloside, deacetylasperulosidic acid and scandoside were formed through hydrolysis and methanolysis (Inouye et al., 1969).

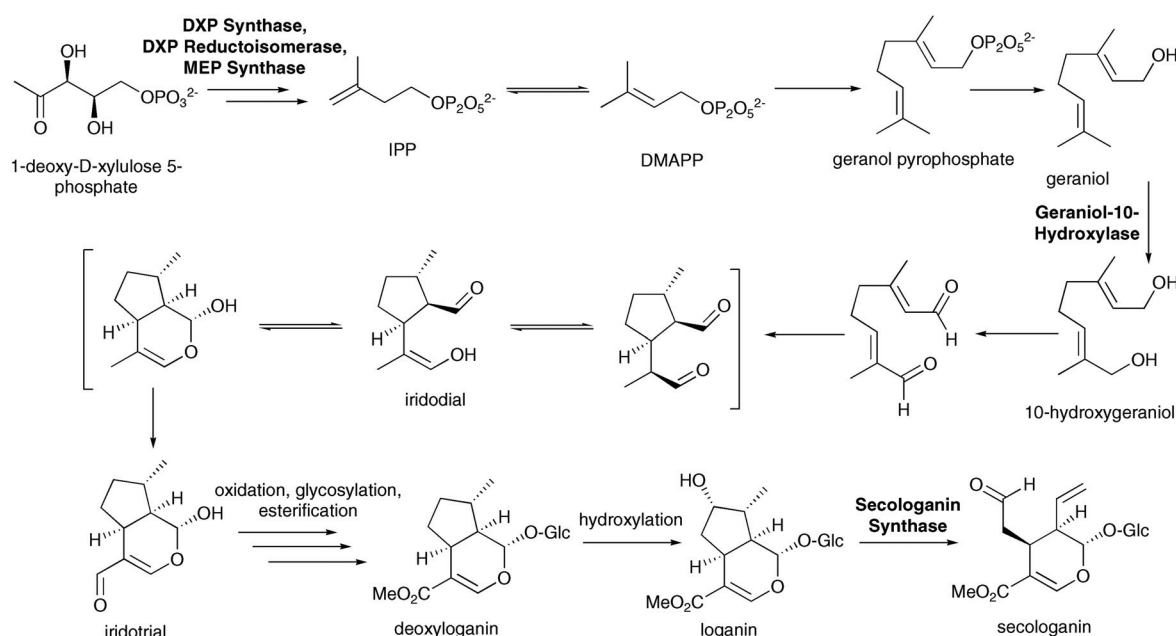


Fig. 3: Biosynthesis of iridoids. Isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP) precursors are synthesized via the non-mevalonate pathway giving rise to the monoterpene geraniol. Upon further oxidations, iridoids such as secologanin, the essential precursor of MIA, are formed (Fig. from O'Connor & Maresh, 2006).

3.1.1 Distribution of iridoids in *Psychotria* s.l.

Due to the focus on alkaloid discovery, data on the distribution of iridoid glycosides in the *Psychotria* alliance is scarce. Inouye et al. (1988) reported the occurrence of asperuloside in the Asian species *Psychotria asiatica* L. (as *P. rubra* (Lour.) Poir.), *P. serpens* L. and *P. manillensis* Bartl. ex DC. Out of 19 neotropical species studied by Lopes et al. (2004), only *Palicourea rigida* Kunth and *Psychotria leiocarpa* Cham. & Schltdl. were found to accumulate iridoids. A comprehensive overview of the structure and diversity of iridoids previously discovered in *Psychotria* and related genera is given by Plagg (2012).

3.1.2 Plant-Herbivore Interaction

In addition to their bitter and deterrent taste, iridoid glycosides display toxicity against invertebrates and pathogens. Iridoid glycosides are activated by either acid hydrolysis or a dedicated β -glucosidases. After cleavage from the glycone moiety, a semiacetal is liberated, which spontaneously rearranges to a highly reactive dialdehyde. The activated aglycones subsequently act as alkylating agents binding to nucleophilic side chains, denaturing and cross linking amino acids, proteins and nucleic acids in the herbivores gut (Dobler et al., 2011; Konno et al., 2009). The consumption of iridoid glycosides thus leads to reduced growth and lower survival rates in herbivores by either reducing food uptake or by taking action in the herbivores gut leading to problems in nutrition uptake. Additionally, aglycones, but not glycosides, are effective against bacteria and fungi (Dobler et al., 2011).

3.1.3 Pharmacological Activities

Structures as well as bioactivity of recently discovered iridoids is reviewed by Dinda et al. (2007a, 2007b, 2009, 2011). Among these, antibacterial, anticancer, antifungal, antiprotozoal and antiviral activities are most prominent.

3.2 Tryptamine Alkaloids

All tryptamine and tryptamine iridoid alkaloids incorporate one or more tryptamine units, derived from the essential amino acid tryptophan via the enzyme tryptophan decarboxylase (TDC, Fig. 4). The enzyme converts tryptophan to tryptamine, which is the direct progenitor of all MIA (El-Sayed & Verpoorte, 2007; O'Connor & Maresh, 2006). Two classes of tryptamine alkaloids may be recognized on base of biosynthetic origin i.e. involvement of an iridoid moiety: Mono-, di- and oligomeric tryptamine alkaloids also classified as cyclotryptamine or pyrrolidinoindoline alkaloids, are formed by methylation and cyclisation of tryptamine. Tryptamine-iridoid alkaloids, also classified as MIA are formed via condensation of tryptamine and the iridoid secologanine. Fig. 4 shows the biosynthesis of different tryptamines starting from the amino acid tryptophan.

3.3 Monomeric tryptamine alkaloids

Tryptamine is involved in *N,N*-dimethyltryptamine (DMT) biosynthesis (Fig. 4), and the enzyme indolethylamine *N*-methyltransferase (INMT) subsequently converts tryptamine to *N*-methyltryptamine and *N,N*-dimethyltryptamine (Kärkkäinen et al., 2005;

Mack et al., 1988). Most likely, NMT represents the branching point to cyclotryptamine alkaloids (see 3.4).

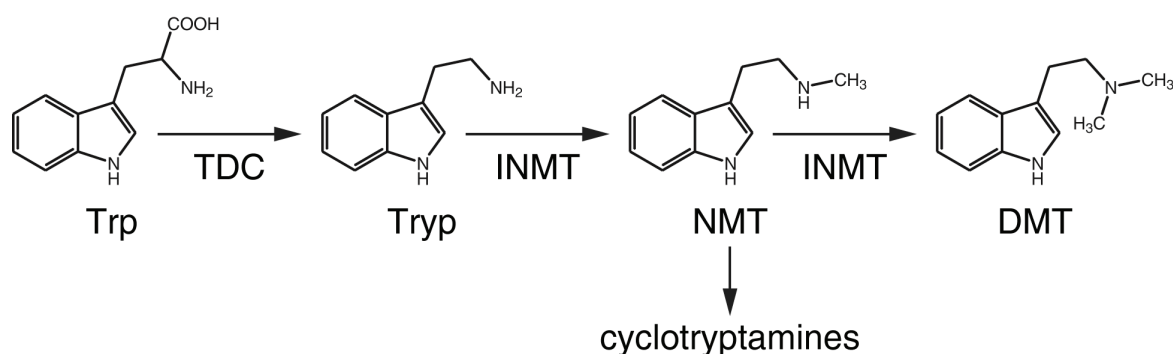


Fig. 4: Biosynthesis of simple tryptamine alkaloids starting from the amino acid tryptophan. Trp: tryptophan, try: tryptamine, NMT: *N*-methyltryptamine, DMT: *N,N*-dimethyltryptamine. TDC: tryptophan decarboxylase, INMT: indolethylamine *N*-methyltransferase.

3.3.1 Distribution in *Psychotria* s.l.

The only species known to accumulate the simple indole alkaloid DMT is *Psychotria viridis* (Rivier & Lindgren, 1972, see comments under section 9.2). Conflicting reports exist on other species like *P. carthagenensis*, which was reported to containing the compound (Rivier & Lindgren, 1972), but was found to be devoid of alkaloids (Leal & Elisabetsky, 1996). Both species may have been confused because they are almost identical in vegetative characters and may be distinguished only by their inflorescence structure (Hamilton, 1989a).

3.3.2 Plant-Herbivore Interaction

Indole is synthesized as a volatile signal in response to herbivore damage, alluring parasitoids to their pray (Hartmann, 2007). Besides its role as a volatile, little is known concerning deterrent, anti-feeding or toxic effects of simple indole alkaloids. However, β -carboline alkaloids have been shown to be detrimental and toxic to coleopteran stored-grain pests (Nenaah, 2011) as well as to lepidopteran pests (Bouayad et al., 2012).

3.3.3 Pharmacological Activities

DMT (Fig. 4) is one of the active principals of the indigenous hallucinogenic beverage ayahuasca, which is prepared and used by several tribes in the Amazon Basin and adjacent areas of South America. Together with stem bark of the vine *Banisteriopsis caapi*, leaves of *Psychotria viridis* are decocted and used to induce hallucinations for religious and medicinal purpose (e.g. Rivier & Lindgren, 1972; Schultes, 1972)

3.4 Cyclotryptamine alkaloids

Cyclotryptamine alkaloids, also classified as pyrrolidinoindoline alkaloids, consist of one or more *cis*-pyrrolidino[2,3-*b*]indoline monomers. Biosynthetically, they are derived from the amino acid tryptophan, from which they are likely formed via cyclisation of *N*-methyl tryptamine monomers (Fig. 4). Two names for these compounds exist, the term cyclotryptamine refers to the biosynthesis of the compound, whereas the term pyrrolidinoindoline refers to the structure of the tricyclic core. Due to their hypothesized biosynthetic origin, they are usually methylated at the nitrogen of the pyrrolidine moiety, whereas the nitrogen of indoline is not (Fig. 5; Steven & Overman, 2007). From these basal units, dimers, trimers and even octamers are formed (Adjibadé et al., 1990a; Ruiz-Sanchis et al., 2011; Saad et al., 1995; Steven & Overman, 2007). Two types of linkage are frequently encountered: C^{3a}/C^{3a'} (between two pyrrolidine rings) and C^{3a}/C^{7'} (between an aromatic and a pyrrolidine ring) resulting in linear structure (Takayama et al., 2004). Fig. 5 shows a selection of cyclotryptamine alkaloids.

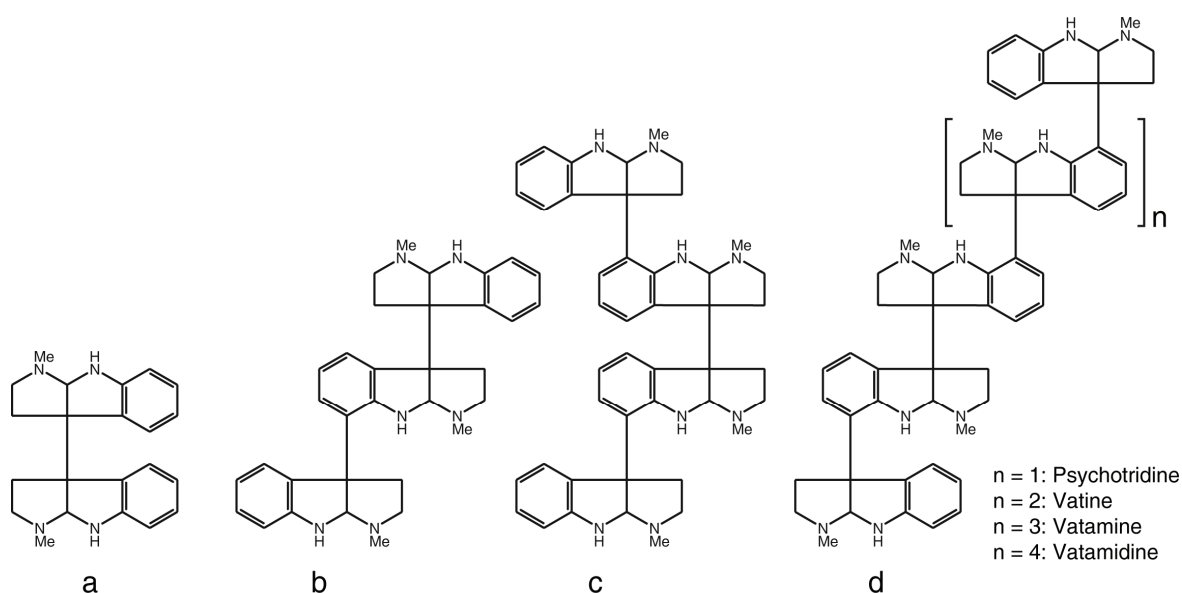


Fig. 5: Selected di- and oligomeric cyclotryptamine i.e. pyrrolidinoindoline alkaloids previously isolated from species of the *Psychotria* alliance. Chimonanthine (a), hodgkinsine (b), quadrigemine C (c) and the penta- to octamers psychotridine, vatine, vatamine and vatamidine (d). Modified from Steven & Overman (2007).

3.4.1 Biosynthesis

Biosynthesis of cyclotryptamine i.e. pyrrolidinoindolines has been studied in *Calycanthus floridus* L. and *Chimonanthus fragrans* Lindl. (both Calycanthaceae) using stable isotopes. After administration of labeled tryptophan and tryptamine, these were incorporated into calycanthine and chimonanthine. The amino acid tryptophan therefore acts as a progenitor in pyrrolidinoindoline biosynthesis (Steven & Overman, 2007). It has

been suggested that further reaction mechanisms involve radical substitutions (Steven & Overman, 2007).

3.4.2 Monomeric cyclotryptamine alkaloids

Monomeric pyrrolidinoindoline alkaloids are reported from a wide range of organisms distributed all over the tree of life. Simple monomers are known to occur in the moulds *Aspergillus fischeri* Wehmer and *Penicillium simplicissimum* (Oudem.) Thom. Trichocomaceae), in the marine bryozoan *Flustra foliacea* (Linnaeus, 1758; Flustridae), the frog *Pseudophryne coriacea* Keferstein, 1868 (Myobatrachidae), the spikemoss *Selaginella moellendorffii* Hieron. (Selaginellaceae) and the seed plants *Allium ramosum* L. (Amaryllidaceae) and *Physostigma venenosum* Balf. f. (Fabaceae) (Ruiz-Sanchis et al., 2011).

3.4.3 Dimeric cyclotryptamine alkaloids

Dimeric pyrrolidinoindoline alkaloids are known from the fungal genera *Amauroascus* (Onygenaceae), *Aspergillus* (Trichocomaceae), *Chaetomium* (Chaetomiaceae), *Corollospora* (Halosphaeriaceae), *Gliocladium* (Hypocreaceae), *Leptosphaeria* (Leptosphaeriaceae), *Penicillium*, (Trichocomaceae) and *Verticillium* (Plectosphaerellaceae), from the marine bryozoan *Flustra foliacea* (Flustridae), but they are more often isolated from plants belonging to Calycanthaceae (incl. Idiospermaceae), Centropalacaceae and Rubiaceae (Ruiz-Sanchis et al., 2011; Steven & Overman, 2007).

Dimeric cyclotryptamine alkaloids linked between the C^{3a} atoms of each pyrrolidinoindoline unit, are divided in **chimonanthine** and **calycanthine** derivatives (Fig. 6). Whilst chimonanthines are indole alkaloids, calycanthines have a quinoline backbone i.e. are quinoline alkaloids that originate from indole alkaloids. It has been shown that alkaloids of the calycanthine type may be artifacts formed from chimonanthines during isolation. Chimonanthines become protonated under acidic conditions, forming an intermediate tetraamino aldehyde which rapidly converts to the stable calycanthine (Fig. 6). It remains uncertain if calycanthines are natural products (Hall et al., 1967; Libot et al., 1988; Steven & Overman, 2007).

Chimonanthines or Calycanthines have been isolated from the skin of the frog *Phylllobates terribilis* (Myers, Daly, and Malkin, 1978; Tokuyama & Daly, 1983; Dendrobatidae), the Calycanthaceae *Calycanthus floridus*, *Chimonanthus praecox* (L.) Link and *Idiospermum australiense* (Diels) S. T. Blake, *Bhesa paniculata* Arn. (Centropalacaceae) and in the Rubiaceae *Argostemma yappii* King, *Pausinystalia*

macroceras (K. Schum.) Pierre ex Beille and in some *Psychotria* spp. (Kam & Choo, 2006; Ruiz-Sanchis et al., 2011; Steven & Overman, 2007). The dimeric pyrrolidinoindolines chimonanthine or calycanthine have also been reported from several *Palicourea* spp.: *Palicourea alpina* (Sw.) DC., *P. domingensis* (Jacq.) DC., *P. fendleri* Standl. and *P. ovalis* Standl. (see Lopes et al., 2004 for references).

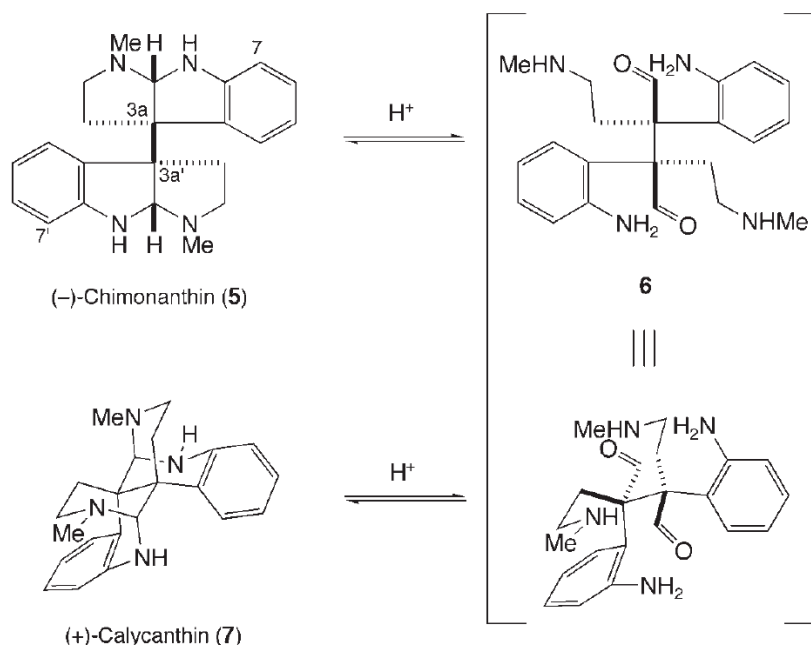


Fig. 6: Spontaneous conversion of chimonanthine to calycanthine under acid conditions. Fig. from Steven & Overman, 2007.

3.4.4 Oligomeric cyclotryptamine alkaloids

All oligomeric cyclotryptamine alkaloids consist of a dimeric $C^{3a},C^{3a'}$ -bispyrrolidino[2,3-*b*]indoline core, to which monomers are added (Steven & Overman, 2007). Oligomeric pyrrolidinoindolinea are less frequent than monomers or dimers, they were discovered in *Idiospermum australiense* (Calycanthaceae), in the Rubiaceae *Hodgkinsonia frutescens* C. T. White, *Psychotria milnei* (A. Gray) K. Schum. (as *Calycodendron milnei* (A. Gray) A. C. Sm.) and in several other *Psychotria* spp. (e.g. Kam & Choo, 2006; Ruiz-Sanchis et al., 2011; Steven & Overman, 2007).

3.4.5 Distribution of cyclotryptamine alkaloids in *Psychotria* s.l.

Because of the uncertainty on the natural occurrence of calycanthine type alkaloids, the following compilation will not differentiate between various cyclotryptamine types. Within *Psychotria*, they are reported from numerous species, particularly from *Psychotria* subg. *Psychotria*: *P. calocarpa* Kurz (Zhou et al., 2010), *P. forsteriana* A. Gray (e.g. Beretz et al., 1985), *P. leptothyrsa* Miq. (as *P. beccarioides* Wernham, Hart et al., 1974),

P. lyciiflora (Baill.) Schltr. (Jannic et al., 1999), *P. malayana* Jack (Hadi & Bremner, 2001), *P. milnei* (A. Gray) K. Schum. (as *Calycodendron milnei* (A. Gray) A. C. Sm., Adjibade et al., 1990a; Libot et al., 1987; Saad et al., 1995), *P. oleoides* (Baill.) Schltr. (Gueritte-Voegelein et al., 1992; Jannic et al., 1999), *P. pilifera* Hutch. (Li et al., 2011) and *P. rostrata* Blume (Lajis et al., 1993; Takayama et al., 2004). Their occurrence is scarce in *Psychotria* subg. *Heteropsychotria*. They were found in *P. colorata* (Willd. ex Roem. & Schult.) Müll. Arg. (Verotta et al., 1998; 1999), *P. glomerulata* (Donn. Sm.) Steyerm. (Solis et al., 1997) and *P. muscosa* (Jacq.) Steyerm. (Verotta et al., 1999) only. Recently, pyrrolidinoindoline alkaloids have also been detected in a species of the genus *Margaritopsis*, *M. cymuligera* (Müll. Arg.) C. M. Taylor (Brand et al., 2012). A comprehensive structural overview of these compounds is given by Plagg (2012).

3.4.6 Plant-Herbivore Interaction

Nothing is known about the role of cyclotryptamine i.e. pyrrolidinoindoline alkaloids in plant defense or biotic interactions. Due to their strong pharmacological activity, toxicity against herbivores should be expected.

3.4.7 Pharmacological Activities

Cyclotryptamine alkaloids show a broad spectrum of biological activities. *Psychotria colorata* is used in the traditional medicine of the Amazonian Caboclos for treating pain (Elisabetsky & Castilhos, 1990). This effect is possibly due to the presence of di- and oligomeric cyclotryptamines (Verotta et al., 1998; 1999) which have been shown to possess similar pain relieving effects as morphine (Elisabetsky et al., 1995). Psycholeine and quadrigemine C were found affecting growth hormone secretion through antagonistic activity at somatostatin receptors (Gueritte-Voegelein et al., 1992; Rasolonjanahary et al., 1995). Besides anti-bacterial and anti-fungal activities, most alkaloids extracted from *P. milnei* (reported under *Calycodendron milnei*) and from *P. rostrata* exhibit cytotoxic activity against rat hepatoma, mice leukemia L₁₂₁₀, proliferating and non-proliferating Vero African green monkey kidney cells, and against human HEp-2 cells (Adjibade et al., 1990b; Mahmud et al., 1993; Saad et al., 1995). Hodgkinsine A further exhibits potent antiviral activity against herpes simplex type 1 and against vesicular stomatitis virus (Saad et al., 1995). Tetrameric and pentameric alkaloids from *P. forsteriana* potently inhibit human platelet aggregation (Beretz et al., 1985).

3.5 Tryptamine-Iridoid Alkaloids

Monoterpene indole alkaloids (MIA) are generally synthesized from the amino acid derived tryptamine and the iridoid secologanin (O'Connor & Maresh, 2006), and may therefore be classified as tryptamine iridoid alkaloids. In the first step, the enzyme tryptophan decarboxylase (TDC) converts the essential amino acid tryptophan to tryptamine (e.g. O'Connor & Maresh, 2006; El-Sayed & Verpoorte, 2007). Subsequently, the enzyme strictosidine synthase (STR or STS) catalyzes a Pictet-Spengler condensation between tryptamine and secologanin (e.g. O'Connor & Maresh, 2006; El-Sayed & Verpoorte, 2007) and is thus quoted a Pictet-Spenglerase (Bernhardt et al., 2010). Fig. 7 shows the biosynthesis of strictosidine.

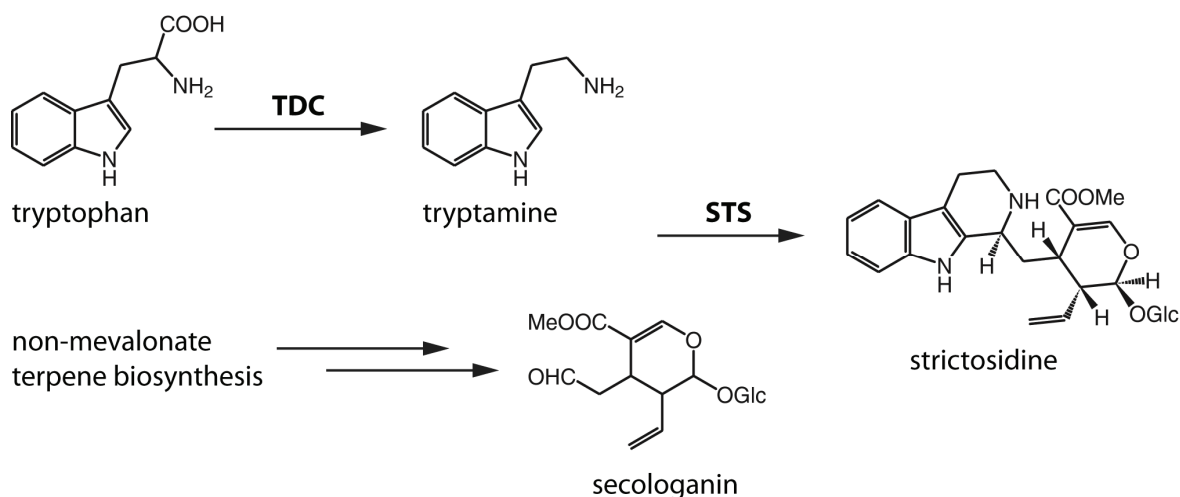


Fig. 7: Biosynthesis of strictosidine. Tryptamine is synthesized from the essential amino acid tryptophan in a tryptophan decarboxylase (TDC) catalyzed reaction, secologanin via the non mevalonate terpene biosynthesis. Strictosidine synthase (STS) catalyses the condensation of both to strictosidine (modified from O'Connor & Maresh, 2006).

STS was previously isolated and characterized from three species out of two families of the Gentianales, CrSTS from Madagascar Periwinkle (*Catharanthus roseus*, Apocynaceae; de Waal et al., 1995; McKnight et al., 1990; Mizukami et al., 1979; Pasquali et al., 1992; Treimer & Zenk, 1979), RsSTS from Indian Snakeroot (*Rauvolfia serpentina* (L.) Benth. ex Kurz, Apocynaceae; Kutchan et al., 1988) and OpSTS from *Ophiorrhiza pumila* Champ. ex Benth. (Rubiaceae; Yamazaki et al., 2003). Their sequence identities are 82% between SrSTS and RsSTS, and 54% or 60 % between CrSTS or RsSTS to OpSTS, respectively (Bernhardt et al., 2010).

Both, CrSTS from *Catharanthus roseus* and RsSTS from *Rauvolfia serpentina* tolerate a variety of substituted tryptamine analogs, but only minor changes to the aldehyde are accepted (Ma et al., 2006; McCoy et al., 2006; Treimer & Zenk, 1979). OpSTS from the rubiaceous *Ophiorrhiza pumila* differs from Apocynaceae STS in additionally accepting a vast range of simple aldehydes (Bernhardt et al., 2010). Due to common ancestry, STS from *Psychotria* spp. is putatively closer related to OpSTS than to apocynaceous STS, which led expect a similarly high substrate promiscuity as evidenced by the discovery of brachycerine (Kerber et al., 2001), croceaines A and B (Düsman et al., 2004) and psychollatine (Kerber et al., 2008), all MIA with loganine instead of a secologanine moiety.

Strictosidine- β -glucosidase (SG or SGD) is the key enzyme in downstream biosynthesis of more complex alkaloids possessing a derived backbone. Strictosidine is deglycosylated releasing a hemiacetal, which is spontaneously converted to the reactive dialdehyde, leading to various pathways (Fig. 8; e.g. Barleben et al., 2007; El-Sayed & Verpoorte, 2007; Geerlings et al., 2000; O'Connor & Maresh, 2006). It was quoted the gateway to more than 2000 structurally diverse compounds, some of which are shown in Fig. 8. Tryptamine iridoid alkaloids found in species of the *Psychotria* alliance show a rather primitive structure when compared with the structurally diverse compounds isolated from *Catharanthus roseus*, *Strychnos*, *Vinca* spp. or other genera of the Gentianales. A characteristic feature is the maintenance of the glucose residue, putatively caused by inoperative SG or reduced activity, blocking downstream modification of strictosidine.

3.5.1 Distribution of tryptamine iridoid alkaloids

Tryptamine iridoid i.e. MIA are found in species of the Apocynaceae, Cornaceae, Gelsemiaceae, Loganiaceae, Nyssaceae and Rubiaceae (O'Connor & Maresh, 2006). Within Palicoureeae and Psychotrieae, they are restricted to *Palicourea* and *Psychotria* subg. *Heteropsychotria*, and are found in numerous species (e.g. Lopes et al., 2004; Plagg et al., 2012; Porto et al., 2009). A comprehensive overview of tryptamine iridoid alkaloids encountered within these groups is given in by Plagg (2012).

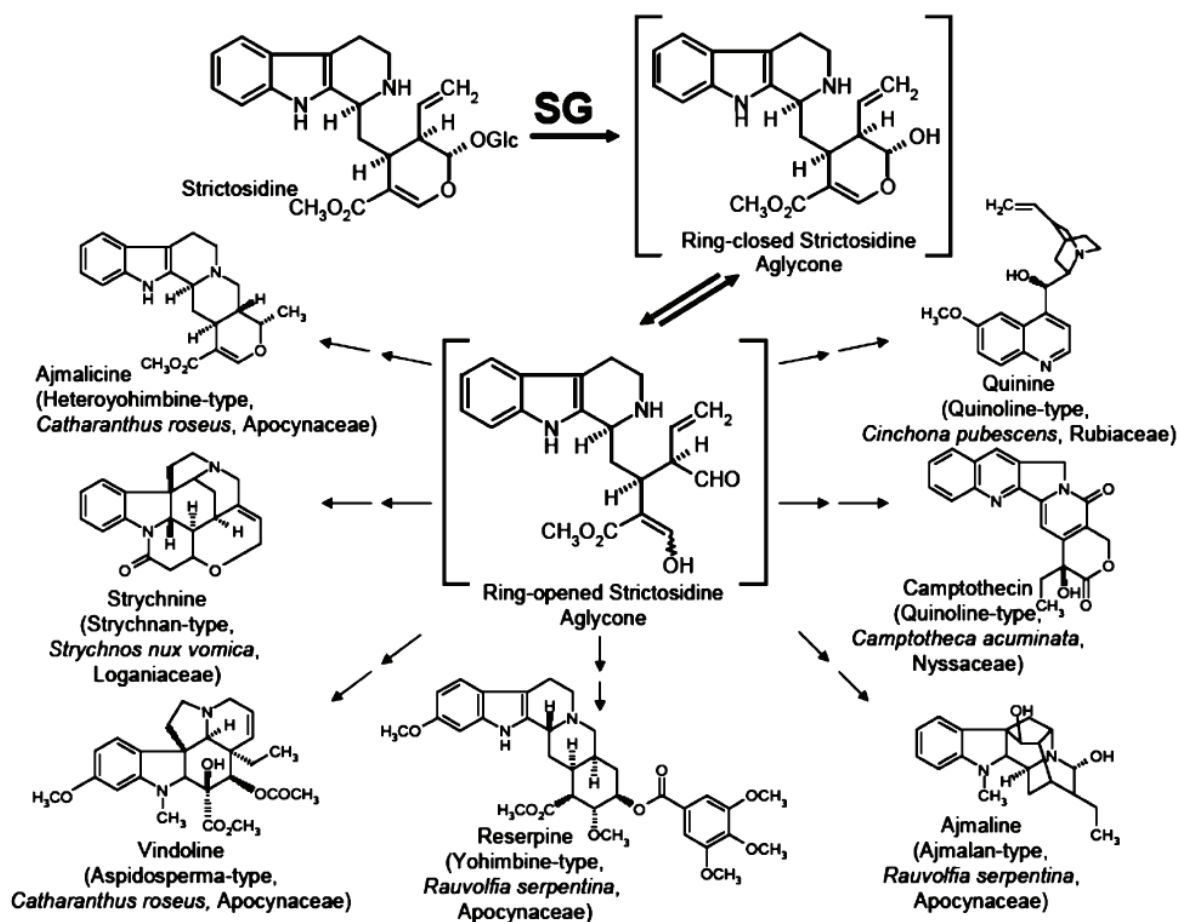


Fig. 8: Strictosidine glucosidase (SG) downstream modification of strictosidine, leading to pharmacologically important compounds. Fig. from Barleben et al., 2007.

3.5.2 Plant-Herbivore Interaction

Whilst antifeedant effects of several compounds like tannins are well understood as having a general effect on herbivores (Barbehenn & Constabel, 2011; Eck et al., 2001; Feeny, 1976), little is known concerning deterrent, anti-feedant or toxic effects of tryptamine iridoid alkaloids. From compounds encountered within the *Psychotria* alliance, only a single compound, strictosidine and its involvement in plant chemical defense was studied (Luijendijk et al., 1996). Strictosidine and its deglycosylation products formed by the enzyme strictosidine glucosidase (see El-Sayed & Verpoorte, 2007) were shown to be active against several microbes and fungi. In contrast, neither strictosidine, nor its aglycone products displayed antifeedant activity against *Spodoptera exigua* larvae.

Defence compounds are often stored as inactive glycosides in the vacuoles of plant cells, these are called phytoanticipins. Upon disruption of the cell, they react with their dedicated β -glucosidase and provide immediate chemical defense against herbivores and pathogens (Morant et al., 2008). Similarly, a possible mechanism of action of strictosidine was shown to involve a cellular pool of both strictosidine and the corresponding

strictosidine β -D-glucosidase. Upon enzyme-substrate reunion, the stable strictosidine is hydrolyzed to a highly reactive dialdehyde capable of protein cross-linking, thus disrupting herbivore feeding (Guirimand et al., 2010). A similar mode of inducing activity after tissue damage is known for cyanogenic glucosides (Zagrobelnya & Møller, 2011), glucosinolates (Winde & Wittstock) and iridoid glucosides (Dobler et al., 2011; Konno et al., 1999).

3.5.3 Pharmacological Activities

Several MIA are prominently employed as pharmaceutical compounds because they possess a broad range of bioactivities such as blocking the adrenergic receptor (yohimbine, *Rauwolfia serpentina*), anti-arrhythmic (ajmalicine and ajmaline, *R. serpentina*), anti-cancer (vinblastine and vincristine; *Catharanthus roseus*), anti-malaria (quinine, *Cinchona calisaya* Wedd.), anti-tumor (camptothecin, *Camptotheca acuminata* Decne.) and convulsant (strychnine, *Strychnos nux-vomica* L.) (e.g. O'Connor & Maresh, 2006).

Compounds found in the *Psychotria* alliance have not yet been applied as pharmaceuticals, but show promising effects in various assays. *P. brachypoda* (Müll. Arg.) Britton (syn. *P. umbellata* Vell.) contains the alkaloids psychollatin and umbellatine (Both et al., 2005; Kerber et al., 2008) exhibiting antidepressant, antimutagenic, antioxidant and anxiolytic as well as analgesic properties (Both et al., 2002, 2005, 2006; Fragoso et al., 2008). Brachycerine, extracted from *P. brachyceras* Müll. Arg., shows antioxidant and antimutagenic effects (Nascimento et al., 2007). Both myrianthosine and strictosidinic acid exhibit anti-inflammatory activities, by influencing chemotactic activity of rat polymorphonuclear leukocytes. The latter compound additionally shows analgesic activity (Reanmongkol et al., 2000; Simões-Pires et al., 2006). 14-oxoprunifoleine and strictosamide isolated from *Psychotria prunifolia* (Kunth) Steyerl. showed selective antiprotozoal effects against *Leishmania amazonensis* (Kato et al., 2012).

3.6 Dopamine-Iridoid Alkaloids

Dopamine- iridoid alkaloids, also classified as isoquinoline alkaloids, are derivatives of the amino acid tyrosine. In a first step, tyrosine is hydroxylated and decarboxylated to dopamine, corresponding to the initial steps of tryptamine-iridoid alkaloid biosynthesis. Condensation with secologanin results in formation of the stereoisomers *N*-deacetylisoipecoside and *N*-deacetylpecoside, which upon deglucosilation and condensation with another dopamine moiety, give rise to emetine, cephaeline and related compounds (Fig. 9, Nomura & Kutchan, 2010).

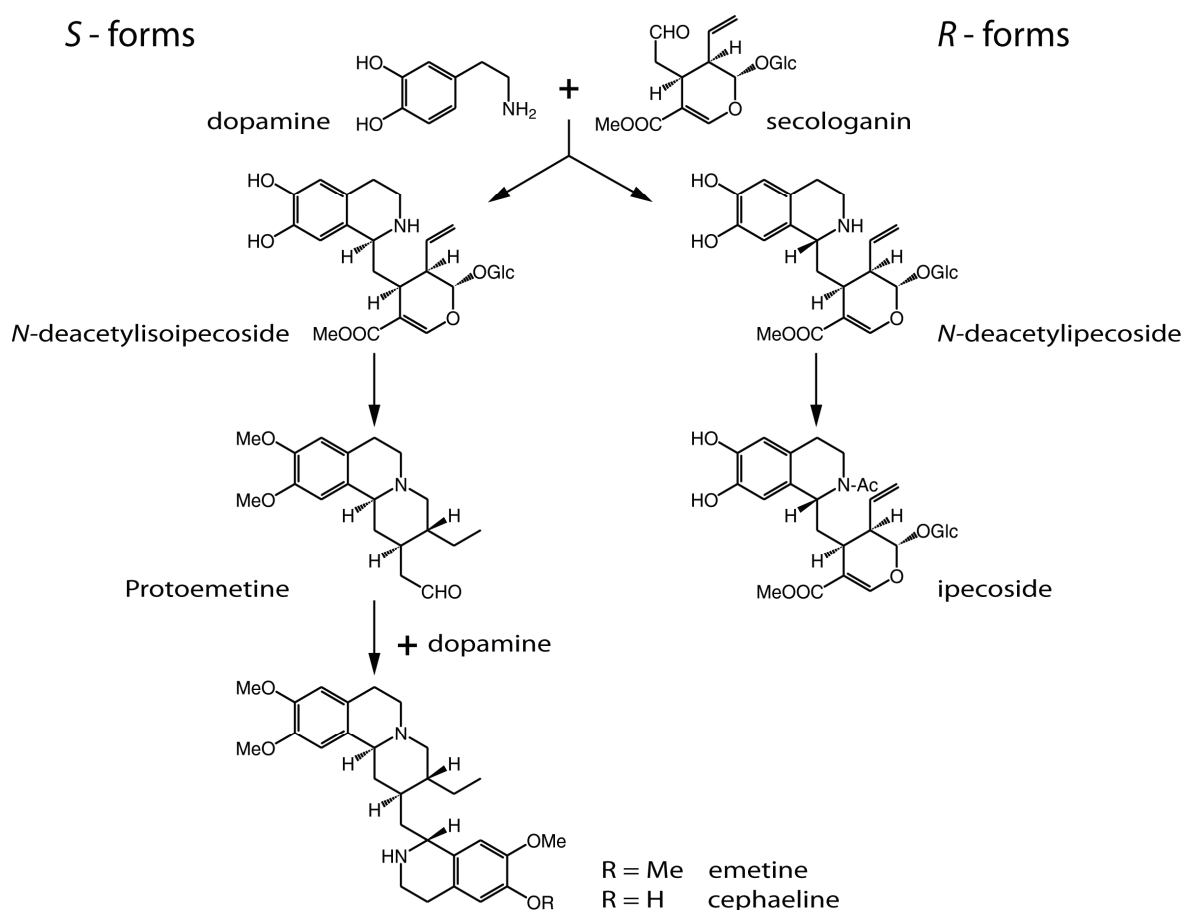


Fig. 9: Dopamine iridoid alkaloid biosynthesis in *Carapichea ipecacuanha*. Stereospecific condensation of dopamine and secologanine leads to ipecoside and emetine derivatives. (Modified and redrawn from Nomura & Kutchan, 2010).

3.6.1 Distribution of dopamine iridoid alkaloids

Within the *Psychotria* alliance, dopamine iridoid alkaloids are known as bioactive compounds from the roots of the medicinal plant ipecacuanha (*Carapichea ipecacuanha*, Rubiaceae-Palicooureeae; e.g. Itoh et al., 1999, 2002; Lee, 2008; Wiegrebbe et al., 1984). They also occur in *Psychotria klugii* Standl. (Muhammad et al., 2003), and they were recently discovered in *C. affinis* (syn. *P. borucana*; Bernhard, 2012; Bernhard et al., 2011). A comprehensive structural overview of dopamine iridoid alkaloids is given by Plagg (2012).

3.6.2 Plant-Herbivore Interaction

Little is known about the role of dopamine iridoid alkaloids as toxic or deterrent compounds in plant-herbivore interactions. Preliminary analysis indicated severe toxic effects of *Carapichea affinis* containing isoquinoline alkaloids, in a bioassay using *Spodoptera littoralis* Boisduval, 1833, the African Cotton Leafworm (Noctuidae; Kainradl, pers. comm.).

3.6.3 Pharmacological Activities

Carapichea ipecacuanha, the origin of the famous emetic and antiamoebic drug ipecacuanha, contains various emetine alkaloids also known as ipecacuanha alkaloids (e.g. Fisher, 1973; Nomura & Kutchan, 2010). Although ipecacuanha and its alkaloids have gained a lot of attention and importance especially in the 18th and 19th century, they are no longer used in treatment of antiamoebic dysentery.

3.7 Tannins

Phenolic compounds vary from simple phenolic acids (e.g. gallic acid) to complex polymeric structures such as lignin. Tannins are naturally occurring oligo- or polymeric compounds of polyphenolic origin. All types of tannins share protein precipitating and linking abilities, which are used to tan animal skins to leather. Depending on their type and origin, tannins can reach a molecular weight up to 20.000 Da (Khanbabaee & van Ree, 2001). They are found in a variety of plant families including widely known sources like oak bark (*Quercus* spp., Fagaceae) Katechu (*Acacia catechu* (L. f.) Willd., Fabaceae-Mimosoideae) and Gambir (*Uncaria gambir* (Hunter) Roxb., Rubiaceae).

Freudenberg (1920) categorized tannins as either hydrolysable or non-hydrolysable, with the latter are also known as proanthocyanidins (PAs) or condensed tannins. Hydrolysable tannins are either glycosidic hexahydroxydiphenoyl units (ellagitannins) or gallic acid derivatives (gallotannins). By contrast, flavonoid derived condensed tannins are oligo- to polymers derived from the condensation of the flavan-3-ol subunits (+)-catechin and (-)-epicatechin (Dixon et al., 2005; Pourcel et al., 2005; Xie et al., 2003). A further group of tannins has later been established, incorporating characters of both groups, and these are termed flavanoellagitannins or complex tannins. A modern and more accurate classification divides tannins into four structural types: gallotannins, ellagitannins, complex and condensed tannins (Fig. 10, Khanbabaee & van Ree, 2001).

Proanthocyanidins, i.e. condensed tannins, are of major importance in the context of the present work (see 7.2.1, 7.3 and 8). Functional aspects of proanthocyanidin biosynthesis have recently been studied in *Arabidopsis thaliana* (L.) Heynh. (Dixon et al., 2005; Xie et al., 2003; Zhao et al., 2010). PAs are synthesized as colorless polymers and are stored in the central vacuole (Stafford, 1988). In dead cells, the cytoplasm and the vacuole disintegrate and PAs are oxidized into brown complexes that cross-link. These oxidation products contribute to the typical brown color of *A. thaliana* testa during seed desiccation (Pourcel et al., 2005).

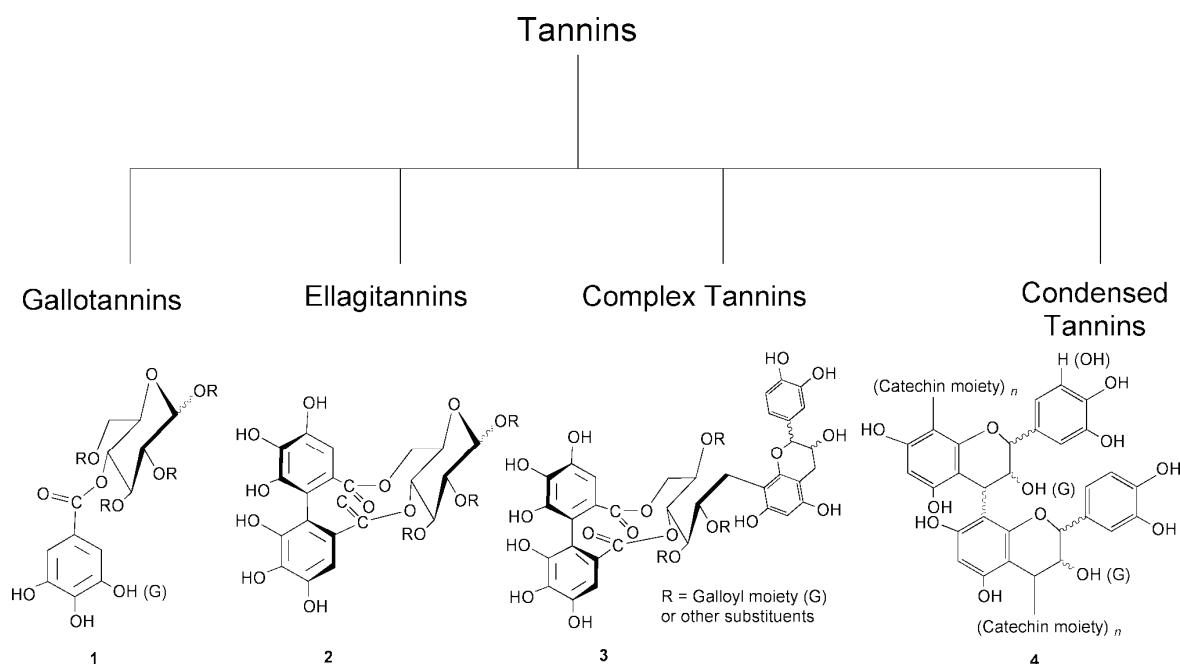


Fig. 10: Structures of the four types of tannins according to Khanbabaee & van Ree (2001).

3.7.1 Distribution of tannins in *Psychotria* s.l.

So far, no tannins have been extracted from *Psychotria* spp. Other polyphenolic compounds encountered in *Psychotria* s.l. include flavonoids from *Psychotria spectabilis* Steyerm. (Benevides et al., 2004) and from *Palicourea rigida* (Rosa et al., 2010). Studying the Vietnamese medicinal plant *Psychotria asiatica* (sub syn. *P. reevesii* Wall. ex Roxb.), Giang et al. (2007) found ^1H - and ^{13}C -NMR signals characteristic for catechin moieties but failed to isolate pure compounds.

3.7.2 Plant-Herbivore Interaction

Among quantitative defense compounds, **polyphenolics** are of outstanding importance because of their ubiquitous occurrence and accumulation at high concentrations. They are frequently found in concentrations ranging from 5-10% dry wt. in plant tissue (Barbehenn & Constabel, 2011) but reaching up to 30 % dry wt. in some cases (Eck et al., 2001). Tannins function as feeding deterrents affecting food-choice and uptake of many vertebrate and invertebrate herbivores and reduce performance and growth rate when incorporated into artificial diet (e.g. Eck et al., 2001; Lindroth & Peterson, 1988). Tannin content is also correlated to reduced herbivory in field studies (Osier et al., 2000; Sagers & Coley, 1995). By contrast, tannins can stimulate feeding and enhance performance in some specialist herbivores (e.g. Kolehmainen et al., 1995; Matsuki & Maclean, 1994; Rank 1992; Rank et al., 1998).

Pre-ingestive and post-ingestive effects of tannin rich diets have been considered as a possible mode of action. Besides pre-ingestive such deterrent or antifeedant effects mediated by adstringent taste, post-ingestive effects are due to metabolic costs in handling tannins. These may be caused by disrupting protein digestion or by direct toxicity (Barbehenn & Constabel, 2011; Barbehenn et al., 2009). Insects generally respond to high tannin content by increasing food consumption to counteract negative effects like high metabolic costs related with tannin uptake (Barbehenn et al., 2009, Osier et al, 2000).

Early studies attributed tannins a mostly antidigestive i.e. antinutritional activity by complexation of katabolic enzymes or dietary proteins, thus decreasing protein digestion and uptake (Feeny, 1968). Various types of tannins have been shown to interfere with mineral nutrient uptake by formation of insoluble complex as demonstrated for iron (Brune et al., 1989). Tannins bind to endogenous enzymes (e.g. proteases) as well as to nutritional proteins in the gut of herbivores, thus inhibiting protein digestibility and consequently decreasing plant nutritional quality. Although this may be the case for mammalian herbivores, these views have been challenged for insect herbivores. Numerous reports failed to demonstrate effects on protein utilization in grasshoppers (Bernays, 1978) or caterpillars (Barbehenn et al., 2009; Karowe, 1981).

By contrast, post-ingestive activities of polyphenolics are more likely to be mediated by their prooxidant activity causing oxidative stress in herbivores. Tannins are activated through oxidation in the insects gut, creating reactive oxygen species (ROS) including quinones, peroxides, semiquinone and hydroxyl radicals. ROS are capable of alkylating proteins by covalently binding to amino acids damaging nutrient proteins and gut-tissue (Barbehenn & Constabel, 2011; Barbehenn et al., 2009). However, in caterpillars of *Lymantria dispar* (Linnaeus, 1758; Noctuidae), high concentrations of tannins (about 15% dry weight) were necessary to generate detrimental amounts of semiquinone radicals, whilst lower concentrations could not overcome antioxidant defense (Barbehenn et al., 2009).

Within the heterogeneous tannins, ellagitannins are more likely causing oxidative stress whilst condensed tannins likely act as feeding deterrents (Barbehenn & Constabel, 2011). However, differences in effect of tannin structures on different groups of insects e.g. caterpillars, beetle larvae or sap-sucking insects remain insufficiently studied.

3.7.3 Pharmacological Activities

Tannins are regarded as functional ingredients of various beverages, foods and herbal remedies and mediate important aspects of their taste, function and bioactivity (Aron & Kennedy, 2008). Tannin containing plant drugs are applied for a variety of indications. They are used for their antioxidant and astringent effects, against diarrhoea and stomach or duodenal tumors, as diuretics, as anticarcinogenic, antiinflammatory, antiseptic, cardiopreventive and haemostatic agents. They show antiviral, antibacterial and antitumour activity (Aron & Kennedy, 2008; Khanbabaee & van Ree, 2001).

3.8 Other Compounds

3.8.1 Coumarins

Coumarins have previously been reported from *Psychotria spectabilis*, from which three compounds have been isolated (Benevides et al., 2005). *P. stachyoides* Benth. yielded scopoletin (Pimenta et al., 2011). Fig. 11 shows these compounds.

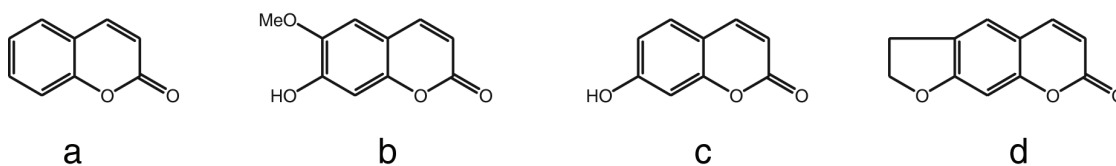
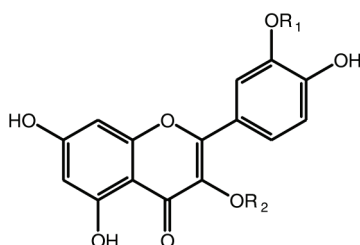


Fig. 11: Coumarins isolated from *Psychotria* spp. Coumarin (a), scopoletin (b), umbelliferone (c) and psoralene (d).

3.8.2 Flavonoids

Flavonoids are widely known for their almost ubiquitous distribution in the plant kingdom, and they have been reported from *Palicourea rigida* (Rosa et al., 2010) and *Psychotria spectabilis* (Benevides et al., 2005). Fig. 12 shows all flavonoids known from the *Psychotria* alliance. Interestingly, all are derivatives of quercetin. Special types of flavonoids, the complex and condensed tannins, are discussed in section 3.7.



$R_1=H$, $R_2=H$:	quercetin
$R_1=H$, $R_2=Glc$:	quercetin-3-O-glucoside
$R_1=H$, $R_2=Rh$:	quercitrin
$R_1=H$, $R_2=Glc-Glc$:	quercetin-3-O-sophoroside
$R_1=Me$, $R_2=Glc$:	isorhamnetin-3-O-glucoside

Fig. 12: Examples of flavonoids known from species of the *Psychotria* alliance.

3.8.3 Naphthoquinone derivatives

Two anthraquinones, an unspecified alizarin methylether and rubiadin have been extracted from *Psychotria stachyoides* (Palicoureeae) together with an alkaloid (Pimenta et al., 2011). Psychorubin, a pyranonaphthoquinone, was isolated from *Notopleura camponutans* (Dwyer & M. V. Hayden) C. M. Taylor (sub syn. *P. camponutans* (Dwyer & M. V. Hayden) Hammel, Palicoureeae; Jacobs et al., 2008; Solis et al., 1995) and from *P. asiatica* (sub syn. *P. rubra* (Lour.) Poir., Psychotrieae; Hayashi et al., 1987). Additionally, 2-azaanthraquinone (also called benz[g]isoquinoline-5,10-dione) has been extracted from *N. camponutans* (Solis et al., 1995). Fig. 13 shows these compounds.

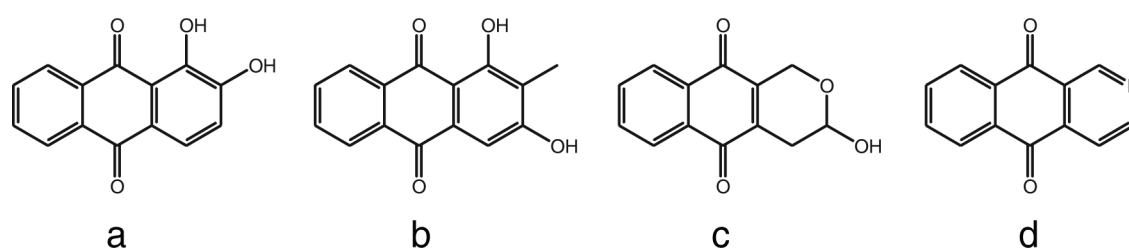


Fig. 13: Naphthoquinone derivatives previously isolated from species of the *Psychotria* alliance. Alizarin (a, an unspecified methylether has been isolated), rubiadin (b), psychorubin (c) and 2-azaanthraquinone (d).

3.8.4 Terpenes:

Megastigmanes, being C₁₃-sesquiterpenoids, have rarely been isolated from leaves of *Psychotria correae* (Achenbach et al., 1995), *P. stachyoides* (Pimenta et al., 2011) and *P. gitingensis* (Tan et al., 2012) and are shown in Fig. 14. Diterpenes are only known from *P. spectabilis*, from where solidagenone and deoxysolidagenone have been extracted (Benevides et al., 2005).

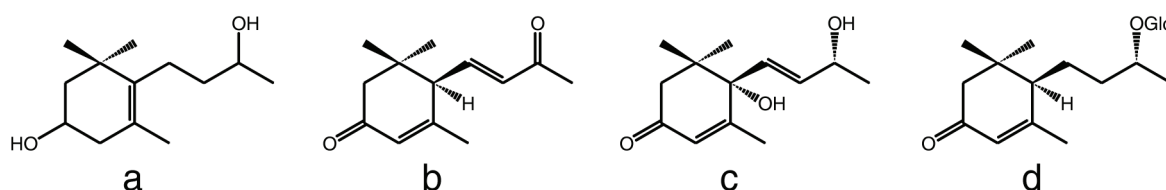


Fig. 14: Different megastigmane sesquiterpenoids isolated from *Psychotria* spp. Megastigm-5-ene-3,9-diol (a), S (+)-dehydrovomifoliol (b), vomifoliol (c) and byzantionoside B (d).

4 Material and Methods

4.1 Plant material

Most plants have been collected in August 2008 and February 2010 in tropical rain forests of Central America. Collections in Costa Rica were made in 2008 by M. Bernhard and Prof. H. Greger, collections 2010 by the author A. Berger. Further species were collected by Brian Sedio in Barro Colorado, Panama or were cultivated at the Botanical Garden, University of Vienna (HBV), originating from various collections mostly made by Prof. M. Kiehn. Voucher specimens of all collected species have been deposited at the herbarium WU (Herbarium der Universität Wien, University of Vienna, Austria).

Additional samples were obtained from herbarium specimens preserved at the herbarium W (Naturhistorisches Museum Wien, Vienna, Austria) and WU. Approximately 1-4 cm² of leaf material was removed from leaves stored in fragment packets affixed to the herbarium sheets, or carefully removed directly from the specimens.

Besides the main advantage in using samples taken from herbarium specimens which is easy access to species outside the own range of collection, problems arising from field techniques for plant collecting have to be kept in mind. Plants collected in remote tropical areas, where a plant drier is not always at hand, are sometimes field-preserved in a way detrimental to phytochemical integrity: Individual specimens are packed between newspaper, sprayed with 50-70% alcohol, pressed into bundles and preserved in sealed plastic bags until drying (Mori et al., 2011). Consequently, polar substances get partly dissolved and become soaked into the newspaper cover, thus reducing the amount of substances detectable in the leaves by comparative TLC and HPLC-analysis. Additionally, chlorophyll gets washed out, giving the specimens a dirty and spotty appearance. When selecting phytochemical samples taken from herbarium specimens, it is therefore favourable to select normally dried specimens, recognizable by annotations on the labels or by their uniform colour.

Table 10 (see appendix) lists all gathered species including scientific authors, collector, collection number, origin of the collection and herbarium number. Fig. 15 shows the country of origin of the analysed material, and Fig. 16 shows the approximate locality for species collected in Costa Rica, respectively. Images of voucher specimens are given in the appendix (Fig. 32-103). If more than one accession per species was collected, a representative one is selected for display. Digitalised images for all voucher specimens

including additional information are accessible under their corresponding species name or herbarium number at <http://herbarium.univie.ac.at/database/search.php>.



Fig. 15: World map showing countries, from where phytochemical samples were obtained. Triangles correspond to the approximate area of collection. BO: Bolivia, BR: Brazil, CD: Democratic Republic of the Congo, CR: Costa Rica (including collections from nearby Panama), EC: Ecuador, ET: Ethiopia, LK: Sri Lanka, PE: Peru, TH: Thailand, US: United States, VE: Venezuela.

4.2 Reference compounds

Crude extracts of *P. elata* and *P. tsakiana* (under the erroneous determination *P. racemosa*) have been studied by Fasshuber (2011) and Plagg (2012). Strictosidine and palicoside have been isolated by them and were used in the present study for co-chromatographic comparison. Similarly, alstrolines and dopamine iridoid alkaloids were available from studies by Bernhard et al (2011, 2012) and Schinnerl et al (2012). Additionally, the CHCl_3 -phase of *Notopleura polyphlebia* was studied by Philipp Kolar during a student course, and 2-aza-anthraquinone was obtained and used for chromatographic comparison. Scopoletin, previously isolated by our working group, was also used as reference substance for co-chromatographic detection. Catechin was purchased from Sigma-Aldrich (USA). A sample of *Ipecacuanhae radix* (*Carapichea ipecacuanha*) was obtained from the drug collection of the Department of Pharmacognosy, University of Vienna and a voucher is deposited at the herbarium WU (WU 0067845).

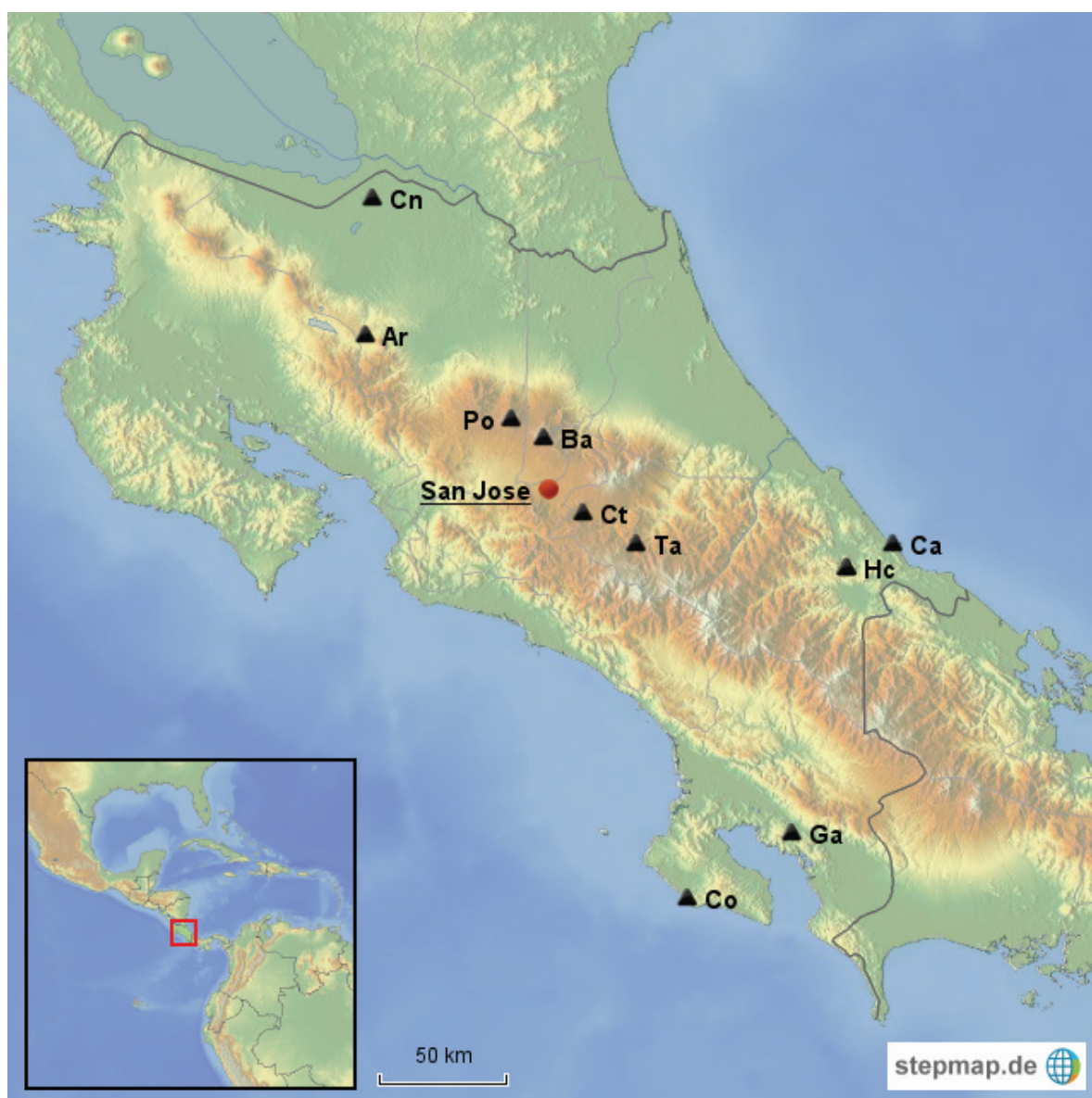


Fig. 16: Map of Costa Rica showing the regions, from where phytochemical samples were obtained. Ar: Arenal, Ba: Barva, Ca: Cahuita, Cn: Caño Negro, Co: Corcovado, Ct: Carpintera, Ga: La Gamba, Hc: Hitoy Cerere, Po: Poas, Ta: Tapanti.

4.3 Analytical methods

4.3.1 Thin layer Chromatography

Comparative thin layer chromatography (TLC) is a basic technique used to separate a mixture of organic compounds. As a stationary phase, various adsorbents are coated on glass plates, aluminum or plastic foil. After applying the sample as a thin spot, a mobile phase consisting of mixtures or pure solvents, is soaked into the stationary phase and separates the sample according to its interaction with the stationary phase. Different mobile phases were used depending on the nature of the samples to be analyzed.

In the course of the present work, pre-coated glass plates (silica gel 60 F254, Merck, Germany) have been used. Two solvent systems have been applied according to the chromatographic problem: $\text{CHCl}_3/\text{MeOH}/\text{H}_2\text{O}$ (80:20:1) and the organic phase of the immiscible $n\text{-BuOH}/\text{H}_2\text{O}/\text{AcOH}$ (5:4:1). Separation was monitored by UV-detection at 254 nm and 366 nm and by spraying with anisaldehyde reagent (85 ml MeOH, 10 ml AcOH, 8 ml H_2SO_4 , 0.5 ml p -anisaldehyde) followed by heating with a hot air gun.

4.3.2 High-Performance Liquid Chromatography

High-Performance Liquid Chromatography (HPLC) is a major analytical technique applied to separate complex mixtures like crude plant extracts. Contrary to TLC, the stationary phase is located within a densely packed column, through which a mobile phase is pumped at high pressure. According to their interaction with the stationary phase, different compounds pass through the column and reach the detector at a specific time. Besides retention time, the detector also provides characteristic UV-spectra of individual compounds. HPLC allows rapid identification of the composition of mixtures by comparing retention times and UV-spectra of individual peaks with authentic samples or with the internal HPLC spectra-database.

In the course of the present work, HPLC analyses were performed on an Agilent 1100 series employing a reversed phase column (Hypersil BDS-C18, 250 x 4.6 mm, 5 μm particle size). The mobile phase was MeOH (B) in an aqueous buffer containing 10 mM ammonium acetate (A) using the following gradient: from 40 % B in A to 100 % B in 15 min, followed by 100 % B for 10 min. Injection volume was 10 μl at a flow rate of 1 mL/min, diode array detection was set to 230 nm. A neutral mobile phase was selected in order to prevent artifact formation during measurements, which in turn was at costs of

broader peaks. For figures of HPLC-chromatograms, a signal range from 3-15 min was selected in most cases.

4.3.3 Nuclear magnetic resonance spectroscopy

Nuclear magnetic resonance spectroscopy (NMR) is a technique that allows structure elucidation of compounds by studying the magnetic properties of certain nuclei (here ^1H and ^{13}C) in a strong magnetic field. All spectra were measured on a Bruker DRX-400 AVANCE or Bruker DRX-600 spectrometer (Bruker, Rheinstetten, Germany) at 400.13 MHz (^1H) and 100.61 MHz (^{13}C). For ^1H , ^{13}C , COSY, TOCSY, NOESY, HMQC, and HMBC, the standard pulse programs of Topspin 1.3 have been applied to record spectra with a range of 7200 Hz (^1H) and 30,000 Hz (^{13}C). CHD_2OD was used as internal standard for ^1H (δ_{H} 3.34) and CD_3OD for ^{13}C (δ_{C} 49.0) spectra. NMR spectra have been measured by Mag. S. Felsing and interpreted by her and Prof. L. Brecker and Mag. H. Fasshuber (Institute of Organic Chemistry, University of Vienna, Austria).

NMR-data and information on structure elucidation of the isolated compounds, will not be given here, but may be found in Fasshuber (2011), Berger et al. (2011a, 2012) or will be published in future papers (Berger et al., in prep.).

4.3.4 Mass spectrometry

Mass spectrometry (MS) is an analytical technique that allows measuring the mass-to-charge ratio of molecules. It is used for determining molecular mass and elemental composition of a given compound, thus aiding and confirming structure elucidations inferred by NMR-data. Mass spectra were recorded and interpreted by E. Lorbeer (Institute of Organic Chemistry, University of Vienna, Austria), employing a high resolution time-of-flight (HR-TOF) mass spectrometer (maXis, Bruker Daltonics) by direct infusion electrospray ionization (ESI).

MS-data of the isolated compounds, will not be given here, but may be found in Fasshuber (2011), Berger et al. (2011a, 2012) or will be published in future papers (Berger et al., in prep.).

4.3.5 Phenolic content, radical scavenging activity and bioassay using *Spodoptera littoralis*

In order to quantify the degree of phytochemical differentiation within the *Psychotria* alliance, total phenolic content and quality, the latter measured as radical scavenging activity, was assessed in a subset of species mentioned in table 6. Phenolic

content was detected using HPLC-UV/VIS profiling and quantified using Folin-Ciocalteu reagent (Sigma-Aldrich), radical scavenging activity was assessed using DPPH reagent (Sigma-Aldrich). In order to assess the ecological relevance of the phytochemical differentiation within species from *Psychotria* and related genera, antifeedant activity of crude extracts on *Spodoptera littoralis* larvae was studied in feeding experiments using an artificial diet.

4.4 Preparative methods

The generally applied procedure to extract and purify single compounds from complex extracts involves methanolic extraction, followed by partitioning through liquid-liquid extraction. A polar phase was selected in most cases and purified by column chromatography, medium pressure liquid chromatography or preparative thin layer chromatography. Depending on the separation problem, a subset of the techniques mentioned below have been selected and combined as needed. Details are mentioned in chapter 5, Extraction and Isolation.

4.4.1 Liquid-liquid extraction

Liquid-liquid extraction is a technique used to separate mixtures of compounds based on their different solubility in two immiscible solvents. It is performed in a separatory funnel by subsequently extracting compounds dissolved or suspended in water with PE, CHCl₃, EtOAc and *n*-BuOH, respectively.

4.4.2 Column Chromatography

Column Chromatography (CC) is a standard preparative method frequently applied for separating mixtures of compounds. In CC, the sample is applied atop a column filled with the stationary phase. The column is rinsed with the mobile phase, which is forced through the column by gravity. Depending on the chromatographic problem, different stationary and mobile phases have been applied.

For roughly separating phases obtained by liquid-liquid extraction, approximately 50 g silica gel (silica gel 60, 0.2-0.5 mm, Merck) was filled into a column with about 80 cm in length and 1.7 cm inner diameter. The extract was coated on silica gel, filled atop of the column and rinsed with solvent mixtures of PE, EtOAc and MeOH with increasing polarity. 100 ml of each solvent mixture was used to elute the compounds from the column and the eluant was collected in 20 x 50 ml fractions.

For further separation, a smaller particle size of the stationary phase was required (silica gel 60, 40-63 μm , Merck). To improve separation, the silica gel was additionally suspended in solvent prior to filling into the column. The dissolved samples were dripped on top of the silica gel and rinsed with solvent mixtures. In most cases, $\text{CHCl}_3/\text{MeOH}$ (80:20) or various mixtures of EtOAc/MeOH have been applied.

Final purification was usually done by size-exclusion chromatography using an isocratic methanolic CC with Sephadex LH-20.

4.4.3 Medium Pressure Liquid Chromatography

Medium Pressure Liquid Chromatography (MPLC) is a preparative technique that combines aspects of both CC and HPLC. It differs from CC by its smaller particle size which requires elevated pressure similar to HPLC and allows rapid and accurate separation of small amounts of pre-purified mixtures. MPLC was performed with mixtures of EtOAc/MeOH or $\text{CHCl}_3/\text{MeOH}$, respectively and the separation was monitored by UV/VIS detection at various wavelengths.

4.4.4 Preparative Thin Layer Chromatography

Preparative Thin Layer Chromatography (prTLC) is similar to analytical TLC, except that a larger amount of sample is added on the plate. Several milligrams of the mixtures to be separated are linearly applied as a small streak located 1.5 cm above the bottom of the plate. After the plate has been developed with solvent, the different pure substances visible as bands were visualized by UV-detection and recovered by scraping the silica gel from the plate and elution with MeOH in a Büchner funnel.

5 Extraction and Isolation

All studied plant materials were air dried, ground and extracted with MeOH (500 mL, 3 x 2 d) at room temperature, filtered and concentrated under reduced pressure to give a crude extract. Extracts were partitioned according to specifications given in Tab. 5. In general, partitioning between CHCl₃, EtOAc, *n*-BuOH and H₂O, respectively, provided the best results due to an almost exclusive enrichment of alkaloid glucosides in the BuOH-phase, whereas sugars and inorganic ions were retained in the H₂O phase. Table 5 lists extracted species and organs, and gives the amounts resulting from extraction and partitioning.

Tab. 5: Detailed information on extraction and phase partitioning.

species	organ	amount	extract	phase partitioning			
				CHCl ₃	EtOAc	BuOH	H ₂ O
<i>Palicourea acuminata</i>	lvs	75 g	14.5 g	3.84 g	0.18 g	n.p.	10.3 g
	stb	16.5 g	1.9 g	0.28 g	0.1 g	n.p.	1.38 g
<i>Psychotria chiriquiensis</i>	lvs	32 g	2.2 g	1.2 g	0.04 g	0.37 g	0.7 g
<i>Psychotria cyanococca</i>	stb	15 g	0.84 g	0.27 g	0.02 g	0.18 g	0.37 g
<i>Psychotria nervosa</i>	lvs	32 g	5.45 g	1.25 g	1.12 g	n.p.	3.14 g
<i>Psychotria suerrensii</i>	stb	25 g	1 g	0.22 g	0.01 g	n.p.	0.71 g
<i>Notopleura polyphlebia</i>	lvs	60 g	4.1 g	1.3 g	0.22 g	0.47 g	2.1 g

Species, organs used, amount of extracted material, crude extracts as well as amount of extracts resulting from phase partitioning are listed. Fractions obtained from phase partitioning were rounded to two digits. lvs: leaves, stb: stem bark, n.p.: not performed.

5.1 *Palicourea acuminata*

For HPLC-UV analysis, small quantities of powdered leaves, stem bark, root bark and fruits of *Palicourea acuminata* (Benth.) Borhidi (syn. *Psychotria acuminata* Benth.) were extracted with MeOH and directly used for chromatographic comparison. For isolation, several accessions of leaves and stem bark respectively, were combined because of similar secondary metabolite accumulation.

Leaves: 800 mg of the aqueous phase was roughly separated by CC (Merck silica gel 60, 0.2-0.5 mm) with solvent mixtures of PE, EtOAc and MeOH with increasing polarity. The fraction eluted with EtOAc/MeOH (70:30) (190 mg) was further separated by isocratic methanolic CC with Sephadex LH-20 to give 61 mg **bahienoside B (2)** and 49 mg **desoxycordifoline (3)**. 780 mg of the EtOAc-phase was roughly separated by CC (Merck silica gel 60, 0.2-0.5 mm) with solvent mixtures of PE, EtOAc and MeOH with increasing

polarity. Fractions eluted with pure EtOAc were combined (51 mg) and purified by isocratic methanolic CC with Sephadex LH-20 to give 5.2 mg **lagamboside (4)** and 3.6 mg of a mixture of **stigmasterol (18)** and **β -sitosterol (19)**.

Stem bark: 600 mg of the aqueous phase was roughly separated by CC (Merck silica gel 60, 0.2-0.5 mm) with solvent mixtures of EtOAc and MeOH with increasing polarity. Similar to the leave extract, an additional 40 mg **4** and 32 mg **6** were obtained by isocratic methanolic CC with Sephadex LH-20. The more polar fractions eluted with EtOAc/MeOH (60:40) were combined (115 mg) and further separated by isocratic methanolic CC with Sephadex LH-20 to yield 21 mg **5-carboxystrictosidine (1)**.

According to HPLC-measurements, EtOAc and CHCl₃ phase of the stem bark extract were combined with subfractions of the EtOAc-phase of the leaves (in total 750 mg) and roughly separated by CC (Merck silica gel 60, 0.2-0.5 mm) with solvent mixtures of PE, EtOAc and MeOH with increasing polarity. Fractions containing compound **5** were further separated by isocratic methanolic CC with Sephadex LH-20 (22.6 mg) and finally purified by MPLC with CHCl₃/MeOH (80:20) to give 5.2 mg of **strictosamide (5)**.

5.2 *Psychotria chiriquiensis*

Leaves: The butanolic phase (370 mg) was roughly separated by CC (Merck silica gel 60, 0.2-0.5 mm) with solvent mixtures of EtOAc and MeOH with increasing polarity. Fractions eluted with EtOAc/MeOH (75:25-70:30) were combined to give 112 mg of impure **lyaloside (6)**. Fractions eluted with EtOAc/MeOH (65:35-55:45) were combined (101 mg) and separated by CC using Sephadex LH-20. The first fractions were combined (55 mg) and further separated by CC (30 g Merck silica gel 60, 40-63 μ m) using a gradient of EtOAc/MeOH (60:40-50:50) to give 1,9 mg of **Chiriq1**, a yet unidentified alkaloid related to 5-carboxystrictosidine.

5.3 *Psychotria cyanococca*

Stem bark: The CHCl₃ and *n*-BuOH-phase were combined (450 mg) and roughly separated by column chromatography (CC, Merck silica gel 60, 0.2-0.5 mm) with solvent mixtures of PE, EtOAc and MeOH with increasing polarity. Fraction VI/1 eluted with 20 % MeOH in EtOAc was combined with the EtOAc-Phase (42 mg) and purified by isocratic methanolic CC with Sephadex LH-20 to give 23.3 mg **lyaloside (6)**.

5.4 *Psychotria suerrensii*

Stem bark: The aqueous phase was roughly separated by CC (Merck silica gel 60, 0.2-0.5 mm) with solvent mixtures of EtOAc and MeOH with increasing polarity. Fractions VII and VIII were combined (135 mg) and purified by isocratic methanolic CC with Sephadex LH-20 to give 43.7 mg **lyalosidic acid (9)**. Fractions eluted with EtOAc/MeOH (40:60) were combined (58 mg) and further separated by isocratic methanolic CC with Sephadex LH-20 to yield 11.6 mg **strictosidinic acid (10)**. Fractions eluted with pure MeOH were combined (109 mg) and further chromatographed by Sephadex LH-20. Fraction 1 and 2 were combined (65,5 mg) and finally purified by MPLC with mixtures of EtOAc/MeOH. Fractions eluted with EtOAc/MeOH (50:50) yielded 15.1 mg pure **ophiorine B (12, Suerr4)**, fractions eluted with 20:80 gave 22.8 mg pure **ophiorine A (11, Suerr5)**.

The CHCl₃-phase was roughly separated by CC (Merck silica gel 60, 0.2-0.5 mm) with solvent mixtures of PE, EtOAc and MeOH with increasing polarity. Fractions eluted with EtOAc/MeOH (95:5 to 90:10) were combined (14.5 mg) and subjected to prTLC to give 6.7 mg of impure **lyaloside (6)**.

5.5 *Notopleura polyphlebia*

Leaves: The butanolic phase (470 mg) was roughly separated by column chromatography (CC, Merck silica gel 60, 0.2-0.5 mm) with solvent mixtures of PE, EtOAc and MeOH with increasing polarity. Fractions eluted with 25-30 % MeOH in EtOAc were combined (250 mg) and chromatographed by isocratic methanolic CC with Sephadex LH-20. Fractions 6 and 7 were combined and subjected to prTLC to yield 2.1 mg of the unnamed megastigmane glucoside **Npoly1 (15)** as well as 2.3 mg of **corchoionoside C (16)**. PrTLC of fraction 13 yielded 3.5 mg of an impure and hence unspecified **p-hydroxybenzoic acid glycoside**. Fractions 14-16 were combined and 24 mg **harounoside (13)** spontaneously precipitated as white crystalline solid. The supernatant (52 mg) was decanted and subjected to CC (Merck silica gel 60, 40-63µm). Beside some more harounoside, 11 mg of a new flavanonol diglycoside **Npoly5 (14)** was obtained.

5.6 *Psychotria nervosa*

Leaves: 800 mg of the EtOAc phase was roughly separated by CC (Merck silica gel 60, 0.2-0.5 mm) with solvent mixtures of PE, EtOAc and MeOH with increasing polarity. Fractions eluted with 5% PE in EtOAc and pure EtOAc were combined (358 mg) and chromatographed by isocratic MPLC using CHCl₃/MeOH (80:20). The first fraction contained most compounds (248 mg) and was submitted to isocratic CC with Sephadex LH-20 employing CHCl₃/MeOH (80:20) to yield 4.1 mg of **rhoifoline (21)**. The remaining mixture of oligo- to polymeric tannins was not further separable neither with MPLC, cyclic MPLC nor other techniques.

5.7 *Psychotria tenuifolia*

Psychotria tenuifolia may stand as an example of a RSCP containing species. Mature fruits were analyzed for presence and composition of soluble RSCP. Seeds were removed from the surrounding pericarp and several were put in a pipette equipped with a cotton wool filter. HPLC-samples were directly obtained by rinsing the seeds in the pipette with MeOH.

6 Results

Six species have been analysed in detail and their secondary metabolite content was elucidated by extraction of several compounds. Among these species were five members of the Palicoureeae and one of the Psychotrieae. Tryptamine iridoid alkaloids were obtained from four species of the Palicoureeae. *Notopleura polyphlebia* gave a diverse array of compounds, of which naphthoquinones and megastigmane sesquiterpenoids are remarkable. A single flavonoid could be isolated from *Psychotria nervosa*, however, accumulation of large amounts of tannins could be detected in *P. nervosa* and all other surveyed species of the Psychotrieae.

The following chapter lists species that have been selected for isolation of compounds, reports their chemical composition and illustrates the distribution of their secondary metabolites in different organs of the respective plant. Furthermore, all isolated compounds are compiled and their distribution in 81 species of the *Psychotria* alliance is shown on base of extensive HPLC/UV-VIS studies contributing to the data matrix, upon which the discussion is based on.

6.1 *Palicourea acuminata* (Palicoureeae)

Several compounds have been obtained from extracts of leaves and stem bark of *Palicourea acuminata*. Fig. 17 shows their structures as well as their occurrence in crude extracts of different organs of *Palicourea acuminata* by means of HPLC-chromatograms. Chromatograms of root bark extracts were similar to those of the stem bark and are not shown here. *P. acuminata* accumulates large amounts of the alkaloids bahienoside B (**2**) and desoxycordifoline (**3**), which are present in all parts of the plant. Stem bark extracts deviated by additionally accumulating 5-carboxystrictosidine (**1**). Beside these three major compounds, small amounts of lagamboside (**4**) and strictosamide (**5**) are present in all organs. The virtually ubiquitous phytosterols stigmasterol (**18**) and β -sitosterol (**19**) were also extracted from the stem bark. These triterpenes lack a conjugated system and are not detectable by a UV/VIS detector. Additionally, lyaloside (**6**) and the coumarin scopoletin (**20**) could be detected in a subfraction of the stem extract.

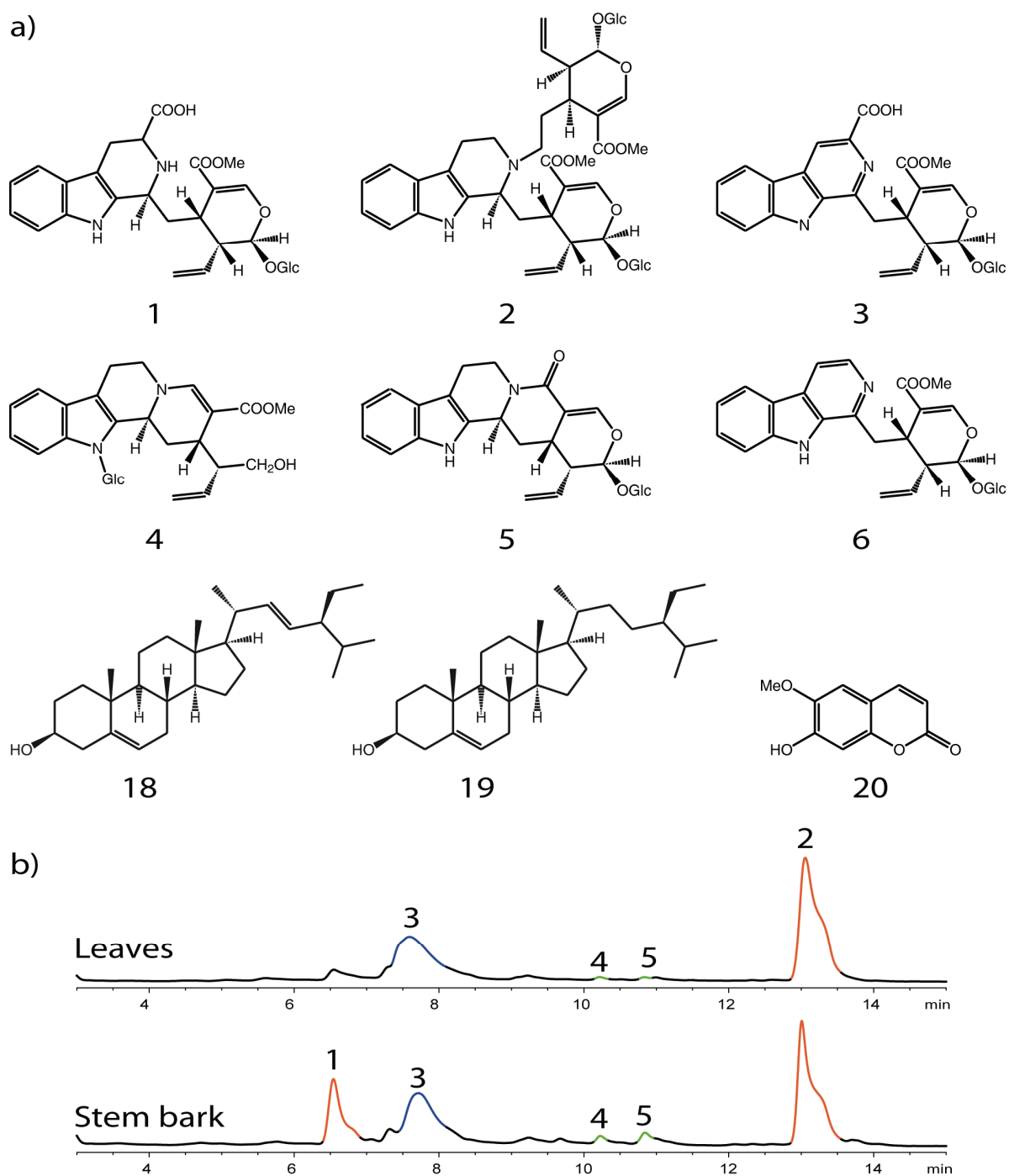


Fig. 17: Structure (a) and distribution (b) of compounds detected in leaves and stem bark of *Paliourea acuminata*. Desoxycordifoline (3), bahienoside B (2), 5-carboxystrictosidine (1), lagamboside (4) and strictosamide (5). Lyaloside (6), stigmasterol (18) and β -sitosterol (19) as well as scopoletin (20).

6.2 *Psychotria chiriquiensis* (Palicoureeae)

The structures of the isolated compound **1**, as well as two further compounds that were detected comparatively are shown in fig 18. Their distribution in crude extracts of leaves, stem bark and fruits of *P. chiriquiensis* by means of HPLC-chromatograms is additionally highlighted. Chromatograms of root bark extracts were similar to those of the stem bark and are not shown here. Leaves, stem and root bark extracts of *P. chiriquiensis* are dominated by large amounts of 5-carboxystrictosidine (**1**). Beside that compound, they show a rather simple composition, with traces of lyaloside (**6**) and a yet unknown compound (Chiriq1) putatively related to 5-carboxystrictosidine. Interestingly, fruits deviated by almost exclusively accumulating asperuloside (**7**), a simple and frequently observed iridoid glucoside, which was detected co-chromatographically employing an authentic sample.

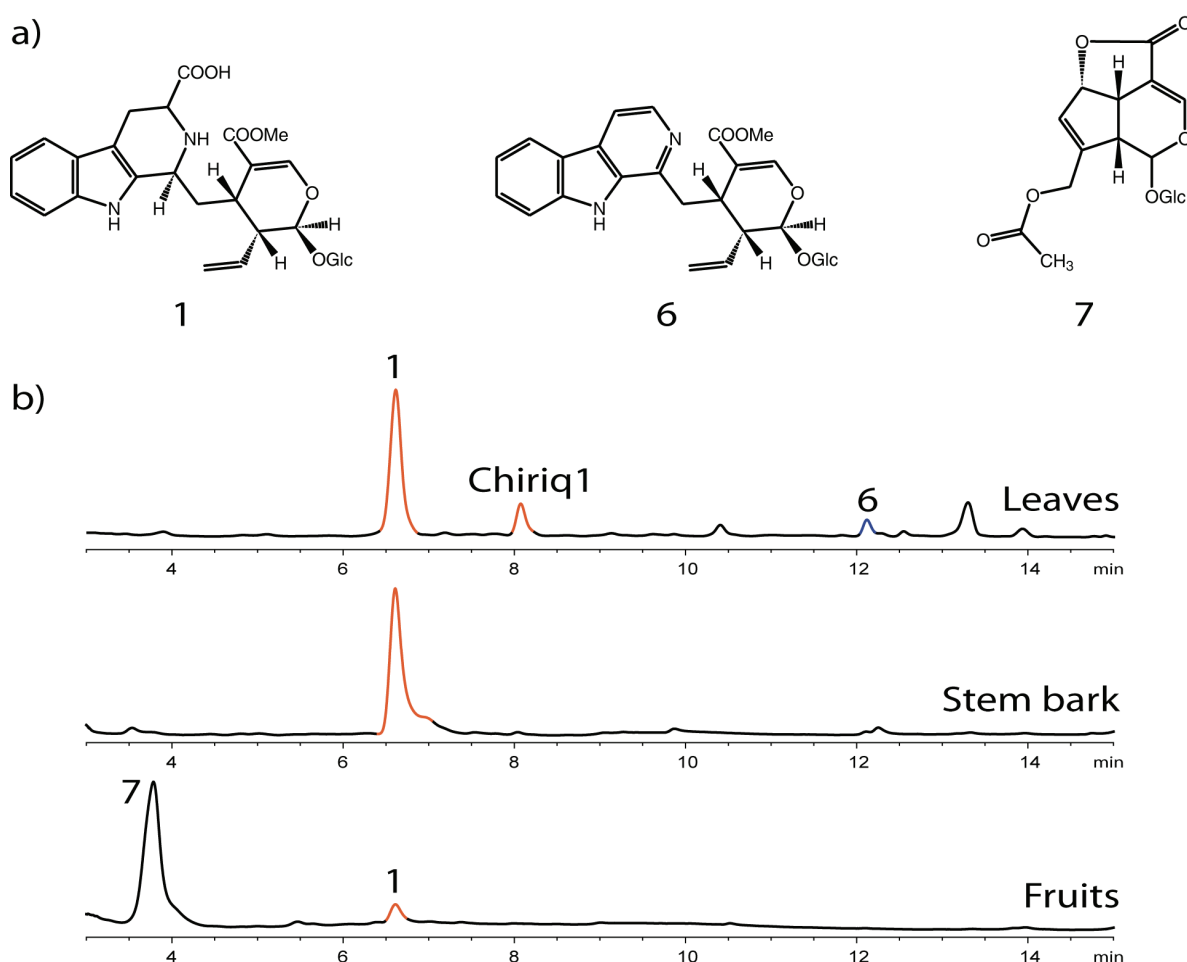


Fig. 18: Structure (a) and distribution (b) of compounds detected in different parts of *Psychotria chiriquiensis*. 5-carboxystrictosidine (**1**), lyaloside (**6**) and asperuloside (**7**).

6.3 *Psychotria cyanococca* (Palicoureeae)

Figure 19 shows the structures of the isolated compound lyaloside (6), as well as compounds that were co-chromatographically detected. Their distribution in crude extracts of leaves and stem bark of *P. cyanococca* by means of HPLC-chromatograms is additionally shown. *P. cyanococca* accumulates significant amounts of the alkaloids 5-carboxystrictosidine (1), desoxycordifoline (3) and lyaloside (6), which are detectable in all parts of the plant. In addition, leaves differed by accumulating large amounts of bahienoside B (2), small amounts of strictosidine (8) and trace amounts of lagamboside (4), which could only be detected in a CC subfraction.

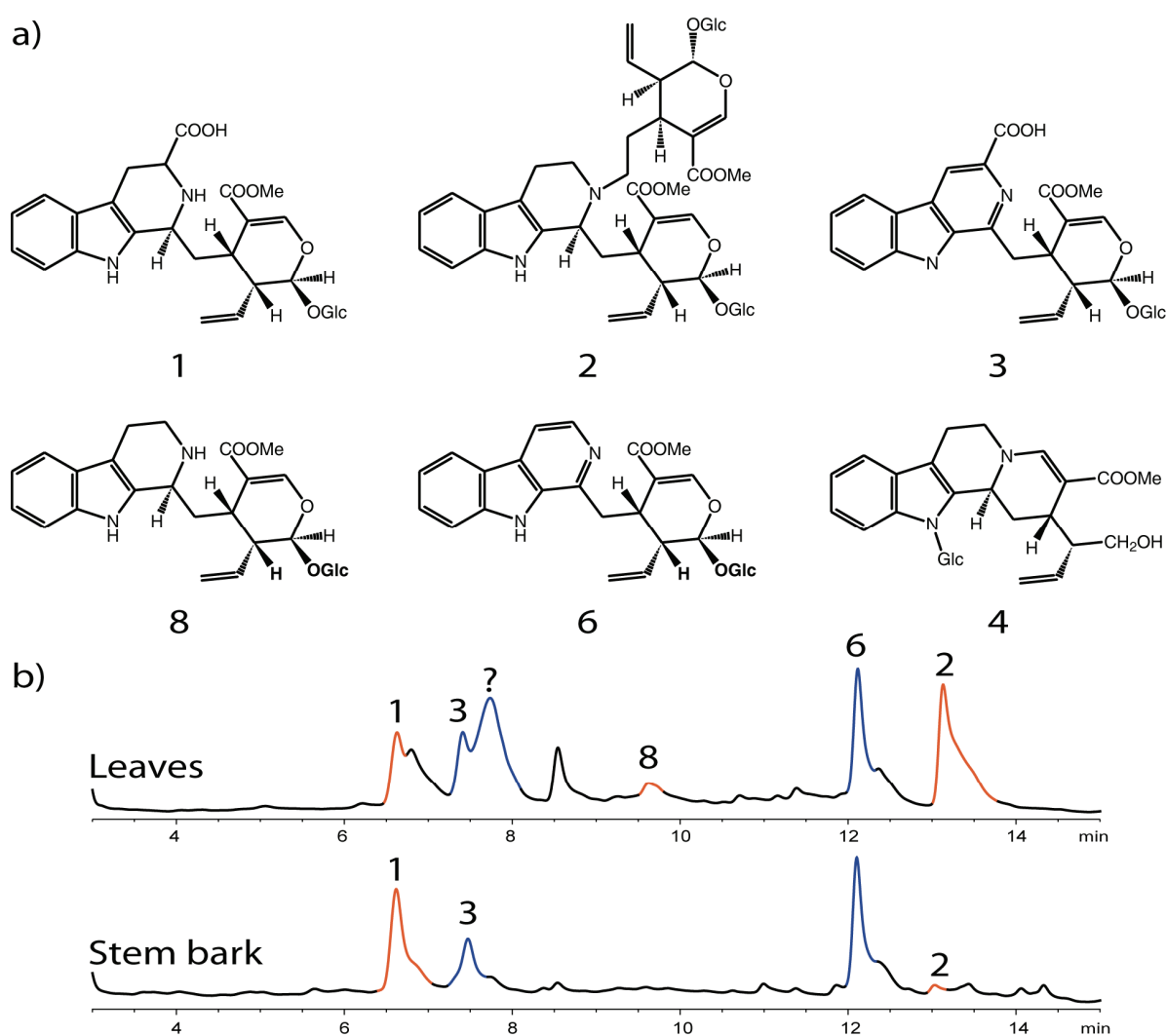


Fig. 19: Structure (a) and distribution (b) of compounds detected in different parts of *Psychotria cyanococca*. 5-carboxystrictosidine (1), bahienoside B (2), desoxycordifoline (3), lagamboside (4), lyaloside (6) and strictosidine (8).

6.4 *Psychotria suerrensis* (Palicoureeae)

The structures of all detected compounds and their distribution in crude extracts of leaves, stem bark and flowers of *P. suerrensis* are shown in Fig. 20. Extracts of flower bracts were similar to those of the flowers and are not shown here. Extracts of all parts were dominated by accumulating lyalosidic acid (**9**) accompanied by traces of the related lyaloside (**6**). Whilst flower extract was the simplest in composition, leave and stem bark extracts deviated by additionally containing ophiorine A (**11**) and B (**12**). The leaf extract is the most complex, where these compounds were accompanied by strictosidinic acid (**10**). Scopoletin (**20**) was additionally detected in a subfraction of the leaf extract.

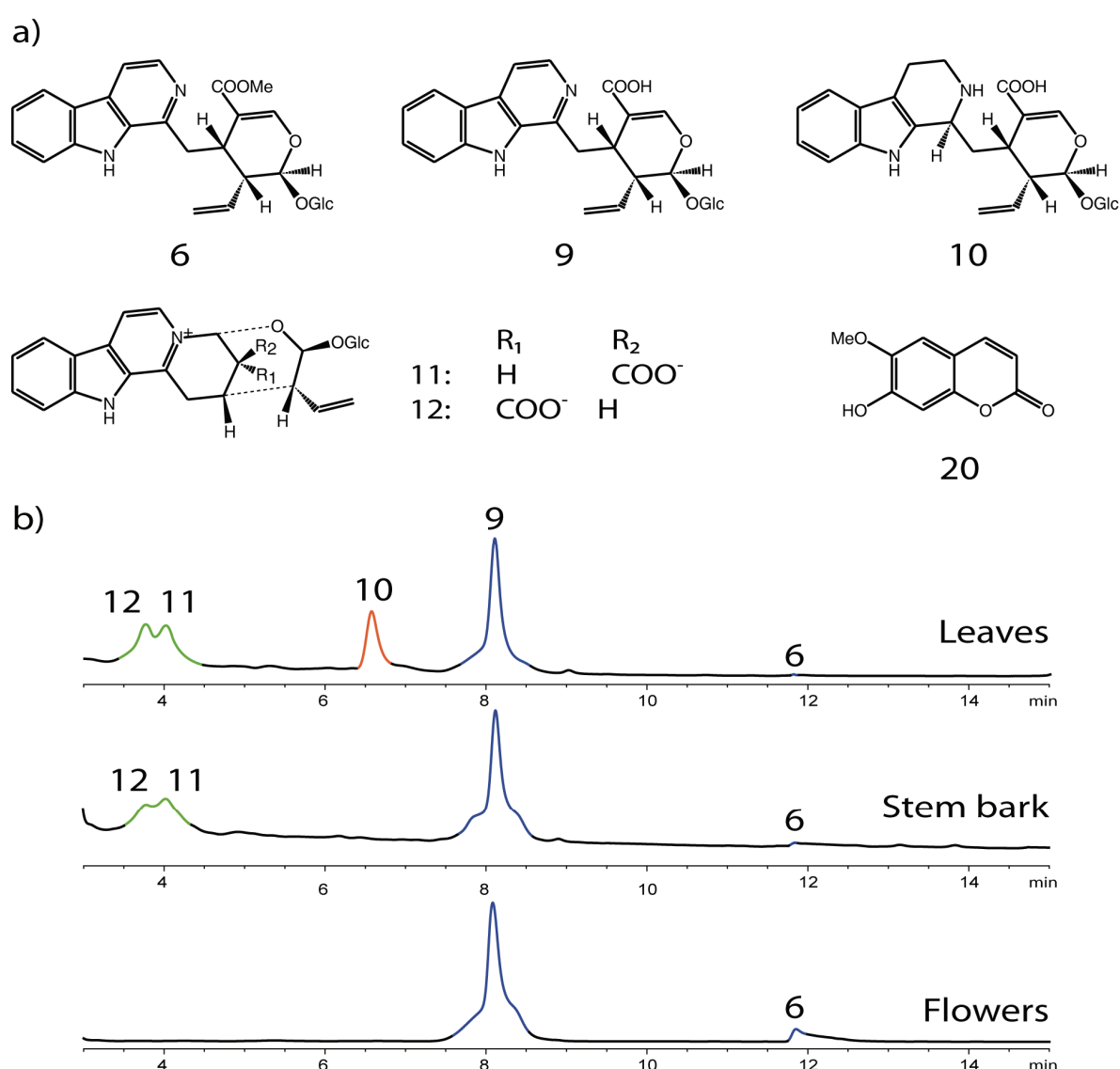


Fig. 20: Structure (a) and distribution (b) of compounds detected in different parts of *Psychotria suerrensis*. Lyalosidic acid (**9**), strictosidinic acid (**10**), ophiorine A (**11**), ophiorine B (**12**), lyaloside (**6**) and scopoletin (**20**).

6.5 *Notopleura polyphlebia* (Palicoureeae)

Figure 21 shows the structures of the isolated compounds and their distribution in crude extracts of leaves and stem bark of *Notopleura polyphlebia* by means of HPLC-chromatograms. Additionally, an impure *p*-hydroxybenzoic acid glycoside was isolated, but its structure could not fully be clarified. It is therefore not included in figure 21. Extracts of *Notopleura polyphlebia* were characterized by accumulating large amounts of harounoside (**13**), which occurs together with 2-aza-anthraquinone (**17**). Leaves additionally gave two megastigmane glucosides (**15** and **16**), besides a new flavanonol diglycoside (Npoly5, **14**) with an unusual di-O-methylaromadendrin 5- diglycoside structure.

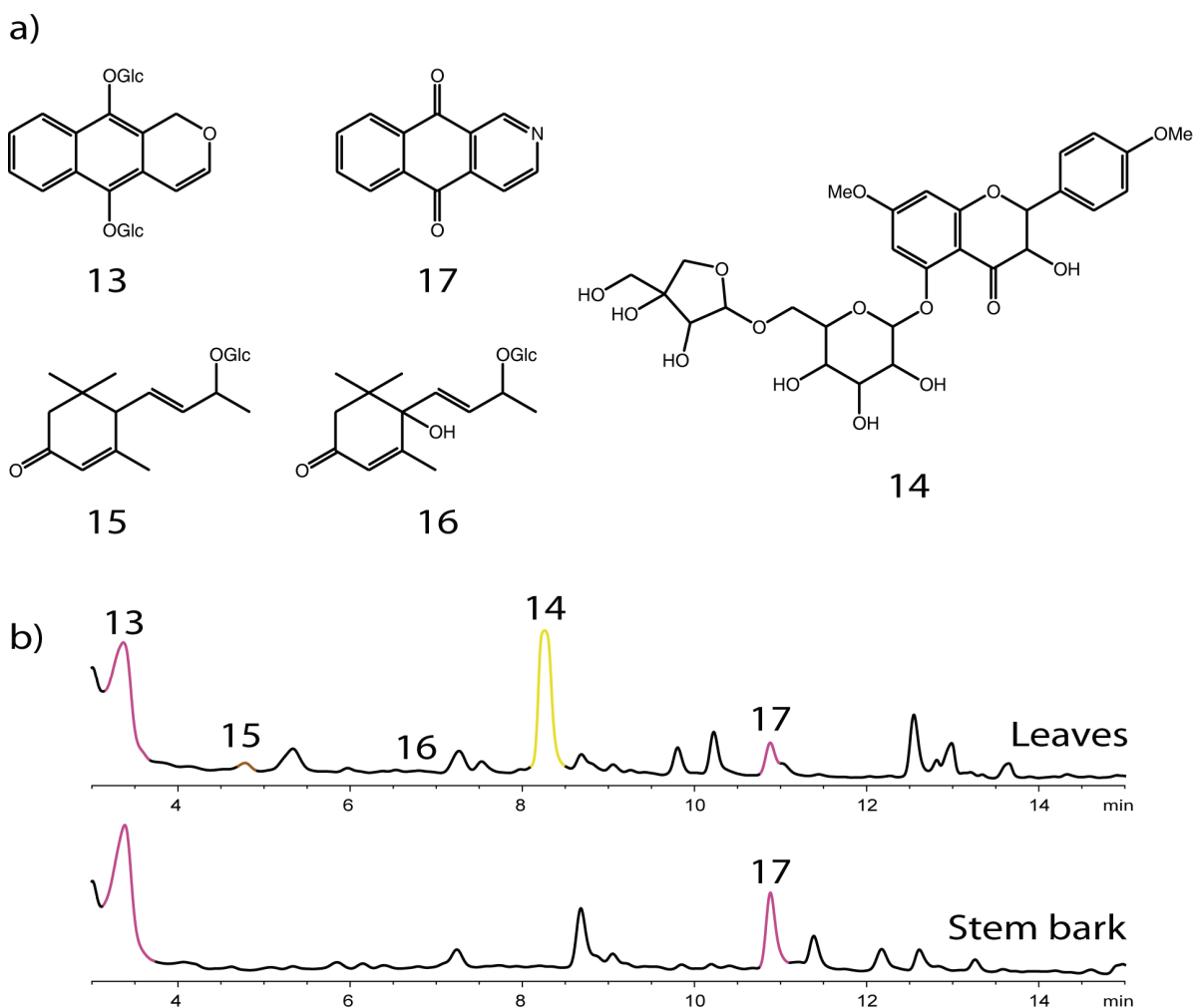


Fig. 21: Structure (a) and distribution (b) of compounds detected in different parts of *Notopleura polyphlebia*. Harounoside (**13**), NPoly5, a new flavanonol diglycoside (**14**), an unnamed megastigmane glucoside (**15**), corchoionoside C (**16**) and 2-aza-anthraquinone (**17**).

6.6 *Psychotria* subg. *Psychotria* (Psychotrieae)

A significant number of *Psychotria* subg. *Psychotria* i.e. *Psychotria* s.str. species including 18 from America, 4 from Asia (*P. adenophylla*, *P. asiatica*, *P. serpens* and *P. zeylanica*), 3 Pacific (*P. hawaiiensis*, *P. hexandra* and *P. mariniana*) and 4 African species (*P. capensis*, *P. kirkii*, *P. orophila* and *P. verschuerenii*) have been studied by HPLC-UV/VIS analysis. Interestingly, no alkaloids could be detected in any of the species of subg. *Psychotria*. Instead, chromatograms revealed large quantities of other compounds, which have previously been overlooked in studies focused on the isolation of alkaloids. Examples of HPLC profiles of leaf extracts from 4 species were selected for illustration (Fig. 22). These represent some of the major centers of diversification within *Psychotria* and include *P. nervosa* (Central America), *P. orophila* (East Africa), *P. serpens* (Asia) and *P. mariniana* (Pacific region).

HPLC analysis showed similar broad “humps” in all species, putatively consisting of numerous overlaying peaks of compounds with closely related structures. All of the compounds present in the whole range of the chromatogram showed a UV/VIS-profile corresponding to that of the dihydroflavonol catechin (see Fig. 22a), indicating accumulation of either complex or condensed tannins, sharing a dihydroflavonol i.e. flavanol backbone. Additionally, pronounced antioxidant activity of crude extracts points to the presence of polyphenolic compounds such as condensed tannins (Berger et al., 2011b). CC fractions were found to be stable in NMR-controlled hydrolysis experiments using acid and base treatment, similarly indicating condensed (non hydrolysable) tannins (H. Fasshuber, unpubl. data). Furthermore, preliminary NMR analyses suggested the presence of oligo- or polymeric catechins (L. Brecker, unpublished data). This is in accordance with preliminary data on the Asian species *P. asiatica*, for which the occurrence of compounds with a catechin moiety was postulated (sub syn. *P. reevesii* Wall.; Giang et al., 2007).

Two experiments were undertaken to exclude the possibility that tannin content in *Psychotria* subg. *Psychotria* is masking alkaloid content in HPLC profiles. The first experiment aimed at removing tannins by acid/base extraction, and was performed for a subset of all species of subg. *Psychotria*. HPLC chromatograms of these extracts did not reveal the presence of alkaloids in any species (data not shown). Secondly, in order to rule out the possibility that tannins bind and/or precipitate alkaloids (e.g. Khanbabaee & van Ree, 2001), equal parts of leaves of a tannin accumulating (*P. marginata*) as well as of an

alkaloid containing species (*P. acuminata*) were combined and extracted. The HPLC chromatogram of the resulting extract corresponded to the sum of both extracts i.e. each peak was retrieved, excluding the possibility that tannins bind and mask alkaloids (data not shown). In addition to tannin accumulation, iridoids are frequently observed in young or unfolding leaves of *Psychotria* subg. *Psychotria* (Tab. 6). *Psychotria mariniana* provides an example for combined iridoid and tannin accumulation (Fig. 22).

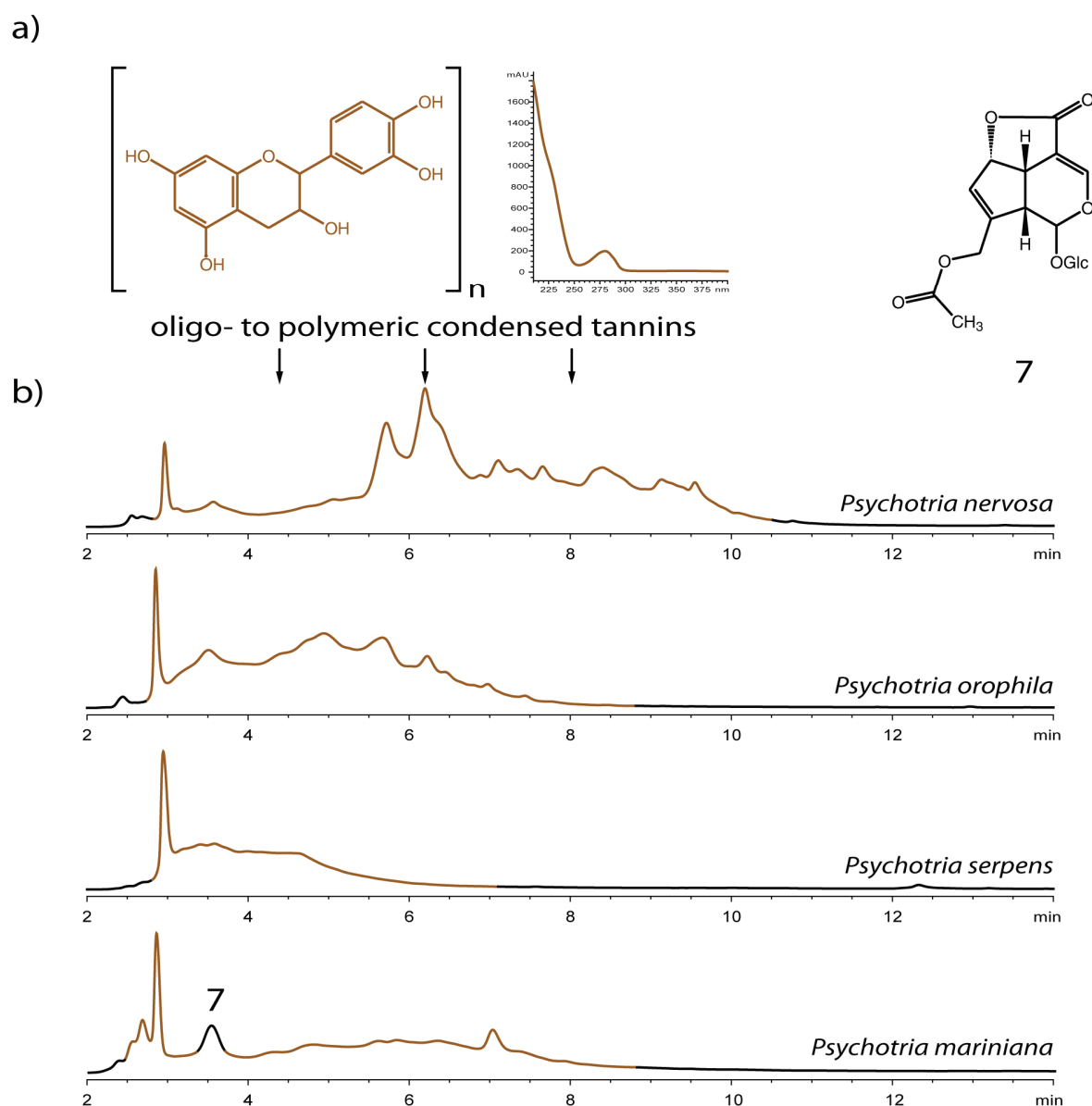


Fig. 22: Structure (a) and distribution (b) of compounds detected in leaves of selected species of *Psychotria* subg. *Psychotria* representing major tropical regions. Structure of a catechin monomer illustrative for oligo-to polymeric tannins as well as asperuloside (7); HPLC chromatograms of *Psychotria nervosa* (CR, Central America), *P. orophila* (ETH, Africa), *P. serpens* (TH, Asia) and *P. mariniana* (USA, Pacific Region). The latter is an example of iridoid accumulation.

6.7 *Psychotria nervosa* (Psychotrieae)

Figure 23 a) shows the structures of the isolated flavonoid rhoifoline (**21**) and a catechin monomer illustrative for oligo- to polymeric condensed tannins. HPLC-chromatograms of crude extracts of leaves and stem bark are additionally shown in Fig. 23 b).

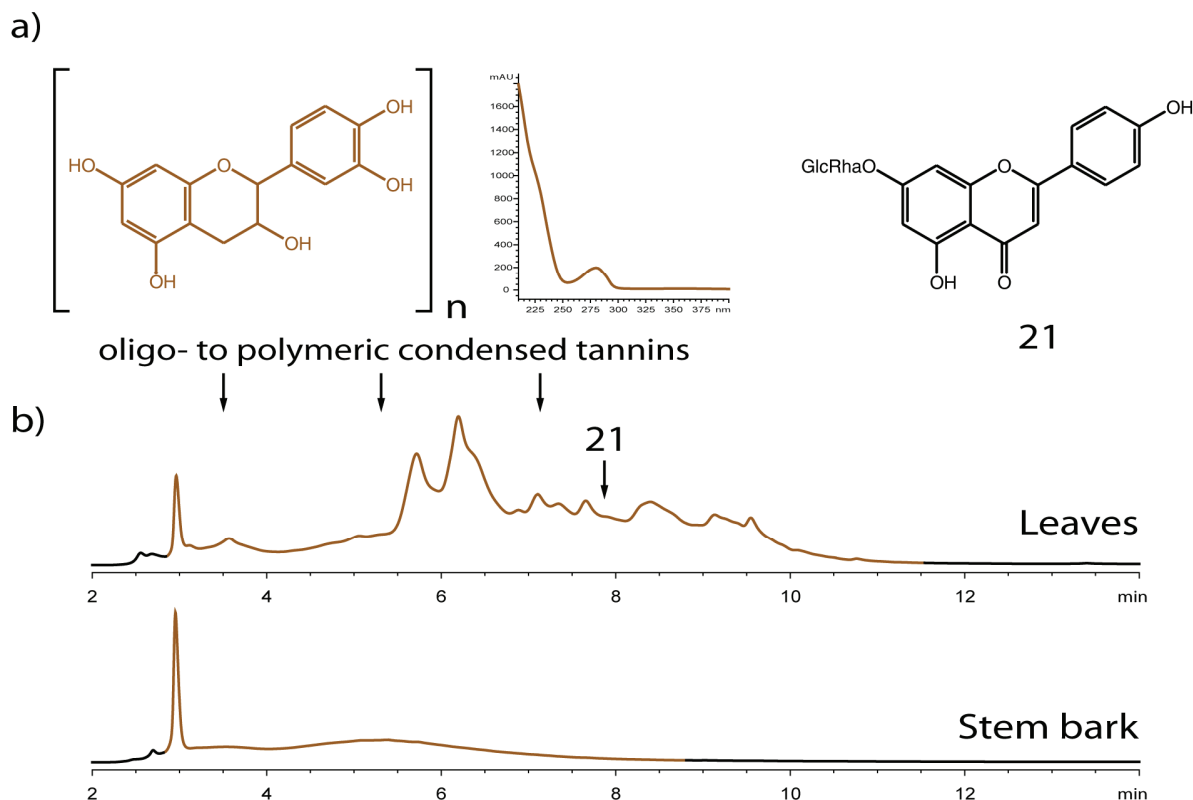


Fig. 23: Structure (a) and distribution (b) of compounds detected in leaves and stem bark of *Psychotria nervosa*. Rhoifoline (**21**) as well as a catechin monomer illustrative for oligo- to polymeric condensed tannins.

6.8 *Psychotria tenuifolia* (Psychotrieae)

In *Psychotria tenuifolia*, mature seeds were analyzed for presence and composition of RSCP. The outer layers of cells representing the seed coat are of a reddish colour giving the seed a rusty appearance. The colourless endosperm may be discriminated in cross section by its inconspicuous colour (Fig. 24 c). Extracts obtained by rinsing seeds with MeOH were studied by means of HPLC-UV/VIS analysis, and profiles were compared with those of other organs of this species. The HPLC chromatogram of the soluble RSCP showed similar composition as leaf and stem bark extract (Fig. 24 b). Furthermore, compounds over the whole range of the chromatogram showed a UV/VIS-profile similar to

the pure compound catechin, as is shown in Fig. 24 a). It is concluded, that the red seed coat pigment is composed of condensed tannins.

Psychotria tenuifolia:

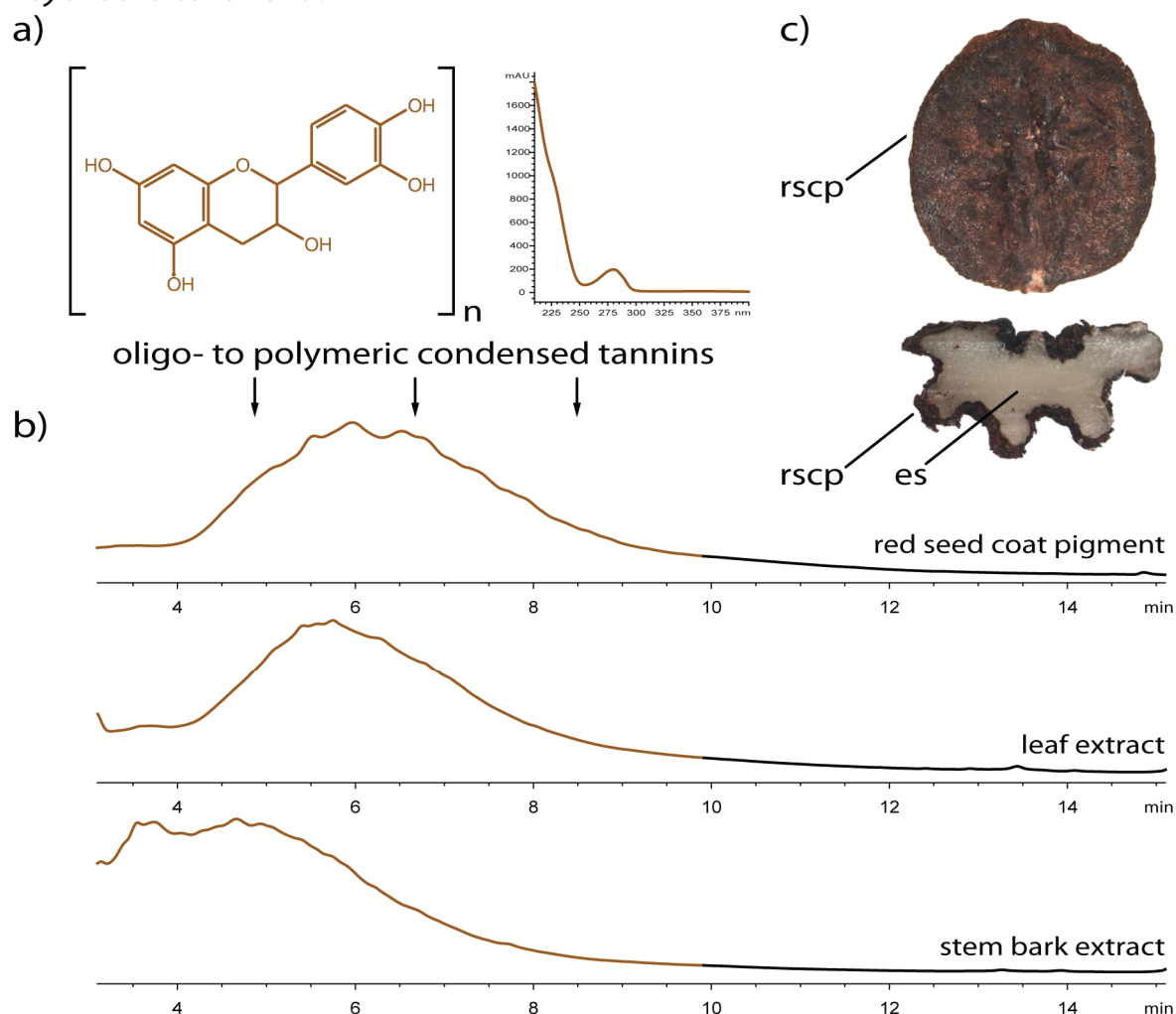


Fig. 24: Structure (a) and distribution (b) of compounds detected in red seed coat pigment (c) and other parts of *Psychotria tenuifolia*. Structure of a catechin monomer illustrative for oligo-to polymeric condensed tannins, adaxial side and cross section of a seed from *P. tenuifolia* showing red seed coat pigment (rscp) and colourless endosperm (es). HPLC-chromatograms of rscp, leaf and stem bark extract showing similar tannin composition.

6.9 Overview of compounds detected

In course of the present study, 81 species of *Psychotria* and related genera of the Palicoureeae as well as from the Lasiantheae were analysed for their phytochemical composition. Among these, 70 are American, 4 African, 4 Asian and 3 are species from the Pacific region. Ten tryptamine-iridoid alkaloids were isolated in the present study, while palicoside and strictosidine were identified on basis of HPLC data. Possible biosynthetic links between these structures are illustrated in Fig. 25, and compounds are grouped according to their structures in a) β -carbolines, b) “simple” strictosidine derivatives, c) *N*-alkylated derivatives and d) compounds resulting from various reactions involving a cyclisation or downstream modification of strictosidine by strictosidine glucosidase activity.

5-carboxystrictosidine (**1**) and strictosidinic acid (**10**) are derived from strictosidine (**8**) by carboxylation or demethylation, respectively (Fig. 25b). Desoxycordifoline (**3**), lyaloside (**6**) and lyalosidic acid (**9**) are formed by desaturation of **1**, **8** and **10**, respectively (Fig. 25a). Bahienoside B (**2**) and palicoside (**21**) originate from **8** by *N*-alkylation with secologanine and a methyl group, respectively (Fig. 25c). Lagamboside (**4**), strictosamide (**5**), ophiorine A and B (**11** and **12**) are formed from **8** by various reactions involving a cyclisation or strictosidine glucosidase activity (Fig. 25d).

Fig. 26 shows compounds originating from other biosynthetic pathways, namely a coumarin, flavonoids, iridoids, monoterpenes, naphthoquinones and triterpenes that have been isolated (**1-6**, **9-12**), or co-chromatographically detected (**17**, **20**) in the present study.

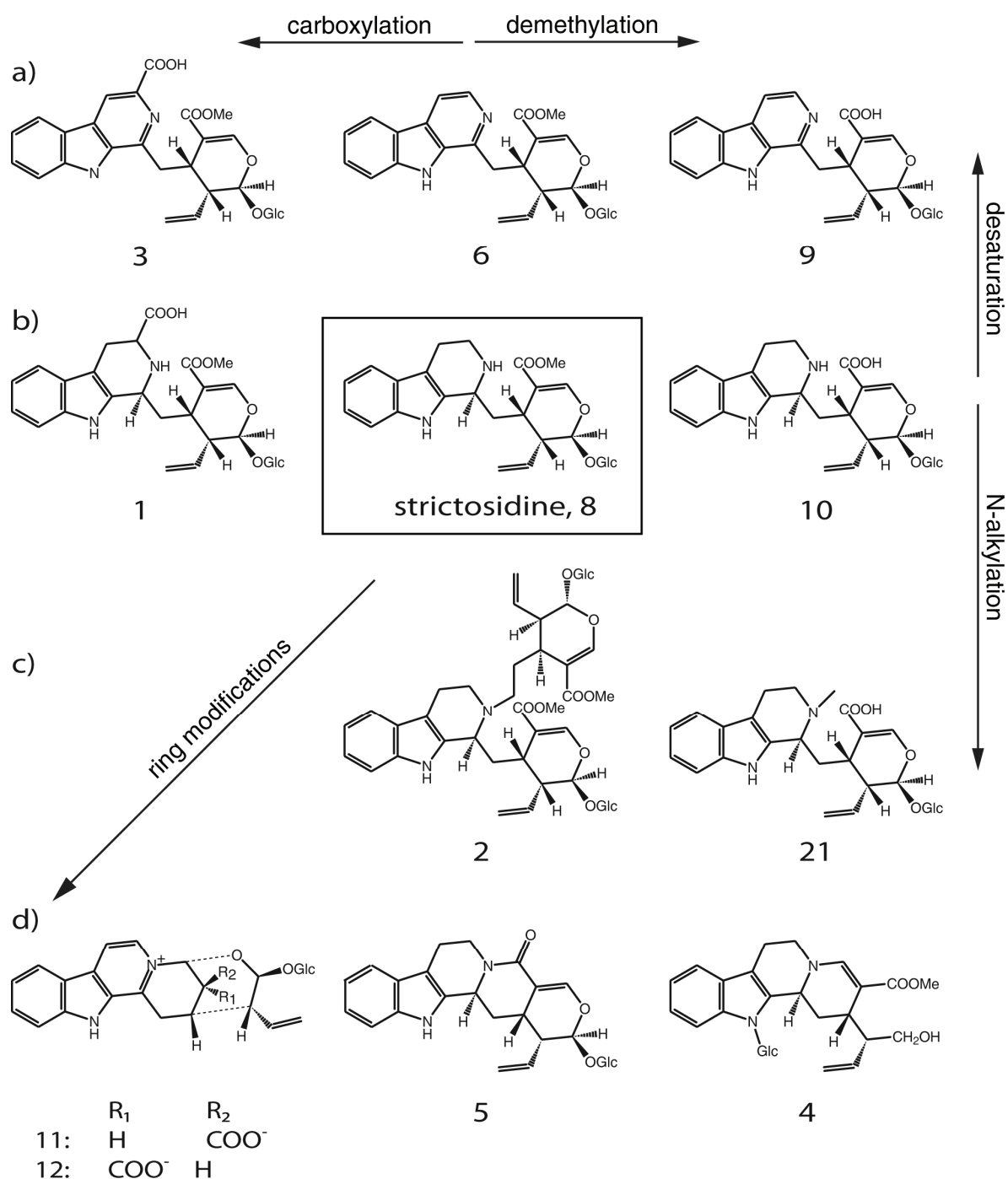


Fig. 25: Tryptamine-iridoid alkaloids found in species of the Psychotria alliance and their hypothetical biosynthetic links to strictosidine (8). 5-carboxystrictosidine (1), bahienoside B (2), desoxycordifoline (3), lagamboside (4), strictosamide (5), lyaloside (6), strictosidine (8), lyalosidic acid (9), strictosidinic acid (10), ophiorine A and B (11 and 12) and palicoside (21). Possible reaction steps are indicated by arrows.

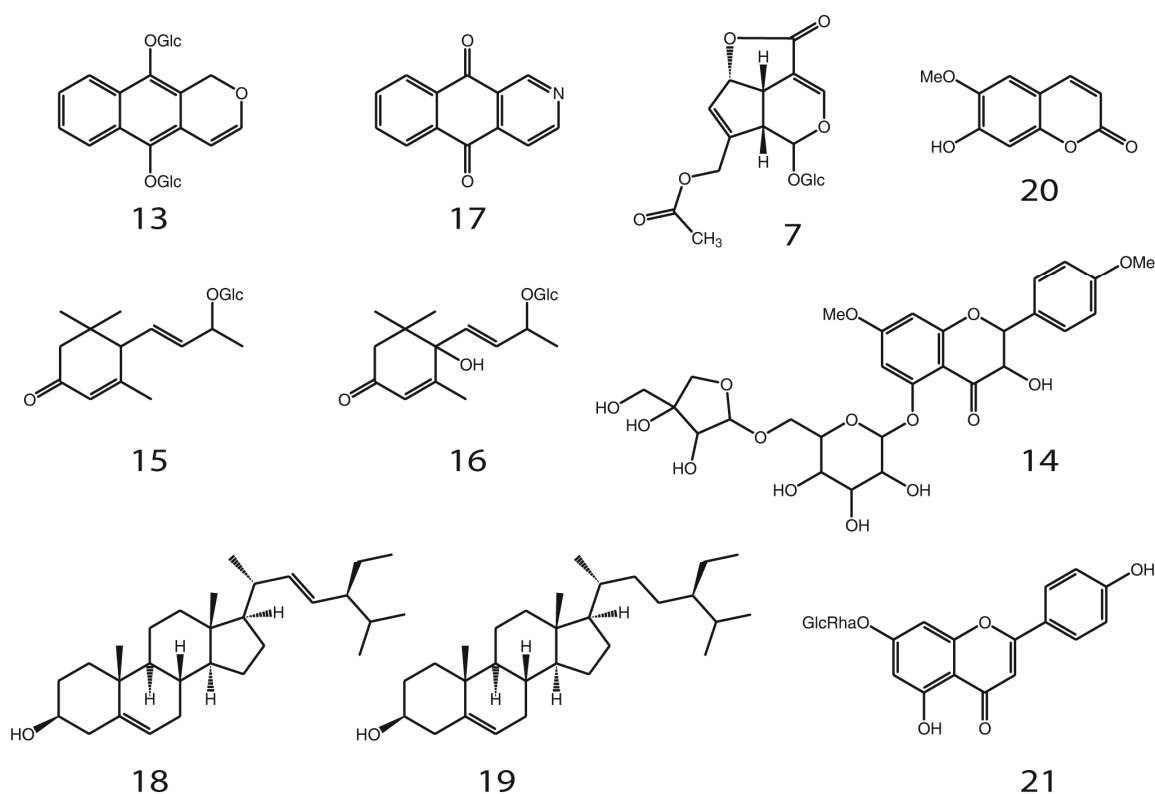


Fig. 26: Further compounds detected within species of the *Psychotria* alliance. Harounoside (**13**), 2-azaanthraquinone (**17**), asperuloside (**7**), scopoletin (**20**), a new aromadendrin 7,4'-diOMe-5-O-apioside-glucoside (**14**), rhoifoline (**21**), unnamed megastigmane (**15**), corchoionoside C (**16**), stigmasterol (**18**) and β -sitosterol (**19**).

6.10 Diversification of chemical profiles within the *Psychotria* alliance

The distribution of all detected compounds is illustrated in Tab. 6, listing all analyzed species, including collector or herbarium of origin of the samples, their tribal placement and the distribution of all detected compounds. Species are arranged in order to reflect both phylogenetic relationships and chemical similarities. It should be mentioned, that *Psychotria* subg. *Heteropsychotria* and *Palicourea* represent a single evolutionary lineage within the Palicoureeae, but nomenclatural changes are still pending. Different tribes are indicated by a horizontal double line, and phytochemically characterized groups are indicated by horizontal lines, respectively. Profiles obtained from different organs and from different accessions by one collector were combined into a single record. All tryptamine iridoid alkaloids are included, however only compound classes are concerned for most other compounds. Compound classes are separated by vertical separation lines. Chemosystematic aspects are discussed in chapter 7

Tab. 6: Secondary metabolite distribution in surveyed species of the *Psychotria* alliance.

Taxon	Collection	Tribe	Tannins	tryptamine iridoid alkaloid										Alstroetine type alkaloids	Dopamine iridoid alkaloids	iridoid		Harounoside	Naphthoquinones	others																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																										
				Strictosidine	Strictosidinic acid	Bahienoside B	5-Carboxystrictosidine	Lagamboside	Strictosamid	Desoxycordifoline	Lyalosidic acid	Lyalosid	Lyalosidic acid deriv.			Ophiorin A & B	Palicoside			unknown derivatives	Asperuloside	Daphylloside	uncertain iridoids	Sitosterin/Stigmasterin	Megastigmane	Scopoletin	Flavonoids, cf. & conf.																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																			
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Taxon	Collection	Tribe	Tannins	tryptamine iridoid alkaloid										Alstroline type alkaloids	Dopamine iridoid alkaloids	iridoid		Harounoside	Naphthoquinones	others					
				Strictosidine	Strictosidinic acid	Bahienoside B	5-Carboxystrictosidine	Lagamboside	Strictosamid	Desoxycordifoline	Lyalosidic acid	Lyalosid	Lyalosidic acid deriv.			Ophiorin A & B	Palicoside			unknown derivatives	Asperuloside	Daphylloside	uncertain iridoids	Sitosterin/Stigmasterin	Megastigmane
<i>Psychotria poeppigiana</i>	AB	Pal															•								
<i>Psychotria poeppigiana</i>	BS	Pal															•								
<i>Psychotria poeppigiana</i>	HG	Pal															• A								
<i>Psychotria elata</i>	AB	Pal		•																					
<i>Psychotria elata</i>	HG	Pal		•			○																		
<i>Psychotria goldmanii</i>	AB	Pal		•					•		○	•	○												•
<i>Psychotria cooperi</i>	AB	Pal		•				○				○													•
<i>Psychotria cooperi</i>	HG	Pal		•																					•
<i>Psychotria suerrensii</i>	AB	Pal			•						•					•								•	•
<i>Psychotria cyanococca</i>	AB	Pal		•		•	•	○		•		•													
<i>Psychotria cyanococca</i>	BS	Pal		•		•				•		•													
<i>Psychotria aubletiana</i>	AB	Pal		•								•												•	•
<i>Psychotria chiriquiensis</i>	AB	Pal					•					•					•								
<i>Psychotria brachiata</i>	AB	Pal										○	•												
<i>Psychotria brachiata</i>	BS	Pal											•			•									
<i>Psychotria solitudinum</i>	AB	Pal											○												
<i>Psychotria deflexa</i>	HG	Pal														•									
<i>Palicourea guianensis</i>	AB	Pal		•																					
<i>Palicourea guianensis</i>	BS	Pal														•									
<i>Palicourea garciae</i>	AB	Pal														•									
<i>Palicourea discolor</i>	AB	Pal		•								•													
<i>Palicourea padifolia</i>	AB	Pal		•								•													•
<i>Palicourea crocea</i>	AB	Pal			•																				
<i>Palicourea lasiorrhachis</i>	AB	Pal														•									•
<i>Palicourea corymbifera</i>	W	Pal			•																				•
<i>Palicourea grandiflora</i>	W	Pal		•					○			○													
<i>Palicourea marcgravii</i>	W	Pal		•		•																			
<i>Palicourea adusta</i>	AB	Pal																						•	•
<i>Palicourea acuminata</i>	HG	Pal		○	•	•	○	○	•		○												•	•	
<i>Palicourea acuminata</i>	BS	Pal			•	•	○		•																
<i>Psychotria cornigera</i>	W	Pal			•		○	○	○																
<i>Psychotria cuspidulata</i>	W	Pal			•			○	•																
<i>Psychotria barbiflora</i>	W	Pal			○						•	○													
<i>Psychotria buchtienii</i>	AB	Pal		•																					
<i>Psychotria buchtienii</i>	HG	Pal		•																					
<i>Psychotria hispidula</i>	AB	Pal		•								○													
<i>Psychotria hispidula</i>	WU	Pal		•								○													
<i>Psychotria hoffmannseggiana</i>	AB	Pal		•								○											○		
<i>Psychotria hoffmannseggiana</i>	BS	Pal		•				○		○															
<i>Psychotria officinalis</i>	W	Pal		•																					
<i>Psychotria platypoda</i>	W	Pal		•																					

see 7.1.1

see 7.1.2

Taxon	Collection	Tribe	Tannins	tryptamine iridoid alkaloid										Alstroline type alkaloids	Dopamine iridoid alkaloids	iridoid		Harounoside	Naphthoquinones	others																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																															
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Phytochemical differentiation between tribes, genera and species groups is indicated by horizontal division lines. Tribal assignment according to Robbrecht & Manen (2006). Pal: Palicoureeae, Psy: Psychotrieae and Las: Lasiantheae. Collectors: AB – A. Berger, BS – B. Sedio, JS – J. Schinnerl, HG – M. Bernhard & H. Greger. Other sample sources: HBV – Botanical Garden, University of Vienna, W & WU – herbarium specimens. “●” – major compounds, “○” – traces, and “A” – artefacts generated during isolation.

7 Chemosystematic Aspects

Results from 81 species of *Psychotria* and related genera of the Palicoureeae as well as from the Lasiantheae are compared and discussed in relation to literature data. Additional phytochemical records used for interpretation of chemical profiles came from the diploma theses of Bernhard (2012), Fasshuber (2011) and Plagg (2012). Due to persistent taxonomic confusion, the identity of several species reported in literature and of the corresponding voucher specimens had to be checked for confirmation of correct species assignment. For that purpose, respective herbarium specimens were studied either physically or as digital image, and have been revised as necessary (see following chapter and appendix). The relevance of phytochemical differentiation in recognising taxonomic groups at various taxonomic levels is discussed separately.

7.1 Relevance for infrageneric classification

Isolation of tryptamine iridoid alkaloids has been the focus of of present study and the distribution of 12 compounds could be studied in species of *Palicourea* s.l. including *Psychotria* subg. *Heteropsychotria*. Most compounds were found to be of scattered occurrence (Tab. 6). Nevertheless for three groups, found to be closely related basing on morphological characters, there is significant evidence to draw chemosystematic conclusions about infrageneric relationships. Several comments on iridoid accumulation are also made in the present chapter.

7.1.1 Section *Didymocarpos*

Section *Didymocarpos* Steyerem. is a group of species including *Palicourea acuminata*, *Psychotria bahiensis*, *P. cerantha*, *P. cuspidata*, *P. jauaensis* and *P. rhodothamna* (Steyermark, 1972) as well as *P. cornigera* and *P. cuspidulata* (Taylor et al., 2004). The group is well defined by their unusual didymous fruit structure, showing a distinctive groove between the two smooth surfaced pyrenes (Steyermark, 1972; Taylor et al., 2004). In the course of the present work, *Palicourea acuminata* was studied in detail and a series of tryptamine iridoid alkaloids have been found (Fig. 17). Among those, the unusual tryptamine bis(iridoid) alkaloid bahienoside B (**2**) and a novel glabratine derivative lagamboside (**4**) deserve special attention (Berger et al., 2012).

The alkaloid bahienoside B (**2**) was first reported from *Psychotria bahiensis*, collected in Trinidad. It was isolated together with 5-carboxystrictosidine (**1**) and several other alkaloids by Paul et al. (2003). *P. bahiensis* was initially regarded as a widespread species

including notable variability (Steyermark, 1972). As additional specimens became available, it became obvious that *P. bahiensis* is an endemic species of the state of Bahia (Brazil), and is not closely related to other species of section *Didymocarpos*. Most of the specimens previously regarded as *Psychotria bahiensis* belongs to either *Palicourea acuminata* or *Psychotria cornigera* (Taylor et al., 2004).

The occurrence of *P. bahiensis* in Trinidad, far outside its range in Bahia, Brazil, and the history of confusion with other species lead to the question, if the plant material studied by Paul et al. (2003) was correctly determined. Consequently, a digital image of their voucher specimen kept at the National Herbarium of Trinidad and Tobago (TRIN-34712) was studied, found misidentified and was revised as belonging to *P. cornigera*. Therefore, all compounds attributed to *P. bahiensis* have actually been isolated from *P. cornigera*.

Based on morphological criteria, the type species of section *Didymocarpos*, *P. bahiensis*, was later excluded from the section (Taylor et al, 2004). A phytochemical sample taken from an authentic specimen of *P. bahiensis* (Irwin et al. 30770, W-1978-010335) matching the type specimen (Salzmann s.n., syntype, W-Rchb 0031193) was additionally checked. None of the alkaloids reported for *P. bahiensis* could be detected by HPLC-UV/VIS analysis (Fig. 27). The species also deviates by a complete lack of alkaloids, showing chromophores indicative for flavonoids. Its exclusion from section *Didymocarpos* is fully supported by phytochemical data.

With the occurrence of bahienoside B (**2**) and desoxycordifoline (**1**) as major compounds in leaf extract of *Palicourea acuminata*, *Psychotria cornigera* and *P. cuspidulata* (Fig. 27), there is enough evidence to hypothesise that these compound characterise the section *Didymocarpos* and supports it as a natural group. Strictosamid (**5**) and lagamboside (**4**) have additionally been found as trace compounds in those species. In stem bark extract of *P. acuminata*, larger quantities of strictosamide (**5**) and lagamboside (**4**) are accumulated together with strictosidinic acid (**1**) (see Fig. 17). One major difference to Paul et al. (2003) is the isolation of desoxycordifoline (**3**), which can not pass acid/base extraction, as proven by tryouts during extraction (data not shown).

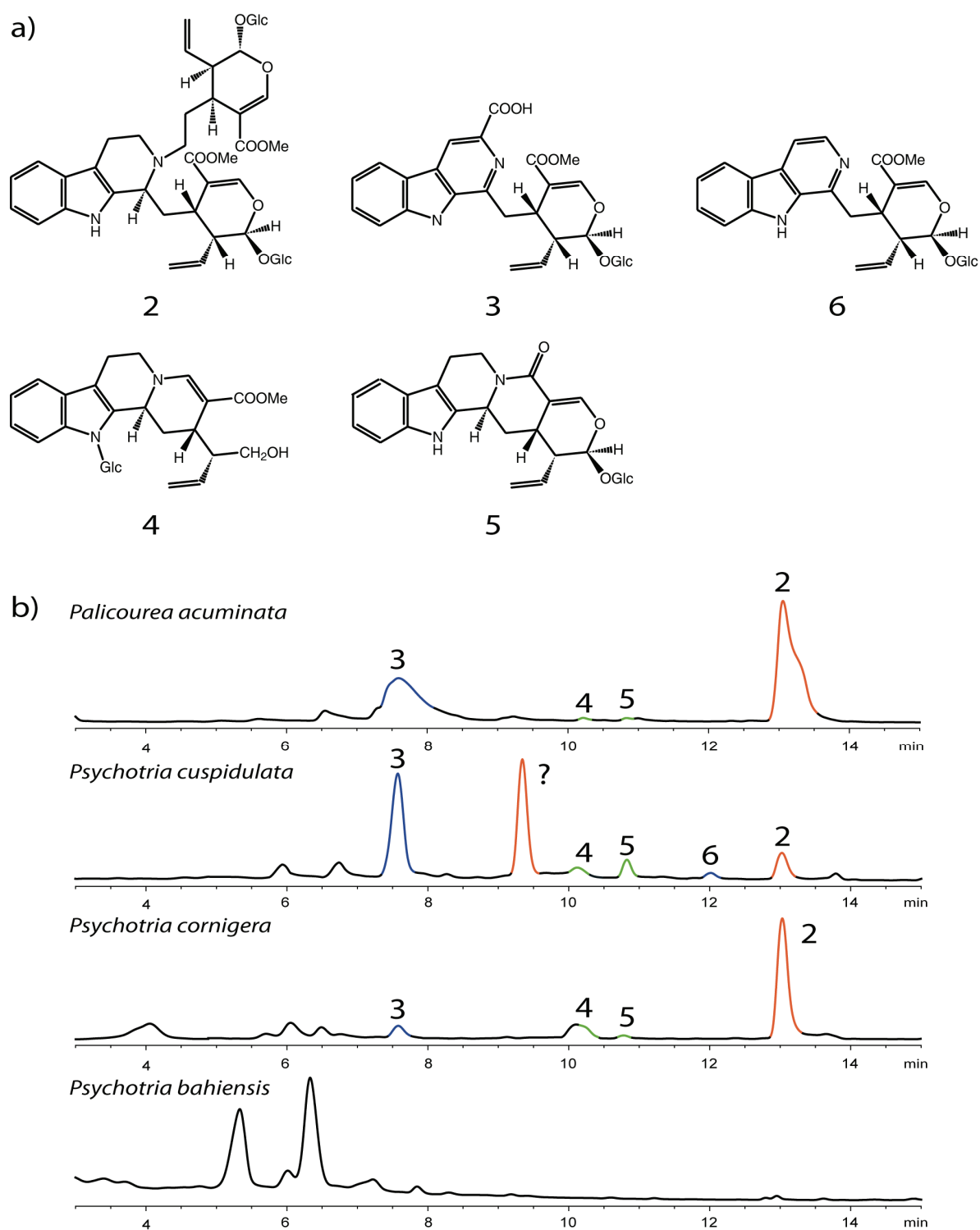


Fig. 27: Structure (a) and distribution (b) of compounds detected in leaf extracts of four species associated with *Psychotria* sect. *Didymocarpos*. Bahienoside B (2) and desoxycordifoline (3) are the main compounds. Small amounts of lagamboside (4) strictosamide (5) and lyaloside (6) are also present. *P. bahiensis* differs in being devoid of any alkaloids.

7.1.2 Section *Pseudocephaelis*, series B. *Pseudocephaelis*

Section *Pseudocephaelis* Steyermark., series B. *Pseudocephaelis* sensu Steyermark (1972) is a group of species including, among others, *Psychotria buchtieni*, *P. hispidula*, *P. hoffmannseggiana* (incl. *P. barbiflora*), *P. gracilentia* (incl. *P. brachybotrya*), *P. officinalis* and *P. platypoda*. The group is characterised by their clustered flowers, which are subtended by one or more bracts, however, individual flowers are lacking own bracts. The whole inflorescence is often congested into head-like clusters, which are surrounded by involucre bracts, giving them a *Cephaelis*-like appearance (Steyermark, 1972).

P. buchtieni, *P. hispidula*, *P. hoffmannseggiana*, *P. officinalis* and *P. platypoda*, were found to accumulate strictosidinic acid (**10**) as the main compound, which may be accompanied by traces of lyalosidic acid (**9**; Fig. 28). The accumulation of large amounts of strictosidinic acid and the occurrence of two compounds (**9**, **10**), which are derived by demethylation of strictosidine is unique among *Palicourea* and *Psychotria* subg. *Heteropsychotria* and indicates the joint occurrence of a special demethylase in these species. The phytochemical pattern is therefore considered as an informative character, characterising these species as a natural group. It is unlikely, that morphologically distinct species containing strictosidinic acid (Tab. 6) belong to this group as well, but the question remains to be answered.

Two samples of *P. barbiflora*, a taxon at present regarded as a synonym of *P. hoffmannseggiana* (Taylor et al., 2004), differed by an inverse ratio of strictosidinic acid (**10**) and lyalosidic acid (**9**), and by accumulating trace amounts of lyaloside (**6**). It remains to be clarified, if the ratio of the former compounds is genetically fixed and related to morphological characters or may be subject of ecological or geographical variation. Chemical results of these two samples of *Psychotria barbiflora* are uniformly distinct from *P. hoffmannseggiana*, and support the recognition of *P. barbiflora* as a separate species as done e.g. by Steyermark (1972).

P. gracilentia is quite similar to other species of the group around *P. hoffmannseggiana*, but may be separated by different bract arrangement (Taylor et al., 2004). However, three different provenances deviated by lacking alkaloids. The question, if those species represent an exception within the chemically uniform group or have to be excluded, remains open to further studies.

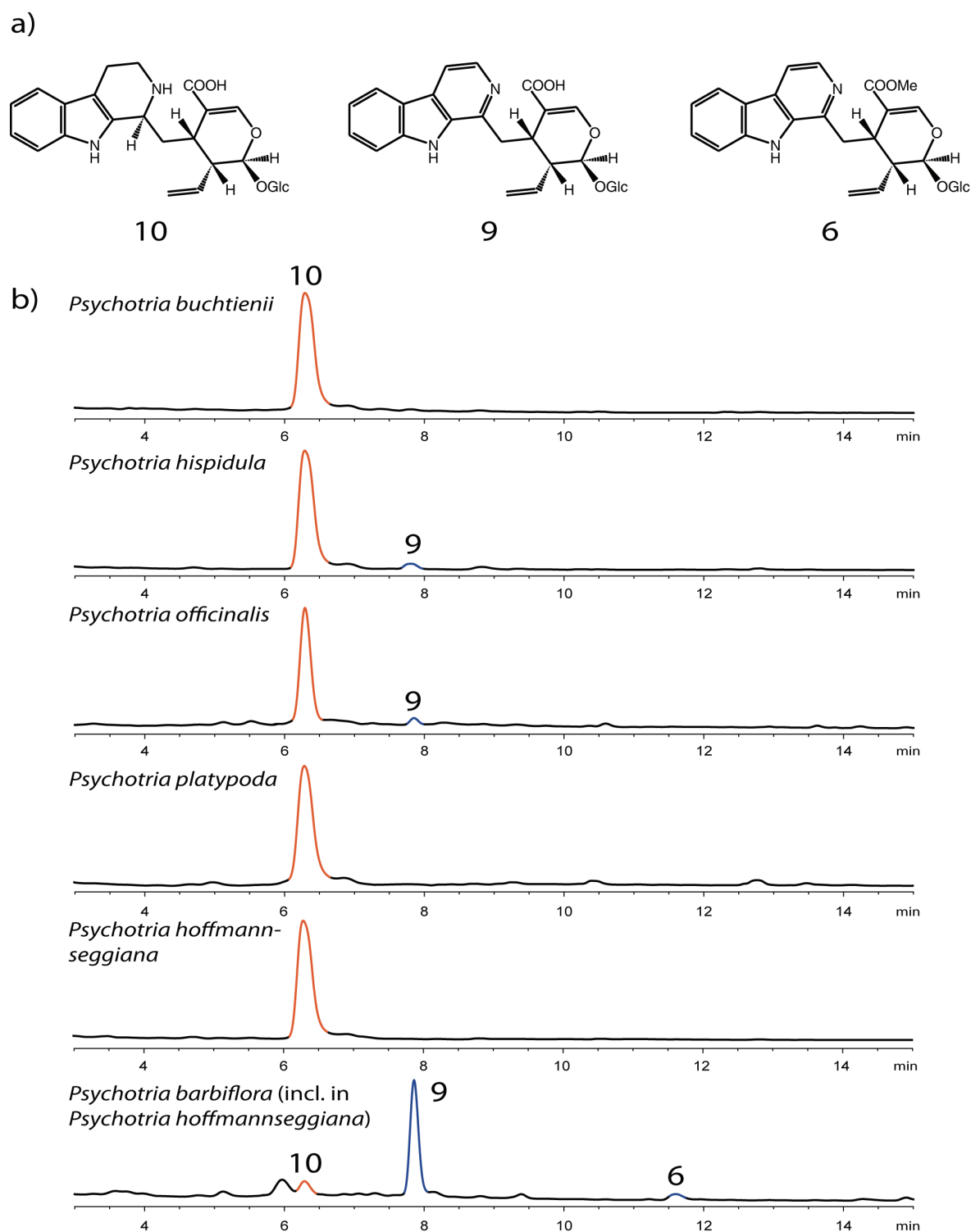


Fig. 28: Structure (a) and distribution (b) of compounds detected in leaf extracts of species of section *Pseudocephaelis*, series B. *Pseudocephaelis*. The group is characterized by accumulating strictosidinic acid (10) with traces of lyalosidic acid (9). *P. barbiflora*, now regarded as a synonym of *P. hoffmannseggiana*, deviates by an inverse ratio of the former compounds, additionally featuring trace amounts of lyaloside (6).

7.1.3 Section *Nonatelia*

The genus *Nonatelia* Aubl. was initially established by Aublet (1775) to harbour species with an unusual five locular ovary. Subsequent authors have not accepted the genus and placed its species within *Psychotria* sect. *Nonatelia* (Aubl.) Müll. Arg. (e.g. Müller, 1881; Steyermark, 1972), an opinion fully supported by molecular phylogeny (Nepokroeff et al., 1999). *Psychotria stenostachya* of the western Amazon basin, the widespread *P. racemosa* and *P. tsakiana* of Costa Rica belong to this group (Taylor et al., 2011). Within *Psychotria*, the section is unique and easily recognised by ovary and stipule structure, as well as conspicuous subparallel tertiary leaf-venation.

Palicoside (*N*-methyl strictosidinic acid, **21**) has been isolated from *Psychotria tsakiana* (Berger et al., 2012; erroneously reported for *P. racemosa* by Fasshuber, 2011 and Plagg, 2012). Whilst the lack of palicoside in several authentic provenances of *P. racemosa* (Berger, A. AB 16021002, WU 0067460; Sedio, B. PYRA; Will, S. 04/08/99/80, WU 0024780) could be confirmed, traces were found in herbarium samples of *P. stenostachya* (Teixeira, L.O.A. et al. 456, W 2009-0001464) (Fig. 29), with whom *P. tsakiana* is apparently closely related and shares inflorescence structure.

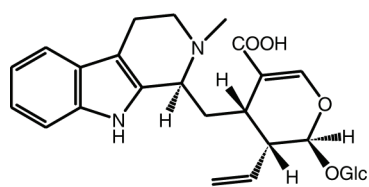
Due to the colour of the herbarium specimen of *P. tsakiana*, it appears likely that it was collected using EtOH to prevent moulding, possibly altering its chemical composition. This is consistent with unusually low peak height in its extract. Leaching of soluble compounds could have made signal intensities low and several peaks of less soluble compounds appearing more prominent, whereas the peak of palicoside (**21**) appears less prominent than it would be in recently collected material, which is tentatively considered similar to those of *P. tsakiana*.

Leaf extracts of three provenances of *P. racemosa* showed strong infraspecific variation in composition with the major peaks at 3, 6 and 9 min. respectively. Peaks between 13 and 14 min. have chromophores similar to widespread plasticisers. They are considered as artefacts from isolation, possibly originating from Eppendorf tubes, wherein quick extraction was performed.

The occurrence of palicoside in *P. stenostachya* and *P. tsakiana* but not in *P. racemosa* is of special interest. Its occurrence helps in distinguishing *P. racemosa*, which is virtually inseparable from the other species when sterile (Taylor et al., 2011). It also demonstrates the limits of chemosystematics in determining infrageneric groups.

Psychotria racemosa was not studied in detail and no compounds were isolated. Preliminary HPLC-UV/VIS analysis indicated the occurrence of flavonoids.

a)



21

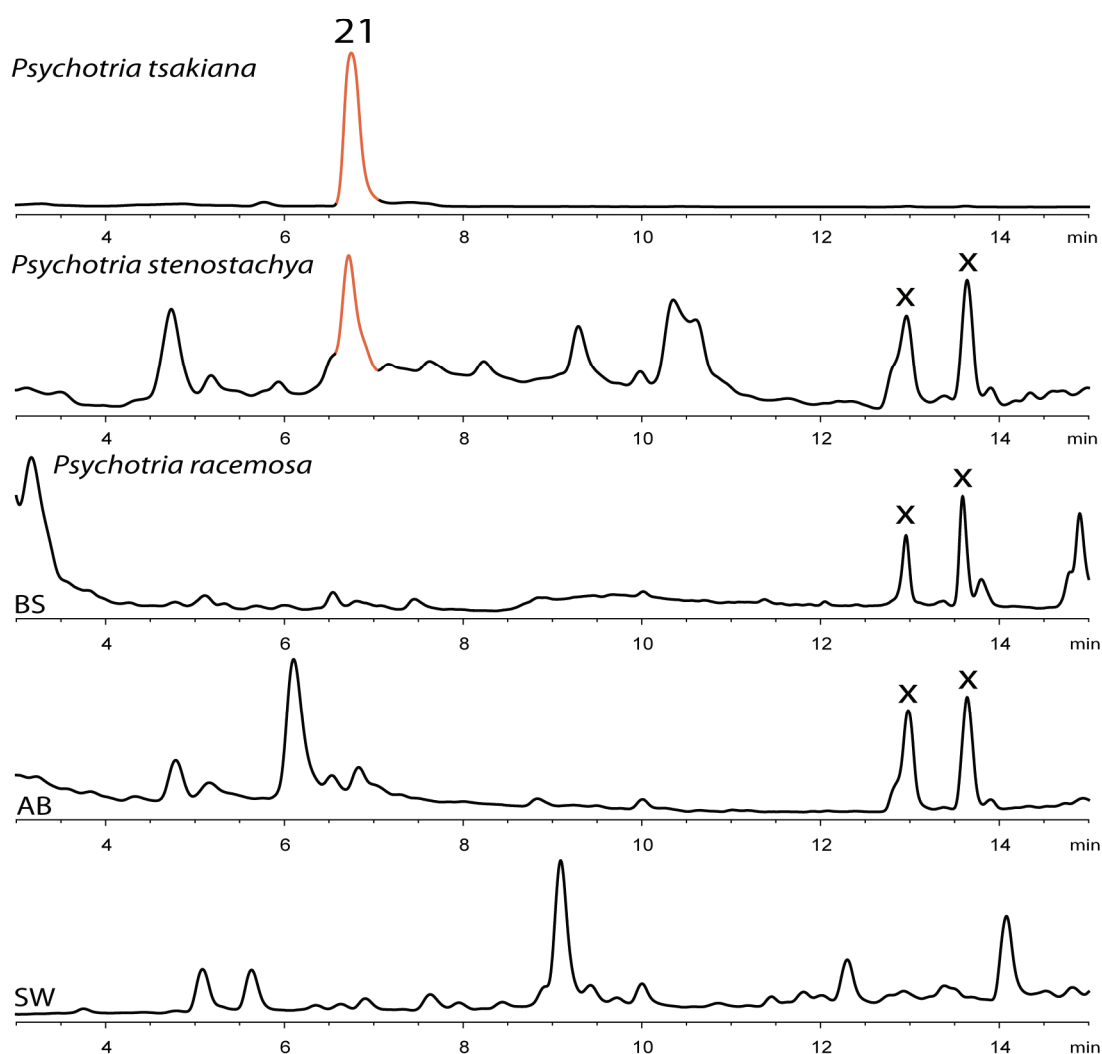
b) *Psychotria tsakiana*

Fig. 29: a) Structure and distribution of palicoside (21). Palicoside occurs in *P. tsakiana* and *P. stenostachya*. Extracts of three provenances of *P. racemosa* (BS: B. Sedio., AB: A. Berger, SW: S. Will) showed strong infraspecific variation, but no alkaloids could be detected. Compounds indicated with “x” show chromophores of plasticisers, and are most likely artifacts of extraction.

7.1.4 Iridoids

Asperuloside was frequently detected in the present study (Tab. 6). In addition, Inouye et al. (1988) found the same compound in three Asian species of *Psychotria* subg. *Psychotria*, which is in good accordance with present results. Asperuloside is especially found in unfolding leaves and gives them a conspicuous blackish colour when dried. Accumulation of asperuloside was detected in species of both Psychotrieae (Fig. 22) and Palicoureeae (Fig. 18), and may be considered a plesiomorphic i.e. basal character of the *Psychotria* alliance. Because of its scattered occurrence in both major clades, it is not considered to exhibit a phylogenetic signal at generic level.

Within subg. *Psychotria*, iridoids are restricted to unfolding leaves. Upon maturing, those are subsequently replaced by polyphenolic compounds. In fully expanded leaves, iridoids are no longer detectable, which may in part be attributed to overlaying signals in HPLC-chromatograms.

Within subg. *Heteropsychotria*, the morphologically similar species *P. pilosa* and *P. mortoniana*, both characterised by their conspicuous pubescence and their bracteose, paniculate inflorescence, share iridoid accumulation. Another iridoid accumulating species, *P. poeppigiana*, shares their conspicuous type of pubescence but differs by its capitate inflorescence, a character, that was shown not to reflect natural relationships (e.g. Steyermark, 1972; Taylor, 1996; Nepokroeff et al., 1999). Fasshuber (2011) reports scandoside methyl ester for “Poeppi1”, a compound isolated from *Psychotria poeppigiana*, whereas Plagg (2012) reports deacetylasperulosidic acid for the same compound. Deacetylasperulosidic acid is the correct identity of “Poeppi1” according to L. Brecker (personal comment). However, when studying their original material, the isolated compound could not be detected in the crude extract. In turn, large amounts of asperuloside were present, which was not recorded by Plagg (2012), who isolated deacetylasperulosidic acid by means of preparative TLC. The compound may be formed from asperuloside via ring cleavage (Inouye et al., 1969) and hence should be considered as an artefact.

The lack of accumulating tryptamine iridoid alkaloids could be either the consequence of a mutation in an enzyme involved in the strictosidine biosynthesis, or the result of down regulation of either strictosidine synthase or one of its substrate producing enzymes. Iridoid accumulation could be of some systematic value in the *Palicourea* s.l. clade, but is far from being understood.

7.2 Relevance for intergeneric classification

7.2.1 *Psychotria* s.str (Psychotrieae)

A significant number of species of *Psychotria* s.str. i.e. *Psychotria* subg. *Psychotria* was studied for a deeper insight of chemical differentiation. No alkaloids could be detected in any species (Tab. 6). With the exception of *P. viridis* containing *N,N*-dimethyltryptamine (e.g. Rivier & Lindgren, 1972), no alkaloids were previously found in neotropical species of subg. *Psychotria*. Thus, present results are in good agreement with literature (e.g. Leal & Elisabetsky, 1996). Additionally, no enzyme activity related to MIA biosynthesis was found in the tannin containing species *P. carthagenensis* (Lopes et al., 2000).

In contrast to chemical uniformity of neotropical *Psychotria* s.str., the taxonomic situation is far more complex in Asia leading to puzzling phytochemical data. Less emphasis has been given to disentangling the generic boundaries of *Psychotria* in the Australasian and Pacific region, and traditionally most species of the tribes Palicoureeae and Psychotrieae have been included in the genus *Psychotria* as exemplified by Backer & Bakhuizen van den Brink (1965) and Smith & Darwin (1988) (Andersson, 2001). With respect to generic differentiations in the *Psychotria* alliance, taxonomic work in the Paleotropics is lagging behind when compared to neotropical taxa. The generic identity of species has still to be figured out, making interpretation of phytochemical data more difficult. Several Asian species currently assigned to subg. *Psychotria* are reported to accumulate cyclotryptamine alkaloids (see introduction, 3.4). Here, these are tentatively regarded to belong to the enigmatic genus *Margaritopsis* (Palicoureeae) as discussed below in section 7.2.5. Consequently, only a limited number of species of true *Psychotria* have been subjected to phytochemical studies. No detailed information has been published so far. However, preliminary NMR data tend suggest the presence of polyphenolic compounds (Giang et al., 2007; Hayashi et al., 1987).

Chemosystematic relevance of tannin accumulation

All species of subg. *Psychotria*, that have been subject in the present analysis, showed strikingly similar HPLC-chromatograms featuring a broad “hump” with a catechin chromophore (Tab. 6, Fig. 22-24). Neither a similar chromatogram nor a chromophore could be detected in species of subg. *Heteropsychotria* or in any other related genus of the *Psychotria* alliance. The putative occurrence of condensed tannins is therefore considered to be of great chemosystematic importance. Consequently, subg. *Psychotria* as well as its

tribe Psychotrieae is considered to be characterised by the accumulation of condensed tannins of the proanthocyanidin type, whereas subg. *Heteropsychotria* as well as the tribe Palicoureeae is characterised by alkaloids of various types (Berger et al., 2012; Lopes et al., 2004; Plagg, 2012; Porto et al., 2009; Schinnerl et al., 2012). When polyphenolics are present in species of the Palicoureeae, these are simple flavonoids (see introduction, 3.8.2 and Fig. 21, 23, and 26).

Red Seed Coat Pigment

Steyermark (1972) divided neotropical representatives of *Psychotria* in two subgenera, *P.* subg. *Psychotria* and *P.* subg. *Heteropsychotria*. The presence or absence of an ethanol soluble red seed coat pigment (RSCP) was one of the major characters employed for delimitation of subg. *Psychotria* or subg. *Heteropsychotria*, respectively (Andersson, 2001, 2002b; Nepokroeff et al., 1999; Robbrecht & Manen, 2006; Steyermark, 1972). The structure and identity of the pigment has never been studied, but its elucidation is of great importance in understanding the significance of RSCP as a diagnostic character.

HPLC-chromatograms of a sample of RSCP were found virtually identical with that of its crude methanolic leaf and stem bark extract by means of chromatogram structure or chromophores (Fig. 24). Similarly to crude leaf extracts, the RSCP is likely of oligo- or polymeric catechin structure. The results are of special importance in systematic studies, because they demonstrate that the presence of RSCP is correlated with vegetative characters. This allows to assess its occurrence by simple HPLC measurements of crude leaf extracts in species, where fruits are not at hand (e.g. in sterile or flowering collections) or not known at all. It also showed that, without direct knowledge, phytochemical characters have been considered for decades in delimitation of systematic groups within *Psychotria*.

The presence of tannins in seed coat has been reported for a variety of commercially important crops such as barley (*Hordeum vulgare* L., Poaceae; Aastrup et al., 1984; Brandon et al., 1982), common bean (*Phaseolus vulgaris* L., Fabaceae; Caldas & Blair, 2009; Díaz et al., 2010), cotton (*Gossypium hirsutum* L., Malvaceae; Halloin, 1982), cowpea (*Vigna* spp., Fabaceae; Lattanzio et al., 2005), faba bean (*Vicia faba* L., Fabaceae; Martín et al., 1991), pea (*Pisum sativum* L., Fabaceae; Troszyńska & Ciska, 2002), sorghum (*Sorghum bicolor* (L.) Moench, Poaceae; Awika & Rooney, 2004; Brandon et al., 1982) and soybean (*Glycine max* (L.) Merr., Fabaceae; Todd & Vodkin, 1993). Furthermore, dark colour of the testa (seed coat) has been related to tannin content in various species (Aastrup et al., 1984; Awika & Rooney, 2004; Brandon et al., 1982; Caldas

& Blair, 2009; Díaz et al., 2010; Halloin, 1982; Lattanzio et al., 2005; Martín et al., 1991; Todd & Vodkin, 1993; Troszyńska & Ciska, 2002). Little is known concerning the distribution of seed coat tannins in species without economic value.

Within the Rubiaceae, the seed coat of the speciose and horticulturally important genus *Ixora* is reported to contain tannins. The seed coat consists of a tannin accumulating single-cell-layered exotesta and an amorphous endotesta consisting of several layers of crushed cells (De Block, 1998). Tannins in seed coats have also been reported in *Arabidopsis thaliana* (Debeaujon et al., 2003).

In *Arabidopsis thaliana*, seed coat browning is caused by the oxidation of flavonoids, particularly proanthocyanidins (Pourcel et al., 2005) contributing to physiological functions such as regulation of seed maturation and dormancy. Tannin content also plays a vital role in seed defence against herbivores and pathogens (Debeaujon et al., 2003; Winkel-Shirley, 1998). Although few studies have directly examined the role of seed coat tannins in plant-herbivore interactions, these compounds provide a certain degree of resistance to bruchid beetles (Chrysomelidae-Bruchinae; Boughdad et al., 1986; Lattanzio et al., 2005) and have a digestibility reducing effect in piglets (van der Poel et al., 1992). Due to the generally accepted role that tannins play in plant-herbivore defence (e.g. Barbehenn & Constabel, 2011), it may be expected that RSCP tannins provide a barrier to phytophagous insects, which may also be the case for seeds of *Psychotria* subg. *Psychotria*.

7.2.2 *Carapichea* (Palicoureeae)

In addition to *Carapichea ipecacuanha*, source of the well known South American vomiting root, dopamine iridoid alkaloids have been isolated from two other species of the *Psychotria* alliance. Muhammad et al. (2003) found similar compounds in *Psychotria klugii* Standl., and Bernhard et al. (2011) and Bernhard (2012) in *Carapichea affinis*, respectively. Fasshuber (2011) lists strictosidinic acid for *Carapichea affinis* (as “*C. offinis*”). However, this compound is not present in the indicated species, but it was isolated from *Psychotria buchtieni*, initially determined as *P. officinalis*. Bernhard et al. (2011) already indicated the chemosystematic importance of dopamine iridoid alkaloids.

Psychotria klugii Standl. was recently shown to be misplaced in *Psychotria*, and it is currently being transferred to the genus *Carapichea* based upon morphological data (C. M. Taylor, personal comment). Reports of dopamine iridoid alkaloids are confined to the genus *Carapichea*, which is in line with the generic reassignment. Based upon consistent

phytochemical data for three species of the *Carapichea* clade, it is reasonable to assume that the accumulation of dopamine iridoid alkaloids represent a consistent and unique chemical character of the group.

Due to taxonomic confusions, emetine and derivatives have accidentally been reported for species other than those mentioned above. In their paper on micropropagation of *Palicourea acuminata*, Lara-Quesada et al. (2003; studied under *Psychotria acuminata*) cite the occurrence of emetine, cephaeline and psychotrine for this species. However, these compounds do not occur in this species (Tab. 6, Fig. 17), and the authors might have mistaken *Cephaelis acuminata* H. Karst for being a synonym of *Palicourea acuminata*. The genus *Cephaelis* is now generally included in *Psychotria* or *Palicourea* (e.g. Steyermark, 1972). However, *Cephaelis acuminata*, a taxon from which dopamine iridoid alkaloid were reported, was never transferred to *Psychotria* and is regarded as a synonym of *Carapichea ipecacuanha* (de Boer & Thulin, 2005; Itoh et al. 1999; 2002). *Palicourea acuminata* is a different taxon with different phytochemistry.

Emetine type alkaloids have also been reported from *Psychotria granadensis* Benth. (Wiegrebbe et al., 1984), a taxon which is now considered a synonym of *Psychotria nervosa*. No alkaloids could be detected in Costa Rican or Panamanian samples of *Psychotria nervosa*, which in contrast accumulated significant amounts of tannins. It seems likely that emetine type alkaloids have erroneously been ascribed to *Psychotria granadensis*, and reports may rather concern *Uragoga granatensis* Baill., a name which is regarded as a synonym of *Carapichea ipecacuanha* (Govaerts et al, 2011).

7.2.3 *Chassalia* (Palicoureeae)

See comments under *Rudgea*.

7.2.4 *Geophila* (Palicoureeae)

A single species, *Geophila repens* was studied by HPLC-UV/VIS analysis, but no alkaloids could be found. Instead, a flavonoid was detected. Until more data is available, the chemistry of *Geophila* remains largely unknown.

7.2.5 *Margaritopsis* (Palicoureeae)

No plant material was available for direct analysis, and no species of the genus is therefore included in Tab. 6. Instead, the following chapter is focussed on literature data evaluation in combination with morphological observations leading to generic re-assignments of several species as *Margaritopsis*.

Cyclotryptamine (= pyrrolidinoindoline) alkaloids have been isolated from a number of species currently assigned to both subgenera of *Psychotria* (as mentioned in the introduction, 3.4). In the Neotropics, reports are confined to species of the Palicoureeae. With the exception of *Margaritopsis cymuligera*, these compounds have been reported from species currently included in subg. *Heteropsychotria*. Contrary, in the Paleotropics and the Pacific region, they seem to be restricted to subg. *Psychotria*. Cyclotryptamine alkaloids are the only alkaloids previously reported from species included in subg. *Psychotria*. Consequently, those alkaloids have been stated to be characteristic compounds for species of subg. *Psychotria* (Lopes et al., 2004).

Challenging the chemosystematic conclusions by Lopes et al. (2004), the differing patterns of cyclotryptamine alkaloids distribution between the Neotropics and Paleotropics, the unclear generic circumscription of the *Psychotria* alliance in the Paleotropics, as well as the general problem with species determination should be mentioned. Relating phytochemical with morphological and DNA phylogenetic data may solve some inconsistencies leading to a much clearer picture of cyclotryptamine alkaloid distribution.

Especially in Asia, Australasia and the Pacific region, *Psychotria* subg. *Psychotria* has been treated in a broader sense, including taxa belonging to both Psychotrieae and Palicoureeae (Andersson, 2001). From Paleotropic species found to contain cyclotryptamine alkaloids, *Psychotria leptothyrsa* (studied as *P. beccarioides*; Hart et al., 1974), *P. milnei* (studied as *Calycodendron milnei*, Libot et al., 1987; Saad et al., 1995), *P. oleoides* (Jannic et al., 1999; Libot et al., 1987) and *P. rostrata* (Lajis et al., 1993; Takayama et al., 2004) show affinities with *Margaritopsis*. Based upon rps16 intron sequence data, *P. oleoides* was shown to belong to the *Margaritopsis* clade (Andersson, 2001). Additionally, rps16 data (GenBank accession number AJ320083, sequence reported by Novotny et al., 2002) clearly place the species in the same group (based upon own alignment using the BLAST algorithm, data not shown). Additionally, *P. leptothyrsa* and *P. rostrata* come close to *Margaritopsis* on base of pyrene morphology (Andersson, 2001). Furthermore, analysed herbarium specimens of *P. rostrata* (*Bakhuizen van den Brink* 6222, W 1924-0007726) and *P. leptothyrsa* (*Ramos. 2-107*, W 1925-0000700) preserved at the Herbarium of the Natural History Museum, Vienna belong to *Margaritopsis* because of their marcescent stipule morphology (own observations). Table 7 summarises cyclotryptamine alkaloid accumulating species that share affinities with *Margaritopsis*, based upon morphological and molecular data. Preliminary reports of an ongoing phylogenetic work on New Caledonian *Psychotria* revealed affinities of many species of

subg. *Psychotria* to *Margaritopsis*, but no details were given (Barrabé et al., 2010; J. Munzinger, personal comment). Beside the already sequenced *P. oleoides*, it is likely that the chemically similar New Caledonian *P. lyciiflora* (Jannic et al., 1999) belongs to *Margaritopsis* rather than to *Psychotria*.

P. milnei from Vanuatu (studied as *Calycodendron milnei*, Libot et al., 1987; Saad et al., 1995) is sometimes assigned to the genus *Calycosia* (*C. milnei*). The few already sequenced species of *Calycosia* are included in the pacific clade of *Psychotria* subg. *Psychotria* and are not related to *Margaritopsis* (Andersson, 2002b). According to morphological data from Andersson (2002b), *Calycosia* has several characters that are unusual within *Psychotria* s.str.: the lack of RSCP (in some species), the occurrence of PGS and unruminated endosperm. With the exception of having caducous stipules, these characters make some species resemble *Margaritopsis*. Therefore, it seems possible that several species, possibly including *Psychotria milnei*, are part of the *Margaritopsis* clade, or may be confused with such, thus fitting in the chemosystematic picture hypothesised above.

Tab. 7. Cyclotryptamine i.e. pyrrolidinoindoline accumulating species showing affinities with the genus *Margaritopsis*, or may easily be confused with such.

species	alkaloids	affinities due to	data source
<i>Margaritopsis cymuligera</i>	Brand et al., 2012		
<i>Psychotria colorata</i>	Verotta et al., 1998	stipule morphology	own observation
<i>Psychotria leptothyrsa</i>	Hart et al., 1974	pyrene morphology; stipule morphology rps16 intron data	Andersson, 2001; own observation; GenBank*
<i>Psychotria milnei</i>	Libot et al., 1987	similarities	own interpretation
<i>Psychotria muscosa</i>	Verotta et al., 1999	confusion with <i>M. kappleri</i> ?	Andersson, 2001
<i>Psychotria oleoides</i>	Libot et al., 1987	cpDNA	Andersson, 2001
<i>Psychotria rostrata</i>	Lajis et al., 1993	pyrene morphology; stipule morphology	Andersson, 2001; own observation

*own phylogenetic reconstruction using MEGA (data not shown). A rps16 intron sequence reported by Novotny et al., 2002 was used, GenBank accession nr. AJ320083.

Assembling the puzzling data on distribution of cyclotryptamine alkaloids and reconsidering the identity of several species showing affinities with *Margaritopsis* (Tab. 7), these alkaloids appear to be chemosystematic markers for the genus *Margaritopsis*. This hypothesis is additionally supported by the recent report of cyclotryptamine alkaloids in *Margaritopsis cymuligera*, the first report of such compounds in a species actually placed under *Margaritopsis* (Brand et al., 2012). Lopes et al. (2004) stated that

cyclotryptamine alkaloids are markers for *Psychotria* subg. *Psychotria*, but considering data of the present report, cyclotryptamine containing species are excluded from subg. *Psychotria* and placed in the *Margaritopsis* clade. Following the hypothesis would leave *P.* subg. *Psychotria* uniformly characterised by condensed tannins. Nevertheless, further testing is necessary and promising especially in the light of possible application of cyclotryptamines, possessing a broad variety of pharmacological effects (e.g. Elisabetsky et al., 1995).

Occurrence of cyclotryptamine alkaloids in subg. *Heteropsychotria*

The reports on the occurrence of cyclotryptamines in other genera of the Palicoureeae are puzzling. They have been reported in *Palicourea ovalis* (García et al., 1997) and in three species of *Psychotria* subg. *Heteropsychotria*, namely in *P. colorata* (Verotta et al., 1998; 1999), *P. glomerulata* (Solis et al., 1997) and *P. muscosa* (Verotta et al., 1999). It could be assumed, that several species of *Margaritopsis* are hidden within subg. *Heteropsychotria*, and indeed in an initial study, Andersson (2001) found two species of *Psychotria* belonging to the *Margaritopsis* clade. Later, Taylor (2005) treated its neotropical representatives and expanded the genus to comprise 27 species, of which *Margaritopsis cymuligera* was recently found to accumulate cyclotryptamine alkaloids (Brand et al., 2012).

Cyclotryptamine alkaloids have been extracted from *Psychotria colorata*, examination of an authentic specimen (filed under *Psychotria colorata* subsp. *megapontica*, W-Rchb 1889-0113559) showed that the species shares persisting stipules that become corky with age with *Margaritopsis*. However, relying on the treatment of neotropical *Margaritopsis* by Taylor (2005) to be complete, the possibility that *Psychotria colorata*, *P. glomerulata* and *P. muscosa* belong to *Margaritopsis* may be considerably low. It appears more likely that the reports of cyclotryptamine alkaloids in those species are exceptional within subg. *Heteropsychotria* and *Palicourea* or may be the result of confusion with several species of *Margaritopsis*.

Verotta et al. (1999) have isolated cyclotryptamine alkaloids from *Psychotria colorata* and *P. muscosa*. Corresponding voucher specimens IAN-166.535 and IAN-166.536 were said to be deposited in the herbarium IAN (Embrapa Amazônia Oriental, Belém, Pará, Brazil). An other duplum of *P. colorata*, “ZC 23, NYBG” was said to be deposited at NY (New York Botanical Garden). However, the curators of both herbaria were not able to locate the specimens in their collections. Consequently, three possibilities arose: The

specimens have not been deposited in the herbarium as stated, or they have been misplaced in the herbarium, or, most likely, their identity has been revised because they have been incorrectly determined. For instance, *Psychotria muscosa* and *Margaritopsis kappleri* are difficult to separate, and share a history of confusion e.g. by Andersson & Rova, 1999 (Andersson, 2001), possibly explaining the isolation of cyclotryptamine alkaloids from material ascribed to *Psychotria muscosa*, when in fact *Margaritopsis kappleri* was studied. *Psychotria colorata* has no similar species within *Margaritopsis*, although some species share its capitate inflorescence structure. However, sterile material of *Psychotria* may be superficially similar to *Margaritopsis* rendering confusions not unlikely.

Contrary, it could be possible that the scarce reports of cyclotryptamine alkaloids in subg. *Heteropsychotria* and *Palicourea* resemble a similar case to the exclusive accumulation of iridoids found in a few species of the largely alkaloid producing clade *Palicourea* s.l. This could be true for *Psychotria glomerulata*, a species from which dimeric cyclotryptamine alkaloids were reported (Solis et al., 1997). It is the only cyclotryptamine accumulating species of subg. *Heteropsychotria*, for which the identity could be confirmed by checking the voucher specimen *Galdames et al.* 599, PMA88389. If a key enzyme in the strictosidine alkaloid biosynthesis may be inoperative due to mutation, a shift to cyclotryptamine alkaloid synthesis may be the result in several species.

7.2.6 *Notopleura* (Palicoureeae)

Naphthoquinone derivatives have been found within species of both major clades i.e. tribes of the *Psychotria* alliance (Fig. 13, 21). Within the Psychotrieae, psychorubin was extracted from *Psychotria asiatica* (sub syn. *P. rubra*, Hayashi et al., 1987). Within the Palicoureeae, such compounds have been found in *Psychotria stachyoides* (Pimenta et al., 2011) and in *Notopleura camponutans* (Jacobs et al., 2008; Solis et al., 1995). The present study adds *N. capacifolia* and *N. polyphlebia* to the list.

Harounoside was first reported from the invasive weed *Mitracarpus hirsutus* (L.) DC. (sub syn. *Mitracarpus scaber* Zucc., Harouna et al., 1995). It is only known from Rubiaceae and its occurrence in the *Psychotria* alliance is now reported for the first time. In HPLC-analysis of crude extracts from *N. capacifolia*, compounds with similar UV-spectra have been detected. Within the *Psychotria* alliance, the alkaloid 2-azaanthraquinone has only been isolated from two species of the genus *Notopleura*, which is in turn devoid of tryptamine iridoid alkaloids. This compound was obtained from *N. camponutans* by bioassay guided fractionation, indicating only the presence, but not the

quantity. Hence, neither specific biosynthetic nor ecological trends may be deduced. In *N. polyphlebia*, 2-azaanthraquinone was a minor compound compared to harounoside.

The chemosystematic significance of the occurrence of naphthoquinone derivatives is difficult to assess. They may be considered as ubiquitous compounds because of being found in species of both major clades i.e. tribes of the *Psychotria* alliance. Within the present study on Costa Rican species of the *Psychotria* alliance, *N. polyphlebia* was the only species, from where naphthoquinone derivatives were isolated. Interestingly, HPLC-chromatograms of most species of *Notopleura* showed very low concentrations of secondary metabolites and HPLC profiles of *N. anomothyrsa* yielded almost no visible compounds. These observations may be partially explained when the drying behaviour of the samples is considered. During the drying process, the freshly cut stem bark of some species conspicuously changed colour, becoming reddish or whitish, respectively, indicating oxidative breakdown or enzymatical reactions of secondary metabolites. These compounds may not be detectable after the reaction occurred, and as no fresh material was available, questions concerning the phytochemical composition of these species remain unanswered.

Although both thoroughly studied species of *Notopleura*, yielded naphthoquinone derivatives, it may be premature to assess their chemosystematic significance until more species have been studied. Whilst the occurrence of naphthoquinone derivatives may not necessarily be a chemosystematic marker for *Notopleura* spp., the absence of alkaloid accumulation is significant as more species have been found negative for alkaloids than positive for naphthoquinones. In any case, the phytochemical profile from *N. polyphlebia* differs from all of the other sampled species, by accumulating large amounts of naphthoquinone derivatives, which may be considered an alternate defence strategy of a species inhabiting moist microsites in rainforest understory.

In addition to naphthoquinones, a new flavanonol has been isolated: 5-O[β -D-apiose-(1 $\rightarrow$ 5 $\rightarrow$)]- β -D-glucose-4',7-di-O-methylaromadendrin. Although 4',7-di-O-methylaromadendrin 5-glycosides are found in unrelated families such as Podocarpaceae and Rubiaceae, they remain rare by means of total occurrence and have only been found in the genera *Podocarpus* (Rizvi & Rahman, 1974) and *Cephalanthus* (Lima & Polonsky, 1973). Unless more data on the diversity and distribution of flavonoids in the *Psychotria* alliance is available, the occurrence of the new flavonoid cannot be evaluated systematically.

7.2.7 *Palicourea* s.l. (Palicoureeae)

By studying a large number of species of *Palicourea* s.l. including *Psychotria* subg. *Heteropsychotria*, the relatively simple composition of plant extracts became evident. In HPLC profiles, extracts of most species are characterised by single or relatively few major peaks and alkaloids were detected in most species (see results, section 6, Tab. 6, Fig. 17-20 & 23). As already pointed out in the introduction 2.8.6 & 2.8.7, *Palicourea* is polyphyletic and derived several times from *Psychotria* subg. *Heteropsychotria* and both groups comprises a single clade that is well characterised by both morphological and DNA phylogenetic data (Andersson & Rova, 1999; Nepokroeff et al., 1999; Robbrecht & Manen, 2006, Taylor, 1996). As expected by their close relationships, no significant differences in accumulation tendencies towards tryptamine iridoid alkaloids could be found between both groups. Their merging is therefore fully supported by phytochemical data which characterises the *Palicourea* s.l. clade by accumulation of tryptamine iridoid alkaloids.

In contrast to the majority of alkaloids found in the Apocynaceae (e.g. *Catharanthus roseus* and *Rauvolfia serpentina*), Loganiaceae (*Strychnos* spp.) Nyssaceae (*Camptotheca acuminata*), or *Ophiorrhiza pumila* (Rubiaceae), alkaloids found in *Psychotria* subg. *Heteropsychotria* and *Palicourea* spp. tend to be of less derived structure i.e. are directly derived from strictosidine featuring a strictosidine backbone without major modifications (e.g. Berger et al., 2012; O'Connor & Maresh, 2006; Plagg, 2012; Porto et al., 2009).

7.2.8 *Rudgea* (Palicoureeae)

With recent reports on *Rudgea cornifolia*, compounds of the genus were studied for the first time, and a novel alstroline derivative, rudgeifoline was isolated (Schinnerl et al., 2012). Alstrolines contain a cyclotryptamine i.e. pyrrolidinoindoline core linked with two secologanine moieties in a tetracyclic system. They are therefore quoted cyclotryptamine iridoid or alstroline type alkaloids. They have recently been discovered in *Alstonia rostrata* (Apocynaceae, Cai et al., 2011) and were previously unknown within the Rubiaceae. In the same study, alstroline A was isolated from *Chassalia curviflora* var. *ophioxyloides* from Thailand.

Due to their tryptamine to secologanin ratio of 1:2 and their tetracyclic core, these compounds are exceptional within the Palicoureeae. They are structurally related to cyclotryptamine alkaloids, but differ by incorporation of an iridoid moiety. Strictosidine

and bahienosides with a 1:1 and 1:2 tryptamine to secologanine ratio, respectively, differ by their tricyclic core.

The genera *Chassalia* and *Rudgea* are closely related according to DNA phylogenetic data (Nepokroeff et al., 1999; Robbrecht & Manen, 2006) and their relationship is underlined by the occurrence of unusual alstroline derivatives, previously unknown in rubiaceous species (Schinnerl et al., 2012).

7.2.9 The polyphyletic genus *Cephaelis*

The genus *Cephaelis* was based on a congested to capitate inflorescence surrounded by conspicuous involucre bracts. *Cephaelis* has been shown to be a polyphyletic assemblage and all its species have initially been assigned to *Psychotria* (Steyermark, 1972). Some were later transferred to other genera such as *Carapichea*, *Notopleura* and *Palicourea* (Andersson, 2002a; Taylor, 2010). Species matching the concept of *Cephaelis* and their studied secondary metabolites are indicated in Tab. 8.

Tab. 8: Species matching the concept of *Cephaelis*. Their name under the genus, their currently accepted name and their secondary metabolite profile are listed.

Species	Synonym under <i>Cephaelis</i>	Secondary metabolites*
<i>Carapichea affinis</i>	<i>Cephaelis affinis</i> Standl.	dopamine iridoid alkloids
<i>Carapichea ipecacuanha</i>	<i>Cephaelis ipecacuanha</i> (Brot.) A. Rich.	dopamine iridoid alkloids
<i>Palicourea tetragona</i>	<i>Cephaelis tetragona</i> Donn. Sm.	iridoids
<i>Psychotria aubletiana</i>	<i>C. axillaris</i> Sw.	tryptamine iridoid alkaloids
<i>P. chiriquiensis</i>	<i>C. chiriquiensis</i> Standl.	tryptamine iridoid alkaloids
<i>P. elata</i>	<i>C. elata</i> Sw.	tryptamine iridoid alkaloid
<i>P. hispidula</i>		tryptamine iridoid alkaloid
<i>P. hoffmannseggiana</i>	<i>C. hoffmannseggiana</i> Willd. ex Roem. & Schult.	tryptamine iridoid alkaloid
<i>P. poeppigiana</i>	<i>C. tomentosa</i> (Aubl.) Vahl	iridoids

*detailed information on accumulated compounds may be found in Tab. 6

Among those, *Palicourea tetragona*, *Psychotria aubletiana*, *P. elata*, *P. chiriquiensis*, *P. hispidula*, *P. hoffmannseggiana* and *P. poeppigiana* are related species according to molecular phylogeny and morphological characters (e.g. Nepokroeff et al., 1999). All these species except *Palicourea tetragona* and *Psychotria poeppigiana* were found to contain tryptamine-iridoid alkaloids, as is the case for most of the species of *Psychotria* subg. *Heteropsychotria* and *Palicourea* (tab. 6). Despite their relation to the alkaloid-containing species, *Palicourea tetragona* and *Psychotria poeppigiana* are lacking alkaloids, but accumulate iridoids instead. *Carapichea affinis* and *C. ipecacuanha* contain tyrosine derived dopamine iridoid alkloids representing a completely different biosynthetic

pathway when compared to tryptamine iridoid alkaloids (Bernhard, 2012; Bernhard et al., 2011; Itoh et al., 1999, 2002; Lee, 2008; Wiegrebe et al., 1984).

Despite similarities in inflorescence morphology, the heterogeneous secondary metabolite profile of species previously classified as *Cephaelis* is a further indicator for the artificial concept of the genus *Cephaelis*. It correlates well with the heterogeneity of the genus, and supports current taxonomic views, not to maintain it as a separate genus.

7.3 Relevance for tribal classification

The tribe Psychotrieae s.l. was subdivided in two major groups i.e. tribes: Psychotrieae s.str. and Palicoureeae (Razafimandimbison et al., 2008; Robbrecht & Manen, 2006). Bremer & Eriksson (2009) followed a more conservative approach and preferred to maintain the tribe Psychotrieae s.l. until more data is available.

Considering the present data on secondary metabolite distribution (Tab. 6, Fig. 30 and 31), the fundamental phytochemical differentiation between both clades becomes obvious. Whereas in the Psychotrieae, all clades were lacking alkaloids but were uniformly characterised by the accumulation of polyphenolic compounds (condensed tannins, “quantitative defence”), these could not be found in the Palicoureeae. The latter group differs by the ability to produce a variety of nitrogen-containing compounds such as complex alkaloids (“qualitative defence”) that could not be detected in the Psychotrieae.

Different major biosynthetic trends between both tribes could be interpreted as differing strategies of chemical defence with putatively strong ecological impact. In the present work, they are considered to be relevant at the level of tribal classifications supporting the system of Robbrecht & Manen (2006).

7.3.1 *Ronabea* (Lasiantheae)

Species of the genus *Ronabea* have been excluded from the *Psychotria* alliance and are now placed in the tribe Lasiantheae. They differ by the sole accumulation of iridoids (Berger et al., 2011a).

7.4 Chemotaxonomic conclusions

Comparison of phytochemical data inferred from both HPLC-UV/VIS analysis and from literature surveys against clades based on morphology and DNA phylogenetic data revealed accumulation patterns typical for segregate genera and tribes of the *Psychotria* alliance. These accumulation patterns are based upon biosynthetically defined secondary metabolites (see Fig. 30 and 31). For a survey of studied species see Tab. 6. The strong correlation with current taxonomy within this alliance suggests that also other hitherto unstudied species would exhibit similar chemical profiles. This has been considered when results were mapped on a simplified molecular phylogeny based on ITS sequence data variation in Fig. 30 of which not all species have been studied in the present work.

In this tree, the major dichotomy is found between the tannin accumulating Psychotrieae and the largely alkaloid accumulating Palicoureeae. On basis of available data on the latter tribe, a genus specific ratio of secologanine, tryptophan derived tryptamine and tyrosine derived dopamine may be expected within compounds. Thus, *Carapichea* is characterized by dopamine-iridoid alkaloids (1:1 or 2:1 ratio), *Rudgea* and *Chassalia* by tryptamine-iridoid alkaloids of the alstroline-type (1:2 ratio), *Psychotria* subg. *Heteropsychotria* and *Palicourea* by tryptamine-iridoid alkaloids of the strictosidine type (1:1 and 1:2 ratio), and *Margaritopsis* by cyclotryptamine type alkaloids (1:0 ratio). Accumulation of naphthoquinones in *Notopleura* is considered in Fig. 30, but is not included in Fig. 31. Data on other genera are largely missing, and no systematic conclusion may be drawn at present. Groups of Fig. 30 and 31 and Tab. 6 have been discussed in detail in the generic treatments on the previous pages.

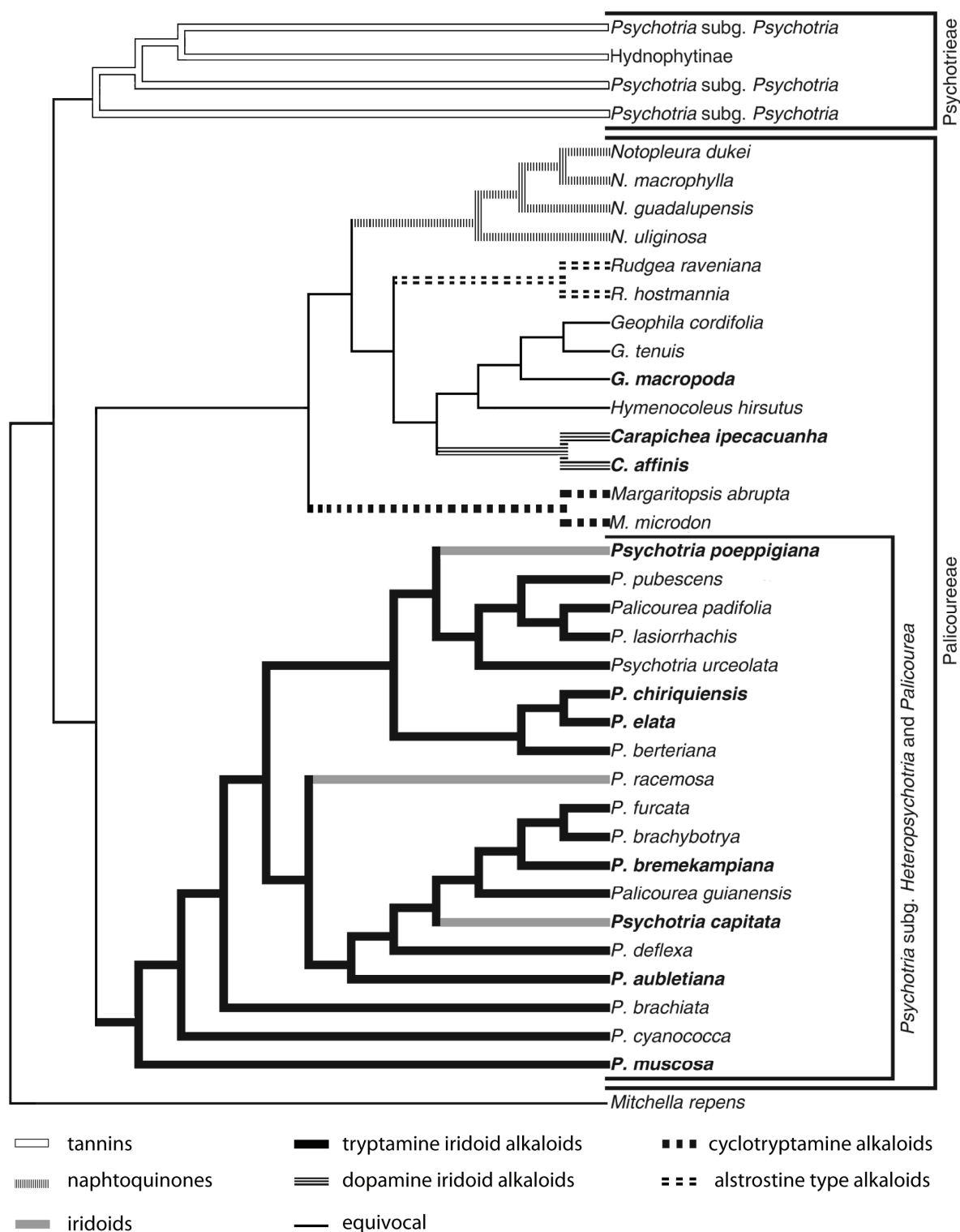


Fig. 30: Phytochemical differentiations between tribes and genera of the *Psychotria* alliance mapped on a simplified phylogenetic tree based on ITS sequence variation. Tree modified from Nepokroeff et al. (1999). Species previously classified as *Cephaelis* are given in bold letters. Unstudied species were included on base of expected similar accumulation tendencies in monophyletic lineages.

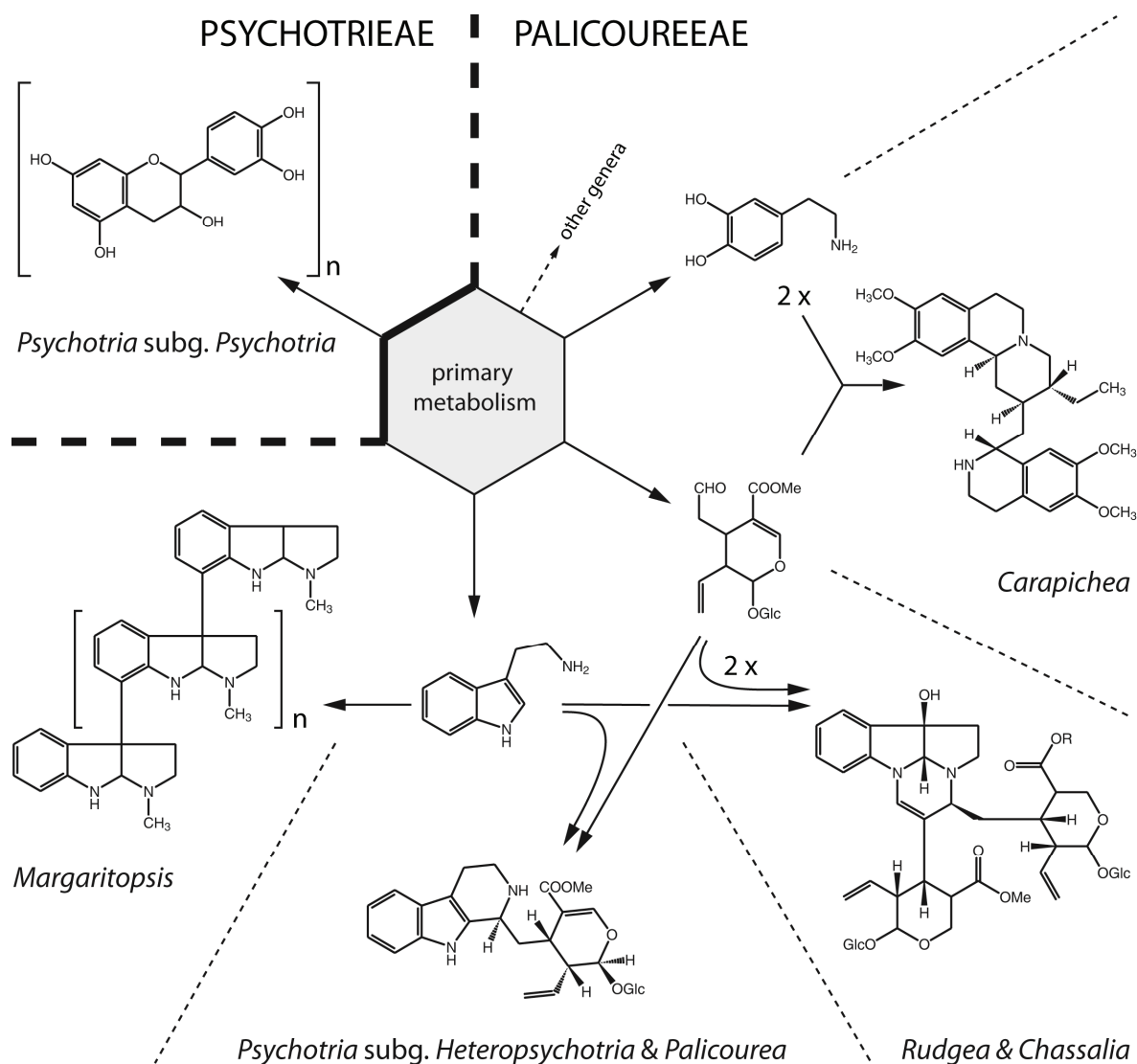


Fig. 31: Simplified biosynthetic scheme indicating major accumulation tendencies within the studied species of the *Psychotria* alliance. Note the compelling dichotomy between the tannin accumulating Psychotrieae and the largely alkaloid accumulating Palicoureeae.

Psychotrieae: *Psychotria* subg. *Psychotria*, condensed tannins indicated as catechin monomer. Palicoureeae: dopamine derived emetine shown for *Carapichea*, other genera show tryptophan derived alkaloids; *Rudgea* and *Chassalia*: alkaloids with alstrostine backbone; *Psychotria* subg. *Heteropsychotria* and *Palicourea*: strictosidine backbone; *Margaritopsis*: cyclotryptamine trimer.

8 Correlation with Plant-Herbivore Interactions

Plants have evolved a series of defence strategies against biotic pressure caused by herbivores and pathogens, towards facilitating survival and improving performance (e.g. Ehrlich & Raven, 1964; Fraenkel, 1959). Besides mechanical adaptations like spines, thorns or trichomes (Fernandes, 1994) and mutualistic interactions such as in myrmecophytes (Eck et al., 2001), chemical defence is generally considered a major strategy against environmental threats (e.g. Farrell & Mitter, 1998; but see Carmona et al., 2011). Defence compounds can act as repellents (Maia & Moore, 2011), toxins (Brem et al., 2002; Kaltenecker et al., 2003; Dobler et al., 2011) or plant digestibility reducing agents (Barbehenn & Constabel, 2011). Whilst toxic compounds are usually present and active in small amounts (“qualitative defence”), digestibility reducing compounds are present in larger quantities (“quantitative defence”), are usually less toxic but show growth reducing effect on herbivores.

According to their ability to tolerate different food plants, herbivores can be divided into a continuum of specialist and generalist herbivores. It is obvious that a generalist herbivore has evolved powerful mechanisms to counteract plant chemical defence in a way to improve food supply. Such an organism is *Spodoptera littoralis* (Noctuidae) being able to feed on a vast number of plant families. It is therefore suitable as a model organism in studying plant-herbivore interactions in a broad variety of plant species (e.g. Brem et al., 2002; Eck et al., 2001; Kaltenecker et al., 2003). Different strategies to cope with herbivores, as based upon chemical differentiation, may be regarded as a functional character. The relevance of the phytochemical diversification within the *Psychotria* alliance - accumulation of either condensed tannins as quantitative defence or of alkaloids as qualitative defence - is discussed in detail. Preliminary analyses and literature data on interesting single species are also addressed.

Tannins might be expected to be highly antioxidative and bioactive against insect pests (Barbehenn & Constabel, 2011). Preliminary analysis of species of different clades within the *Psychotria* alliance revealed significant differences regarding both, total phenolic (tannin) content and DPPH radical scavenging activity by at least ten fold (data not shown). Whereas total phenolic content was generally low within the Palicoureae, it was found significantly higher in species of the Psychotrieae. Radical scavenging activity, an effect obviously correlated with phenolic content, was similarly higher in species of the Psychotrieae. The data indicates the heterogeneity of species of the *Psychotria* alliance

towards total phenolic content and DPPH radical scavenging activity and is in good accordance with the HPLC- inferred phytochemical dichotomy between tannin accumulating species of the Psychotrieae (*P.* subg. *Psychotria*) and largely alkaloid accumulating species of the Palicoureeae (Tab. 6, Fig. 30 and 31). These trends towards accumulation of compounds may be interpreted as contrasting strategies in dealing with herbivores, which was tested in feeding experiments using *Spodoptera littoralis* larvae.

When incorporated in an artificial diet at 2.5 mg/g, crude extracts of *Psychotria* subg. *Psychotria* consistently reduced larval weight to less than 10 % relative to control within a week (data not shown). It is concluded that taxa of the Psychotrieae, which accumulate large amounts of tannins (a “quantitative” carbon based defence strategy“), displayed a significantly higher antifeedant activity when compared to species of the Palicoureeae, which are almost bare of activity. In a similar study, Eck et al. (2001) found tannin containing extracts of *Macaranga* spp. (Euphorbiaceae) exhibiting dose dependant growth rate reductions of *Spodoptera littoralis* larvae. More data will be available in the diploma thesis of Rupert Kainradl (Univ. Vienna; in prep.), who will perform continued studies on *Psychotria* extracts and their activity on *Spodoptera littoralis* larvae.

Specialist herbivores are rarely observed in *Psychotria*. These include *Trichaea* (Crambidae) leaf-rolling caterpillars and several species of eumolpine beetles (Chrysomelidae), apparently feeding on subg. *Psychotria* only (B. Sedo, personal comment). These groups of insects are thought to have specialised on subg. *Psychotria* because of their ability to adapt to tannin content, underlining the importance of secondary metabolite accumulation in shaping plant-herbivore interactions.

Interestingly, extracts of *Psychotria solitudinum* showed almost no detectable compounds in the HPLC profiles indicating that other unknown defense strategies must exist. This species is characterised by considerably thick leaves, drying stiff chartaceous (Burger & Taylor, 1993), and it was the only species in the range of collection, almost devoid of leave herbivory (A. Berger, own observation). Leaf toughness, caused by cell walls providing a mechanical barrier to herbivores, is generally considered a defensive trait in many tropical species (Lowman & Box, 1983; Read et al., 2009; Sagers & Coley, 1995). Toughness of leaves is relatively constant within a given species and it is even used as a diagnostic character in species determination within *Psychotria* s.l. (Burger & Taylor, 1993). Due to limited resources in competitive environments, one could expect a trade-off between mechanical and chemical defence (but see Read et al., 2009).

Species of the Palicoureeae synthesise putatively toxic alkaloid glycosides of various types (“qualitative” nitrogen based defence strategy), but they have not been found to accumulate tannins so far. Nevertheless, in our generalist *Spodoptera littoralis* model, strictosidine type alkaloid containing extracts of *Psychotria* subg. *Heteropsychotria* and *Palicourea* failed to display significant growth reducing activity (A. Berger, unpubl.). These preliminary results support a previous study, where neither strictosidine, nor its aglycone products displayed antifeeding activity against *Spodoptera exigua* larvae (Luijendijk et al., 1996).

Besides insect herbivores, mammals are a second major group devastating to plants (Coley & Barone, 1996). In mammals, toxicity was reported for several species of the genus *Palicourea*. *P. aenofusca* (Müll. Arg.) Standl., *P. corymbifera* (Müll. Arg.) Standl., *P. juruana* K. Krause, *P. grandiflora* (Kunth) Standl. and *P. marcgravii* A. St.-Hil. are said to exhibit bovine toxicity. *Palicourea*-induced intoxications of cattle are common in pastures on cut down forests and degraded land where food sources are scarce. Some of the species may be leading to sudden death when ingested (Górniak, 1986; Lee et al., 2012; Ribeiro de Assis et al., 2006; Tokarnia et al., 2000).

Palicourea corymbifera is significantly toxic to mice and causes symptoms related to the central nervous system (Ribeiro de Assis et al., 2006). Similarly, aqueous extracts of *P. marcgravii* displayed toxicity in rats leading to dopamine-related nervous excitability and convulsions (Górniak et al., 1990). In several species, toxicity is related to monofluoroacetic acid (Lee et al., 2012), but this compound could not be confirmed for *P. corymbifera* (Ribeiro de Assis et al., 2006). Moreover, it was concluded that the toxicity is related to other compounds present in the leaves. Due to the fact, that aqueous extracts displayed toxicity, a glycosidic nature of the toxins may be expected.

On basis of present results, accumulation of tryptamine-iridoid alkaloids (MIA) may be expected in further species of the genus *Palicourea*. The reportedly toxic species *P. corymbifera*, *P. grandiflora* and *P. marcgravii* have been subjected to HPLC-UV/VIS analysis and several tryptamine-iridoid alkaloids could be detected (Tab. 6). Compounds with related structure are known to interact with receptors of the central nervous system and may contribute to the toxicity of *Palicourea* spp. (Both et al., 2005, 2006).

Both chemical defense strategies described for the *Psychotria* alliance may also be viewed under the aspect of metabolic costs as limiting factor in plant defence (Herms & Mattson, 1992). This theory is based upon a trade-off between resource investment in growth, reproduction and defence, as both strategies compete for limited resources. High tannin content is negatively correlated with growth, suggesting metabolic costs of tannin accumulation in *Psychotria horizontalis* (Sagers & Coley, 1995). In tropical environments, metabolic cost of alkaloid formation may exceed those of tannins especially in the light of nitrogen poor laterite soils, but metabolic costs of alkaloid formation are not sufficiently well studied to answer the question. Simon et al. (2010) studied resource and growth costs of cyanogenic glycoside defence under varying nitrogen supply in *Eucalyptus cladocalyx* F. Muell. (Myrtaceae). Plants were found to allocate resources to carbon based defence under limited nitrogen supply. However, no significant correlation between secondary metabolite accumulation and several growth parameters was obvious, and it was concluded that the overall costs are too low to detect.

In the *Spodoptera littoralis* model, the beneficial role of tannins in plant defence, by far exceeds those of alkaloids. A beneficial shift in defence strategy may have occurred in subg. *Psychotria*, maximising antifeeding effects while reducing costs of defence through changing from nitrogen containing and resource expensive compounds to cheaper tannins exhibiting a broader effect on generalist herbivores.

9 Correlations with Ethnobotanical Aspects

9.1 Ethnopharmacology

Table 9 lists selected species of *Psychotria* and related genera and their application in ethnobotany and ethnomedicine. As it may be expected that bioactive compounds are responsible for the respective uses, these reports will be correlated with present results on distribution of secondary metabolites in the *Psychotria* alliance. It is assumed that chemical profiles are similar in related taxa, which allows to hypothesise the presence of possible bioactive compounds even in those species not yet studied.

Tab. 9: Species of *Psychotria* and related genera and their application in ethnobotany and ethnomedicine of various tribes of tropical regions.

species	Indication & usage	used in/by *	literature
<i>Chassalia curviflora</i>	Malaria, ringworms. for washing wound	Traditional Vietnamese Medicine	Khánh, 1996
<i>Geophila repens</i>	Fungal infections	Ketchwas	Schultes, 1985
<i>Notopleura anomothyrsa</i>	Fever	Teribes of Bocas del Toro, Panama	Gupta et al., 2005
<i>Palicourea angustifolia</i>	Rheumatism	Region Sibundoy	Schultes & Raffauf, 1990
<i>Palicourea buntingii</i>	Shortness of breath	Region of Putumayo	Schultes & Raffauf, 1990
<i>Palicourea condensata</i>	Fish Poison	Region of Putumayo	Schultes & Raffauf, 1990
<i>Palicourea corymbifera</i>	Coughs and chest ailments	Desano Indians	Schultes, 1985
<i>Palicourea crocea</i>	Antirheumatic	Kubeo Indians	Schultes & Raffauf, 1990
<i>Palicourea grandiflora</i>	Rat poison	Upper Río Negro	Schultes, 1985
<i>Palicourea grandifolia</i>	Fish poison	Kofán Indians	Schultes, 1985
<i>Palicourea guianensis</i>	Stomach-ache, vermifuge, styptic and coagulant	Indians of the Río Vaupés & Miritiparaná, Kubeo Indians	Schultes, 1985; Schultes & Raffauf, 1990
<i>Palicourea lasiantha</i>	Diarrhoea, emetic effects, minor fish poison, inducing delayed birth	Region of Leticia and Mocoa	Schultes, 1985; Schultes & Raffauf, 1990
<i>Palicourea macrobotrys</i>	Fish poison	Karijona Indians	Schultes & Raffauf, 1990
<i>Palicourea nitidella</i>	Rodenticide	Region of Río Guainía	Schultes, 1985
<i>Palicourea subspicata</i>	Fish Poison	Region of Putumayo	Schultes, 1985
<i>Palicourea tetraphylla</i>	Fish poison	Tikuna Indians	Schultes, 1985
<i>Palicourea triphylla</i>	Fish Poison	Region of Río Apaporis	Schultes, 1985
<i>Palicourea virens</i>	Skin disease, fungal infections	Bora Indians	Schultes & Raffauf, 1990
<i>Palicourea acuminata</i> (Studied under <i>Psychotria acuminata</i>)	Nervous disorders, insomnia and headache, against problems with urinating, also considered poisonous	Natives of Belize; Cuna Indians, Peru; Leticia area and Río Apaporis	Arvigo & Balick, 1993; Duke & Vasquez, 1994; Schultes, 1985
<i>Psychotria adenophylla</i>	Breast pains	Traditional Vietnamese Medicine	Khánh, 1996
<i>Psychotria alba</i>	Lumbago, Depurative	San Martin Quechuas, Peru	Sanz-Biset et al. 2009
<i>Psychotria asiatica</i> (syn.	Throat inflammation,	Traditional	Giang et al., 2007; Khánh,

Correlations with Ethnobotanical Aspects

<i>P. reevesii</i> , <i>P. rubra</i>)	dysentery and rheumatic fever, treating scalding, wounds and snakebites	Vietnamese Medicine	1996
<i>Psychotria brachiata</i>	Problems in breathing	Tikuna Indians	Schultes, 1985
<i>Psychotria capensis</i>	Emetic, gastric complaints	Zulu, Xhosa and Sotho	McGaw et al., 2000
<i>Psychotria capitata</i>	Severe colds	Karapana Indians of Río Apaporis	Schultes, 1985
<i>Psychotria carthagenensis</i>	Depurative, against rheumatism	San Martin Quechuas, Peru	Sanz-Biset et al., 2009
<i>Psychotria colorata</i>	pain	Amazonian Caboclos, Brazil	Elisabetsky & Castilhos, 1990
<i>Psychotria deflexa</i>	against fever	Cuna Indians, Peru	Duke & Vasquez, 1994
<i>Psychotria denmsinervia</i>	Lumbago	Baka pygmies, Cameroon	Betti, 2004
<i>Psychotria egensis</i>	Emetic	Yukuna Indians of the Río Miritipará	Schultes, 1985
<i>Psychotria emetica</i>	Chicken pox, fever	Teribes of Bocas del Toro, Panama	Gupta et al., 2005
<i>Psychotria ernestii</i>	Depurated	San Martin Quechuas, Peru	Sanz-Biset et al. 2009
<i>Psychotria flavida</i>	Wound healing	Tribal people of Tirunelveli hills, India	Ayyanar & Ignacimuthu, 2009
<i>Psychotria flaviflora</i>	Treating burns	Natives of La Pedrera	Schultes & Raffauf, 1990
<i>Psychotria gracilentia</i>	For clear visions	Andoke Indians	Schultes & Raffauf, 1990
<i>Psychotria horizontalis</i>	Ayahuasca preparation	Peru	Duke & Vasquez, 1994
<i>Psychotria humboldtiana</i>	Reduce swellings of feet	Tikuna Indians	Schultes, 1985
<i>Psychotria marginata</i>	Sore parts, in ayahuasca preparation	Cuna Indians, Peru	Duke & Vasquez, 1994
<i>Psychotria medusula</i>	Pain of burns, herpes sores	Karijona Indians	Schultes, 1985
<i>Psychotria montana</i>	Bacillary dysentery and to wash wounds	Traditional Vietnamese Medicine	Khánh, 1996
<i>Psychotria nervosa</i>	Dysentery	Region of Mocoa	Schultes, 1985
<i>Psychotria officinalis</i>	Fish Poison	Region of Leticia, Putumayo and Soratama, Colombia	Schultes, 1985
<i>Psychotria pilosa</i>	Vomits, fever, strong diarrhoea, cramps, chills	Yanesha Indians, Peru	Valadeau et al., 2010
<i>Psychotria poeppigiana</i>	Pulmonary ailments, haemorrhoids, strong pain in the back, uterine haemorrhage, vomits, fever, lack of appetite; as antitussive	Region of Río Cauca, Kabuyari Indians, Colombia; Yanesha, Chami and Wayäpi Indians, Peru	Duke & Vasquez, 1994; Schultes, 1985; Schultes & Raffauf, 1990; Valadeau et al., 2010
<i>Psychotria psychotriifolia</i>	Fever	Teribes of Bocas del Toro	Gupta et al., 2005
<i>Psychotria racemosa</i>	Rat poison	Region of Putumayo	Schultes, 1985
<i>Psychotria rufescens</i>	Dysentery	Region of Mocoa	Schultes, 1985
<i>Psychotria siamica</i>	As tonic	Mien people, Thailand	Panyaphu et al., 2011
<i>Psychotria stenostachya</i>	Ayahuasca preparation	Peru	Duke & Vasquez, 1994
<i>Psychotria tenuicaulis</i>	Uterine haemorrhage	Yanesha Indians, Peru	Valadeau et al., 2010
<i>Psychotria tenuifolia</i>	Washing wounds, swellings and rashes, to facilitating healing of sore spots and ulcers	Natives of Belize	Arvigo & Balick, 1993
<i>Psychotria viridis</i>	Healing rituals	Widely applied among various tribes	Rivier & Lindgren, 1972; Schultes, 1985
<i>Psychotria zevallosii</i>	Added to tobacco	Region of Putumayo	Schultes, 1985

*If not stated otherwise, the region of usage is located in the northwestern Amazon, Colombia

9.1.1 *Psychotria* subg. *Psychotria*

Within the present study, species of subg. *Psychotria* have been shown to accumulate large amounts of polyphenolic compounds i.e. tannins (Tab. 6, Fig. 22-24), a substance class with widely accepted biological activity such as antioxidant, protein precipitating and crosslinking activity (Barbehenn & Constabel, 2011; Khanbabaee & van Ree, 2001).

Putatively tannin containing species of subg. *Psychotria* are widely used in traditional medicine of tropical America, Africa and Asia (Arvigo & Balick, 1993; Ayyanar & Ignacimuthu, 2009; Betti, 2004; Duke & Vasquez, 1994; Khánh, 1996; Schultes, 1985; Schultes & Raffauf, 1990). Interestingly, most species are reported as being applied topically in context of skin infections or wound healing: *P. marginata* is taken as a hot leaf decoction to wash sore parts (Duke & Vasquez, 1994). *P. montana* is used against bacillary dysentery and to wash wounds (Khánh, 1996). Leaves of *P. asiatica* are used externally to cure wounds, internally for the treatment of throat inflammation, dysentery and rheumatic fever (reported under syn. *P. reevesii*; Giang et al., 2007) and for treating scalding, wounds and snakebites (studied under syn. *P. rubra*; Khánh, 1996). Roots of *P. flavida* are topically applied for treating wounds (Ayyanar & Ignacimuthu, 2009). *P. nervosa* (reported under its syn. *P. rufescens*) is a reputed cure for dysentery (Schultes, 1985; Schultes & Raffauf, 1990). Leaves of *P. tenuifolia* are used for washing wounds, swellings and rashes, and a poultice is applied topically to facilitating healing of sore spots and ulcers (Arvigo & Balick, 1993). Antibacterial activity was additionally shown for extracts of single species e.g. *Psychotria capensis* and *P. microlabastra* (McGaw et al., 2000; Khan et al, 2001).

The activity of tannins on a given pathogen's proteins and enzymes may be responsible for their usage in treating topical infections or wounds, inhibiting bacterial surface proteins and harmful extracellular enzymes.

9.1.2 *Palicourea* s.l.

A large number of species of *Palicourea* s.l., including species of *Psychotria* subg. *Heteropsychotria*, are used in traditional medicine (e.g. Arvigo & Balick, 1993; Schultes, 1985; Schultes & Raffauf, 1990). Whereas species of subg. *Psychotria* have been shown to uniformly accumulate polyphenolic compounds, metabolites of subg. *Heteropsychotria* and *Palicourea* are structurally diverse alkaloids (Tab. 6). Therefore, only two species treated in the present work will be discussed in detail.

An extract of *Palicourea acuminata* is applied in treating nervous disorders, insomnia and headache (Arvigo & Balick, 1993). This species accumulates tryptamine iridoid alkaloids of various types (Fig. 17). Their structure is evidently related to the neurotransmitter serotonin (5-hydroxytryptamine). The effects of these alkaloids on the central nervous system were studied in a mouse model and psychollatine (also known as umbellatine) was reported to act as a 5HT_{2A/C} receptor modulator (Both et al., 2005). It was also suggested to act via involvement of NMDA glutamate receptors (Both et al., 2006). Because of their similarities in the indolic backbone, all tryptamine-iridoid alkaloids related to strictosidine are likely to bind to serotonin receptors effecting the serotonergic system. Psychollatine differs from most alkaloids of *P. acuminata* only in its iridoid moiety, whereas its tryptamine derived alkaloid moiety, the likely site of importance in receptor binding, is identical. In a similar manner to psychollatine, compounds of *P. acuminata* could affect the serotonergic or glutamate receptor system. However, these effects need to be proven in appropriate model systems.

Psychotria poeppigiana, a species accumulating the iridoid asperuloside (Tab. 6), is often referred to as being an effective treatment of pulmonary ailments in both Columbia and Peru. It is also used as an antitussive (Duke & Vasquez, 1994; Schultes, 1985; Schultes & Raffauf, 1990). It is furthermore reported to relief pains in the back, which may be assigned to rheumatic disease (Valadeau et al., 2010). Asperuloside, the main compounds present in leaves of *Psychotria poeppigiana*, possesses antirheumatic activity, which might be related to its application in pain relieving (Li et al., 2006). The apparently widespread use of *Psychotria poeppigiana* in treating respiratory ailments led expect some degree of activity, however no studies explaining those applications have been conducted. Iridoids show a broad range of bioactivity (Dinda et al., 2007a, 2007b, 2009, 2011). Among these, antibacterial, anti-inflammatory and antiviral effects of iridoids could be of some importance in treating respiratory ailments.

9.2 Ayahuasca preparation

N,N-dimethyltryptamine (DMT) is one of the active principles of the indigenous hallucinogenic beverage ayahuasca, which is prepared and used by several tribes in the Amazon Basin and adjacent areas of South America (e.g. Luna, 1986; Schultes 1972, Rivier & Lindgren, 1972). Together with stem bark of the vine *Banisteriopsis caapi* (Spruce ex Griseb.) C. V. Morton (Malpighiaceae), DMT-containing leaves of *Psychotria viridis* Ruiz & Pav. are decocted and used to perform rituals in order to induce

hallucinations for religious and medicinal purpose (e.g. McKenna et al., 1984; Rivier & Lindgren, 1972; Schultes, 1986).

P. viridis contains the well known hallucinogenic substance *N,N*-dimethyltryptamine (DMT, Fig. 4) which, when orally consumed, is not active by itself. DMT is structurally related to the neurotransmitter serotonin and thus selectively binds on 5-HT_{2A} and 5-HT_{2C} receptors in the central nervous system acting as agonist (Smith et al., 1998). *B. caapi* contains the β -carboline type alkaloids harmine, harmaline and tetrahydroharmine as active agents (e.g. McKenna et al., 1984; Rivier & Lindgren, 1972; Schultes, 1986). These β -carbolines are potent inhibitors of human detoxifying enzyme monoamine oxidase A (MAO-A) thus preventing orally ingested DMT from oxidative deamination before inducing its activity at the central nervous system (Buckholtz & Boggan, 1977; McKenna et al., 1984). Consequently, only the synergetic combination of effects from these two species may cause the desired effect.

Since the discovery of ayahuasca by Richard Spruce (Spruce, 1873, 1908; Villavicencio, 1858), its knowledge has nowadays spread outside of traditional communities, and has experienced a recent popularisation (e.g. Halpern, 2004; Tupper, 2008). Consequently, it has raised the interest in plant species containing the psychoactive alkaloid DMT especially in the light of drug tourism and possible therapeutic potential (McKenna, 2004; Riba & Barbanoj, 2005; Santos et al. 2007; Winkelmann, 2005). An increasing amount of information about different plant species assumed to have psychoactive properties is publicly available in the internet (e.g. Trout, 2004, <http://www.erowid.com>) but mostly lacking proper scientific support which may result in possible intoxications through misidentification of species and/or usage of yet unstudied species. Although several studies report the use of different species of *Psychotria* in indigenous communities (e.g. Rivier & Lindgren, 1972; Sanz-Biset et al., 2009), the chemical background of the use of these plants remains largely unstudied.

In addition to the widely used *Psychotria viridis*, several other species were reported to be used in ayahuasca rituals by indigenous communities. The Culina Indians use an unidentified species of *Psychotria* as well as *P. carthagenensis* in ayahuasca recipes (Rivier & Lindgren, 1972). The San Martin Quechuas use *P. alba*, *P. carthagenensis* and *P. ernestii* as additives (Sanz-Biset et al., 2009). Species additionally mentioned to be incorporated into ayahuasca are *P. alba* (Duke & Vasquez, 1994), *P. carthagenensis* (Rivier & Lindgren, 1972; Schultes & Raffauf, 1990), *P. horizontalis* (Duke & Vasquez,

1994), *P. marginata* (Duke & Vasquez, 1994), *P. nervosa* (Ted, 2002; www.botanicalspirit.com), *P. poeppigiana* (Schultes & Hofmann, 1980; Schultes et al., 2001; Rättsch, 2007 relying on personal comment from Robert Montgomery), “*P. psychotriaefolia*” (Schultes & Hofmann, 1980), *P. psychotriifolia* (Der Marderosian et al, 1970) and *P. stenostachya* (Duke & Vasquez, 1994).

Following the chemotaxonomic approach, species of the *Psychotria* alliance were screened for the occurrence of alkaloids and especially DMT in light of ethnopharmacology and possible misuse of species in non traditional usage. However, alkaloid content could be reported for most species of subg. *Heteropsychotria* and *Palicourea* (Tab. 6), no DMT could be detected in any sampled species. In contrast to literature data and information available from internet sources, the present study indicates *Psychotria viridis* as the only species of *Psychotria* s.l. known to contain the hallucinogenic principle DMT.

Misreports may be explained by several facts, primarily by confusion about plant names. Thus, plants may have been studied under a species name, which is not presently recognised, but should be regarded as synonym of *Psychotria viridis*. This is the case for *P. psychotriaefolia* nom. ined., an unpublished synonym of *P. viridis*. Additionally, simple misidentification has led to the accidental report of the presence of this compound in another species, when in fact *P. viridis* was used. Rivier & Lindgren (1972) reported the occurrence of a substantial amount of DMT in *Psychotria carthagenensis*. Later, different samples of *P. carthagenensis* were shown to be devoid of alkaloids (Leal & Elisabetsky, 1996) highlighting problems of misidentifications of *Psychotria* species. A similar case of misidentification is found in plants identified as *P. psychotriifolia* (Der Marderosian et al, 1970) when accidentally *P. viridis* was studied as clarified by Schultes (1986).

If identification of a species is correct, it is possible that different populations of a given species accumulate different compounds under varying conditions (“chemoraces”). Material for sampled species of the present study was almost exclusively collected in Costa Rica whereas most ethnobotanical studies were carried out in the Amazon Basin. It is furthermore possible that several plants are used as additives merely for superstitious or magical-religious reasons without a definite biological function, or that they may contain compounds of yet unknown structure and function. It may be speculated that the polyphenolic content of species from subgen. *Psychotria* compensates for some of the side effects of Ayahuasca, due to their antioxidant and radical scavenging activity.

At present, *P. viridis* remains the only known source of psychoactive DMT in the alkaloid-rich *Psychotria* alliance and even within the family of Rubiaceae. Considering the vast number of known species of *Psychotria* and related genera, it remains likely that ethnobotanical and phytochemical surveys may reveal further species whether ethnobotanically used or not, containing the psychoactive compound DMT.

10 Summary

81 species of *Psychotria* and related genera of the *Psychotria* alliance have been analysed for their phytochemical composition employing the extraction of compounds and their comparative HPLC-UV/VIS analysis. Major classes of compounds detected comprise cyclotryptamine iridoid alkaloids, tryptamine iridoid alkaloids, dopamine iridoid alkaloids and tannins. These phytochemical differentiations were interpreted in relation to literature and current taxonomy. Species of the Psychotrieae (*Psychotria* subg. *Psychotria*) showed high total phenolic content, good radical scavenging activity and were found to accumulate condensed tannins only, which could not be detected in the Palicoureeae. By contrast, species within the Palicoureeae were dominated by alkaloids derived from various pathways and their different combinations of building blocks turned out to be genus-specific. Furthermore, infrageneric groups were recognised based upon the distribution of several alkaloids within *Palicourea* s.l. In preliminary bioassays against larvae of the generalist herbivore *Spodoptera littoralis*, good activity correlated with the distribution of tannins, indicating the ecological relevance of phytochemical differentiations.

Compounds encountered within the Palicoureeae, may be grouped in tyrosine derived dopamine and tryptophan derived tryptamine alkaloids, and by the ratio of these building blocks to the iridoid secologanine. Compounds of *Carapichea* are dopamine iridoid alkaloids with a ratio of 1:1 or 1:2. Alkaloids of other genera within the tribe were formed via tryptamine. With the exception of bahienosides, all alkaloids of *Palicourea* s.l. (including *Psychotria* subg. *Heteropsychotria*) showed a tryptamine to secologanine ratio of 1:1. Cyclotryptamine alkaloids from the genus *Margaritopsis* are defined by a ratio of 1:0. Alstrostines are cyclotryptamine iridoid alkaloids characterised by a 1:2 ratio and are found in both *Chassalia* and *Rudgea*. *Notopleura* differs in accumulating naphthoquinones.

Present results largely confirm the current taxonomic views within *Psychotria* and its relatives. Based upon the detected chemical structures and literature data, a biosynthetic scheme has been developed and correlated with distribution of major compounds in this group. All of the studied plant groups exhibit consistent chemical profiles regarding major accumulation tendencies. Hence, it might be expected that related, yet unstudied species, synthesize secondary metabolites from the same biosynthetic family, indicating predictive value in these cases. Confirmation of the hypothetical biosynthetic trends and distributions of the present study, and the ecological impact of secondary metabolite differentiation, warrants further and enhanced investigation.

Deutsche Zusammenfassung

Im Rahmen der vorliegenden Diplomarbeit wurden 81 Arten der Verwandtschaft um die Gattung *Psychotria* phytochemisch untersucht. Eine Vielzahl von Verbindungen wurde isoliert und deren Verbreitungsmuster wurden durch vergleichende HPLC-UV/VIS Messungen aufgeklärt. Die Verbreitung der gefundenen Stoffklassen wurde in Relation zur Literatur gesetzt und innerhalb der Gattungen und Triben auf ihre systematische Bedeutung hin analysiert. Während sich Arten der Psychotrieae (*Psychotria* subg. *Psychotria*) durch Akkumulation von kondensierten Gerbstoffen, sogenannten Tanninen in Kombination mit erhöhtem Phenolgehalt und starker radikalabfangender Wirkung kennzeichnen, fehlen diese in Arten der verschiedenen Gattungen der Palicoureeae. Im Gegensatz dazu akkumulieren Arten der Palicoureeae Alkaloide verschiedener Biosynthesewege. Um die ökologische Bedeutung der unterschiedlichen Stoffgruppen zu untersuchen, wurden Fraßversuche mit Raupen der Kleinen Baumwolleneule (*Spodoptera littoralis*) durchgeführt. Gerbstoffe beinhaltende Arten zeigten die höchste Aktivität, was die ökologische Bedeutung der phytochemischen Differenzierung deutlich macht.

Nach vorliegenden Ergebnissen zu den Palicoureeae, lassen sich die Gattungen *Carapichea*, *Chassalia*, *Notopleura*, *Margaritopsis*, *Palicourea* s.l. (inklusive *Psychotria* subg. *Heteropsychotria*) und *Rudgea* jeweils durch das Auftreten bestimmter Stoffklassen charakterisieren. Als gattungsspezifisch werden Alkaloide und das Verhältnis deren einzelner Bausteine Dopamin, Tryptamin und Secologanin erachtet. Demnach ist die Gattung *Carapichea* durch Dopamin Iridoid Alkaloide mit einem Dopamin-Secologanin-Verhältnis von 1:1 bis 1:2 charakterisiert, wohingegen sich die Alkaloide aller anderen Gattungen aus Tryptamin und Secologanin aufbauen. Bei *Margaritopsis* geschieht dies in einem Verhältnis von 1:0, bei *Palicourea* s.l. in der Regel im Verhältnis 1:1, bei *Rudgea* und *Chassalia* im Verhältnis von 1:2. Arten der Gattung *Notopleura* unterscheidet sich durch das Vorkommen von Naphthoquinonen. Basierend auf den Ergebnissen, wurde ein konsistentes Biosyntheschema entwickelt, welches die Vorhersage der chemischen Profile nicht untersuchter Arten ermöglichen soll. Weiters lassen sich innerhalb der Gattung *Palicourea* s.l. einzelne Artengruppen durch bestimmte Tryptamin-Iridoid Alkaloide charakterisieren.

Obwohl die vorliegende Arbeit klare Biosynthesetrends aufzeigt und diese mit der derzeit gängigen Taxonomie der Verwandtschaft gut übereinstimmen, sollten die vorliegenden Ergebnisse in weiterführenden Studien mit größerer Stichprobenzahl bestätigt und deren ökologische Bedeutung noch tiefgreifender untersucht werden.

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13 Appendix

13.1 Corrigenda

Confusions about plant origin and compound names of previous studies came up in the context of the present work and needs to be clarified. Relevant comments and corrections of previous diploma thesis are pointed out in the following.

Confusion of specimens: Bernhardt et al. (2011) cite the specimen HG 207084 (WU) as voucher for their study on *Carapichea affinis*. However, there is no such specimen, neither in the stated herbarium nor in the private collection of the authors. The orthographically similar HG 2207084 (WU 0044553) is a specimen of *Psychotria mortoniana*, in which these compounds are not present (Tab. 6). Instead, specimens representing *C. affinis* were collected by H. Greger under the numbers HG 1907081 (WU 0044534), -82 (WU 0044537 & -38), HG 2007085 (WU 0044536), -86 (WU 0044533 & 0044535). Compounds reported for *C. affinis* are detectable in these accessions (Tab. 6) and actually represent the authentic voucher specimens of Bernhardt et al. (2011).

Confusion of names: Fasshuber (2011) reports asperuloside from “*Psychotria remis*”. However, no such species exists, and the compound was in fact isolated from *Ronabea emetica* (Berger et al., 2011a).

Reanalysis of *Psychotria buchtienii*: Plagg (2012) reports strictosidinic acid as well as strictosidine for *Psychotria buchtienii*. This could not be confirmed upon reanalysis of the same material which did not show strictosidine. Instead, the HPLC-chromatograms of crude extracts of *P. buchtienii* as well as those of pure “strictosidine” shown in Plagg (2012) did not show a peak at characteristic retention time nor with characteristic chromophore of strictosidine. Instead, different retention times and discrepancies in the UV/VIS spectra of the compound indicated as strictosidine point to the sole presence of strictosidinic acid. It is concluded, that strictosidine is not present in this species (Tab. 6).

13.2 List of taxa and voucher specimens

Table 10 gives an alphabetical list of all taxa treated in the present survey. Additional informations on taxonomic authors, collectors of the plant material and voucher specimens, approximate location including country, province and region or nearby city, and herbarium numbers is included.

Tab. 10: Alphabetical list of all surveyed collections including additional information.

taxon	collector	origin	herbarium nr.
<i>Carapichea affinis</i> (Standl.) L. Andersson	Bernhard, M. & Greger, H. HG 1907081	Costa Rica. Puntarenas. La Gamba.	WU 0044534
<i>Carapichea affinis</i> (Standl.) L. Andersson	Bernhard, M. & Greger, H. HG 1907082	Costa Rica. Puntarenas. La Gamba.	WU 0044537-8
<i>Carapichea affinis</i> (Standl.) L. Andersson	Bernhard, M. & Greger, H. HG 2007085	Costa Rica. Puntarenas. La Gamba.	WU 0044536
<i>Carapichea affinis</i> (Standl.) L. Andersson	Bernhard, M. & Greger, H. HG 2007086	Costa Rica. Puntarenas. La Gamba.	WU 0044533, 0044536
<i>Carapichea ipecacuanha</i> (Brot.) L. Andersson	Anonymous collector s.n.	Drug collection.	WU 0067845
<i>Chassalia curviflora</i> var. <i>ophioxylodes</i> (Wall.) Deb & Berger, A. AB 3107111		Thailand. Chiang Mai.	WU 0064562
<i>Chassalia curviflora</i> var. <i>ophioxylodes</i> (Wall.) Deb & Greger, H. HG 1073		Thailand. Chanthaburi.	WU 0045615-6
<i>Chassalia curviflora</i> var. <i>ophioxylodes</i> (Wall.) Deb & Greger, H. HG 1074		Thailand. Trat.	WU 0045614
<i>Geophila repens</i> (L.) I. M. Johnst.	Berger, A. AB 28021003	Costa Rica. Puntarenas. Corcovado.	WU 0067347, MO (1/2)
<i>Notopleura aggregata</i> (Standl.) C. M. Taylor	Berger, A. AB 07031001	Costa Rica. Puntarenas. Virgen de	WU 0067348, MO (2/2)
<i>Notopleura anomothyrsa</i> (K. Schum. & Donn. Sm.) C. M. Taylor	Berger, A. AB 07021001	Costa Rica. Limón. Hitoy Cerere.	WU 0067350, MO (2/2)
<i>Notopleura anomothyrsa</i> (K. Schum. & Donn. Sm.) C. M. Taylor	Berger, A. AB 13021006	Costa Rica. Alajuela. Arenal.	WU 0067349
<i>Notopleura capacifolia</i> (Dwyer) C. M. Taylor	Berger, A. AB 05031003	Costa Rica. Puntarenas. Virgen de	WU 0067351, MO (1/2)
<i>Notopleura capacifolia</i> (Dwyer) C. M. Taylor	Bernhard, M. & Greger, H. HG 2907082	Costa Rica. Puntarenas. La Gamba.	WU 0044587-8
<i>Notopleura polyphlebia</i> (Donn. Sm.) C. M. Taylor	Berger, A. AB 05031001	Costa Rica. Puntarenas. Virgen de	WU 0067355
<i>Notopleura polyphlebia</i> (Donn. Sm.) C. M. Taylor	Berger, A. AB 19021001	Costa Rica. Puntarenas. La Gamba.	WU 0067353-4, MO (3/3)
<i>Notopleura polyphlebia</i> (Donn. Sm.) C. M. Taylor	Bernhard, M. & Greger, H. HG 2907084	Costa Rica. Puntarenas. La Gamba.	WU 0044559
<i>Notopleura tolimensis</i> (Wernham) C. M. Taylor	Berger, A. AB 13031003	Costa Rica. Cartago. La Carpintera.	WU 0067356
<i>Notopleura uliginosa</i> (Sw.) Bremek.	Berger, A. AB 05031002	Costa Rica. Puntarenas. Virgen de	WU 0067357
<i>Notopleura uliginosa</i> (Sw.) Bremek.	Berger, A. AB 13021007	Costa Rica. Alajuela. Arenal.	WU 0067358, MO (1/2)
<i>Palicourea acuminata</i> (Benth.) Borhidi	Berger, A. AB 16021011	Costa Rica. Puntarenas. La Gamba.	WU 0067430
<i>Palicourea acuminata</i> (Benth.) Borhidi	Sedio, B. PYAC	Panama. Panamá. Barro Colorado	

taxon	collector	origin	herbarium nr.
<i>Palicourea acuminata</i> (Benth.) Borhidi	Bernhard, M. & Greger, H.	Costa Rica. Puntarenas. La Gamba.	WU 0044525, 0044528
<i>Palicourea acuminata</i> (Benth.) Borhidi	Bernhard, M. & Greger, H.	Costa Rica. Puntarenas. La Gamba.	WU 0044524
<i>Palicourea acuminata</i> (Benth.) Borhidi	Bernhard, M. & Greger, H.	Costa Rica. Puntarenas. La Gamba.	WU 0044523, 0044527
<i>Palicourea acuminata</i> (Benth.) Borhidi	Bernhard, M. & Greger, H.	Costa Rica. Puntarenas. La Gamba.	WU 0044530-1
<i>Palicourea acuminata</i> (Benth.) Borhidi	Bernhard, M. & Greger, H.	Costa Rica. Puntarenas. La Gamba.	WU 0044529
<i>Palicourea acuminata</i> (Benth.) Borhidi	Bernhard, M. & Greger, H.	Costa Rica. Puntarenas. La Gamba.	WU 0044526, 0044532
<i>Palicourea adusta</i> Standl.	Berger, A. AB 10021002	Costa Rica. Heredia. Barva.	WU 0067360-3, MO (5/5)
<i>Palicourea adusta</i> Standl.	Berger, A. AB 11021001	Costa Rica. Alajuela. Poás.	WU 0067359, MO (2/2)
<i>Palicourea corymbifera</i> (Müll. Arg.)	Till, W. 3066	Peru. Huancavelica. Cerros Sira.	WU 0067340-1
<i>Palicourea crocea</i> (Sw.) Roem. &	Berger, A. AB 06021004	Costa Rica. Limón. Hitoy Cerere.	WU 0067364, MO (1/2)
<i>Palicourea crocea</i> (Sw.) Roem. &	Berger, A. AB 12021002	Costa Rica. Alajuela. Los Chiles, Rio	WU 0067365-6, MO (3/3)
<i>Palicourea discolor</i> K. Krause	Berger, A. AB 01021006	Costa Rica. Cartago. Tapanti.	WU 0067367-9, MO (4/4)
<i>Palicourea garciae</i> Standl.	Berger, A. AB 01021001	Costa Rica. Cartago. Tapanti.	WU 0067370-2, MO (3/4)
<i>Palicourea grandiflora</i> (Kunth) Standl.	Ehrendorfer, F. 74922-1-4	Brazil. Amazonas. Near Manaus.	WU 0067344
<i>Palicourea guianensis</i> Aubl.	Berger, A. AB 05021004	Costa Rica. Limón. Cahuita.	WU 0067373
<i>Palicourea guianensis</i> Aubl.	Berger, A. AB 16021010	Costa Rica. Puntarenas. La Gamba.	WU 0067374
<i>Palicourea guianensis</i> Aubl.	Sedio, B. PALG	Panama. Panamá. Barro Colorado	
<i>Palicourea lasiorrhachis</i> Oerst.	Berger, A. AB 01021005	Costa Rica. Cartago. Tapanti.	WU 0067378, MO (2/2)
<i>Palicourea lasiorrhachis</i> Oerst.	Berger, A. AB 02021003	Costa Rica. Cartago. Monte Sky.	WU 0067375
<i>Palicourea lasiorrhachis</i> Oerst.	Berger, A. AB 02021004	Costa Rica. Cartago. Monte Sky.	WU 0067376-7, MO (3/3)
<i>Palicourea marcgravii</i> A. St.-Hil.	Bang, M. Plantae Bolivianae 495	Bolivia. La Paz, Yungas.	WU 0067343
<i>Palicourea padifolia</i> (Willd. ex Roem. & Schult.) C. M. Taylor & Lorence	Berger, A. AB 02021001	Costa Rica. Cartago. Monte Sky.	WU 0067379-82, MO (3/5)
<i>Palicourea tetragona</i> (Donn. Sm.) C. M. Taylor & Lorence	Berger, A. AB 16021001	Costa Rica. Puntarenas. La Gamba.	WU 67383-4, MO (3/3)
<i>Palicourea tetragona</i> (Donn. Sm.) C. M. Taylor & Lorence	Bernhard, M. & Greger, H.	Costa Rica. Puntarenas. La Gamba.	WU 0044578-9
<i>Palicourea tetragona</i> (Donn. Sm.) C. M. Taylor & Lorence	Bernhard, M. & Greger, H.	Costa Rica. Puntarenas. La Gamba.	WU 0044580

taxon	collector	origin	herbarium nr.
<i>Psychotria adenophylla</i> Wall.	Greger, H. HG 1067	Thailand. Trat.	WU 0067391-2
<i>Psychotria adenophylla</i> Wall.	Greger, H. HG 1071	Thailand. Trat.	WU 0067390
<i>Psychotria aubletiana</i> Steyerl.	Berger, A. AB 13031002	Costa Rica. Cartago. La Carpintera.	WU 0067431
<i>Psychotria bahiensis</i> DC.	Irwin, H.S. et al. 30770	Brazil. Bahia. Serra da Agua de	W 1978-0010335
<i>Psychotria barbiflora</i> DC.	Amaral, I.L. 112489	Brazil. Pará.	W-2006-0017004
<i>Psychotria barbiflora</i> DC.	Wulfschlägel, 243.	Suriname. Marowijne.	W-1889-0074043
<i>Psychotria brachiata</i> Sw.	Berger, A. AB 13021001	Costa Rica. Alajuela. La Fortuna.	WU 0067432-3, MO (2/3)
<i>Psychotria brachiata</i> Sw.	Sedio, B. PYb1	Panama. Panamá. Barro Colorado	
<i>Psychotria buchtienii</i> (H. Winkl.) Standl.	Berger, A. AB 21021002	Costa Rica. Puntarenas. La Gamba.	WU 0067434-6, MO (4/4)
<i>Psychotria buchtienii</i> (H. Winkl.) Standl.	Bernhard, M. & Greger, H. HG 19070811	Costa Rica. Puntarenas. La Gamba.	WU 0044572
<i>Psychotria buchtienii</i> (H. Winkl.) Standl.	Bernhard, M. & Greger, H. HG 2007088	Costa Rica. Puntarenas. La Gamba.	WU 0044570-1
<i>Psychotria buchtienii</i> (H. Winkl.) Standl.	Bernhard, M. & Greger, H. HG 24070810	Costa Rica. Puntarenas. La Gamba.	WU 0044566-7
<i>Psychotria buchtienii</i> (H. Winkl.) Standl.	Bernhard, M. & Greger, H. HG 24070813	Costa Rica. Puntarenas. La Gamba.	WU 0044564-5
<i>Psychotria buchtienii</i> (H. Winkl.) Standl.	Bernhard, M. & Greger, H. HG 2407088	Costa Rica. Puntarenas. La Gamba.	WU 0044561-3, 0044569
<i>Psychotria buchtienii</i> (H. Winkl.) Standl.	Bernhard, M. & Greger, H. HG 2607087	Costa Rica. Puntarenas. La Gamba.	WU 0044560, 0044568
<i>Psychotria campylopoda</i> Standl.	Boom, B.M. 9428	Venezuela. Bolívar. Cerro	W 2007-0021889
<i>Psychotria capensis</i> (Eckl.) Vatke	Greger, H. HG 1066	United Kingdom, CUBG	WU 0044541
<i>Psychotria capitata</i> Ruiz & Pav.	Berger, A. AB 16021009	Costa Rica. Puntarenas. La Gamba.	WU 0067439
<i>Psychotria capitata</i> Ruiz & Pav.	Berger, A. AB 22021003	Costa Rica. Puntarenas. La Gamba.	WU 0067437-8
<i>Psychotria capitata</i> Ruiz & Pav.	Sedio, B. PYC1	Panama. Panamá. Barro Colorado	
<i>Psychotria capitata</i> Ruiz & Pav.	Bernhard, M. & Greger, H. HG 19070805	Costa Rica. Puntarenas. La Gamba.	WU 0044520
<i>Psychotria capitata</i> Ruiz & Pav.	Bernhard, M. & Greger, H. HG 19070806	Costa Rica. Puntarenas. La Gamba.	WU 0044521
<i>Psychotria capitata</i> Ruiz & Pav.	Bernhard, M. & Greger, H. HG 19070812	Costa Rica. Puntarenas. La Gamba.	WU 0044522
<i>Psychotria carthagenensis</i> Jacq.	living collection	Cult. HBV	living collection
<i>Psychotria</i> cf. <i>sylvivaga</i> Standl.	Berger, A. AB 01021002-A	Costa Rica. Cartago. Tapanti.	WU 0067426, MO (1/2)
<i>Psychotria chagrensis</i> Standl.	Berger, A. AB 05021003	Costa Rica. Limón. Cahuita.	WU 0067396-7, MO (3/3)
<i>Psychotria chagrensis</i> Standl.	Berger, A. AB 16021006	Costa Rica. Puntarenas. La Gamba.	WU 0067394
<i>Psychotria chagrensis</i> Standl.	Sedio, B. PYCH	Panama. Panamá. Barro Colorado	
<i>Psychotria chagrensis</i> Standl.	Bernhard, M. & Greger, H. HG 2207088	Costa Rica. Puntarenas. La Gamba.	WU 0044576

taxon	collector	origin	herbarium nr.
<i>Psychotria chagrensis</i> Standl.	Bernhard, M. & Greger, H. HG 2307082	Costa Rica. Puntarenas. La Gamba.	WU 0044577
<i>Psychotria chagrensis</i> Standl.	Bernhard, M. & Greger, H. HG 2607082	Costa Rica. Puntarenas. La Gamba.	WU 0044573-4
<i>Psychotria chagrensis</i> Standl.	Bernhard, M. & Greger, H. HG 2607084	Costa Rica. Puntarenas. La Gamba.	WU 0044575
<i>Psychotria chiriquiensis</i> (Standl.) C. M. Taylor	Berger, A. AB 02021008	Costa Rica. Cartago. Monte Sky.	WU 0067440-2, MO (2/4)
<i>Psychotria convergens</i> C. M. Taylor	Berger, A. AB 10021001	Costa Rica. Heredia. Barva.	WU 0067398, MO (2/2)
<i>Psychotria cooperi</i> Standl.	Berger, A. AB 22021002	Costa Rica. Puntarenas. La Gamba.	WU 0067443, MO (2/2)
<i>Psychotria cooperi</i> Standl.	Bernhard, M. & Greger, H. HG 3007085	Costa Rica. Puntarenas. La Gamba.	WU 0044539-40
<i>Psychotria cornigera</i> Benth.	Zimmermann, G. 36	Venezuela. Amazonas. La	WU 0063826
<i>Psychotria cuspidulata</i> (K. Krause) Standl.	Palacios, W. & Neill, D. 739	Ecuador. Pastaza. Río Curaray	WU 0063825
<i>Psychotria cyanococca</i> Seem. ex Dombroin	Berger, A. AB 06021001	Costa Rica. Limón. Hitoy Cerere.	WU 0067445-6, MO (3/3)
<i>Psychotria cyanococca</i> Seem. ex Dombroin	Berger, A. AB 13021002	Costa Rica. Alajuela. Arenal.	WU 0067449
<i>Psychotria cyanococca</i> Seem. ex Dombroin	Berger, A. AB 15021006	Costa Rica. Puntarenas. La Gamba.	WU 0067449
<i>Psychotria cyanococca</i> Seem. ex Dombroin	Berger, A. AB 16021003	Costa Rica. Puntarenas. La Gamba.	WU 0067448, MO (2/2)
<i>Psychotria cyanococca</i> Seem. ex Dombroin	Sedio, B. PYPI	Panama. Panamá. Barro Colorado	WU 0044584
<i>Psychotria deflexa</i> DC.	Bernhard, M. & Greger, H. HG 2207081	Costa Rica. Puntarenas. La Gamba.	WU 0044582
<i>Psychotria deflexa</i> DC.	Bernhard, M. & Greger, H. HG 22070810	Costa Rica. Puntarenas. La Gamba.	WU 0044582
<i>Psychotria deflexa</i> DC.	Bernhard, M. & Greger, H. HG 2207082	Costa Rica. Puntarenas. La Gamba.	WU 0044583
<i>Psychotria deflexa</i> DC.	Bernhard, M. & Greger, H. HG 24070817	Costa Rica. Puntarenas. La Gamba.	WU 0044581
<i>Psychotria elata</i> (Sw.) Hammel	Berger, A. AB 01021007	Costa Rica. Cartago. Tapanti.	WU 067450-1
<i>Psychotria elata</i> (Sw.) Hammel	Bernhard, M. & Greger, H. HG 2407081	Costa Rica. Puntarenas. La Gamba.	WU 0044517-9
<i>Psychotria goldmanii</i> Standl.	Berger, A. AB 02021009	Costa Rica. Cartago. Monte Sky.	WU 0067452-4, MO (4/4)
<i>Psychotria gracilentia</i> Müll. Arg.	Sedio, B. PYB2-2	Panama. Panamá. Barro Colorado	
<i>Psychotria gracilentia</i> Müll. Arg.	Cid Ferreira, C.A. 4083	Brazil. Amazonas. Uaxini.	W 2008-0022287
<i>Psychotria gracilentia</i> Müll. Arg.	Teixeira, L.O.A. et al. 942	Brazil. Rondônia. Humaitá-Lá brea.	W 2009-0001433
<i>Psychotria graciliflora</i> Benth.	Berger, A. AB 13021003	Costa Rica. Alajuela. Arenal.	WU 0067399-01, MO (4/4)
<i>Psychotria grandis</i> Sw.	Sedio, B. PYG3	Panama. Panamá. Barro Colorado	
<i>Psychotria hawaiiensis</i> (A. Gray) Fosberg	MK-890731-2/2b	USA. Hawaii. Big Island.	WU 0066071

taxon	collector	origin	herbarium nr.
<i>Psychotria hexandra</i> H. Mann	MK-920805-1/2	USA. Hawaii. Kauai.	WU 0066070
<i>Psychotria hispidula</i> Standl. ex Steyerl.	Berger, A. AB 16021008	Costa Rica. Puntarenas. La Gamba.	WU 0067455-7, MO (3/4)
<i>Psychotria hispidula</i> Standl. ex Steyerl.	MK-300786-1-10	Costa Rica. Cartago. Turrialba.	WU 0066074-5
<i>Psychotria hoffmannseggiana</i> (Willd. ex Roem. & Schumacher)	Berger, A. AB 28021001	Costa Rica. Puntarenas. Corcovado.	WU 0067458, MO (2/2)
<i>Psychotria hoffmannseggiana</i> (Willd. ex Roem. & Schumacher)	Sedio, B. PYFU	Panama. Panamá. Barro Colorado	
<i>Psychotria horizontalis</i> Sw.	Berger, A. AB 28021004	Costa Rica. Puntarenas. Corcovado.	WU 0067402
<i>Psychotria horizontalis</i> Sw.	Sedio, B. PYHO-1	Panama. Panamá. Barro Colorado	
<i>Psychotria kirkii</i> Hiern.	Berger, A. & Gilli, C. s.n.	Ethiopia. Oromiya. Near Bitata.	ETH
<i>Psychotria ligustrifolia</i> (Northr.) Millsp.	living collection	HBV	living collection
<i>Psychotria limonensis</i> K. Krause	Berger, A. AB 05021002	Costa Rica. Limón. Cahuita.	WU 0067403-4, MO (3/3)
<i>Psychotria limonensis</i> K. Krause	Sedio, B. PYLI-1	Panama. Panamá. Barro Colorado	
<i>Psychotria manausensis</i> Steyerl.	Lieras, E. & Monteiro, O.P. P19669	Brazil. Amazonas. Manaus-Porta	W 2009-0001999
<i>Psychotria marginata</i> Sw.	Berger, A. AB 06021005	Costa Rica. Limón. Hitoy Cerere.	WU 0067406-8, MO (3/4)
<i>Psychotria marginata</i> Sw.	Berger, A. AB 16021004	Costa Rica. Puntarenas. La Gamba.	WU 0067405, MO (2/2)
<i>Psychotria marginata</i> Sw.	Sedio, B. PYMA	Panama. Panamá. Barro Colorado	WU 0044589, 90
<i>Psychotria marginata</i> Sw.	Weissenhofer, A. HG 3007083	Costa Rica. Puntarenas. La Gamba.	
<i>Psychotria mariniana</i> (Cham. & Schltdl.) Fosberg	MK-920907-1/5	USA. Hawaii.	WU 0066072
<i>Psychotria micrantha</i> Kunth	Berger, A. AB 15021005	Costa Rica. Puntarenas. La Gamba.	WU 0067409
<i>Psychotria micrantha</i> Kunth	Sedio, B. PYMI	Panama. Panamá. Barro Colorado	
<i>Psychotria mertoniana</i> Standl.	Bernhard, M. & Greger, H. HG 1907084	Costa Rica. Puntarenas. La Gamba.	WU 0044551-2
<i>Psychotria mertoniana</i> Standl.	Bernhard, M. & Greger, H. HG 2207083/8	Costa Rica. Puntarenas. La Gamba.	WU 0044549
<i>Psychotria mertoniana</i> Standl.	Bernhard, M. & Greger, H. HG 2207084	Costa Rica. Puntarenas. La Gamba.	WU 0044553
<i>Psychotria mertoniana</i> Standl.	Bernhard, M. & Greger, H. HG 2207084/8	Costa Rica. Puntarenas. La Gamba.	WU 0044550
<i>Psychotria mertoniana</i> Standl.	Bernhard, M. & Greger, H. HG 2407087	Costa Rica. Puntarenas. La Gamba.	WU 0044554-6
<i>Psychotria nervosa</i> Sw.	Berger, A. AB 05021001	Costa Rica. Limón. Cahuita.	WU 0067411-2, MO (2/3)
<i>Psychotria nervosa</i> Sw.	Berger, A. AB 05021008	Costa Rica. Limón. Cahuita.	WU 0067410
<i>Psychotria officinalis</i> (Aubl.) Raeusch. ex Sandwith	Zerny, H. s.n.	Brazil. Pará. Near Belem.	W 1959-0010741
<i>Psychotria orophila</i> E. M. A. Petit	Berger, A. & Gilli, C. s.n.	Ethiopia. Oromiya. Kibre Mengist.	ETH
<i>Psychotria orosiana</i> Standl.	Berger, A. AB 01021004	Costa Rica. Cartago. Tapanti.	WU 0067413-5, MO (4/4)

taxon	collector	origin	herbarium nr.
<i>Psychotria parvifolia</i> Benth.	Berger, A. AB 02021005	Costa Rica. Cartago. Monte Sky.	WU 0067416-7, MO (3/3)
<i>Psychotria pilosa</i> Ruiz & Pav.	Berger, A. AB 15021003	Costa Rica. Puntarenas. La Gamba.	WU 0067468, MO (2/2)
<i>Psychotria pilosa</i> Ruiz & Pav.	Bernhard, M. & Greger, H. HG 2607086	Costa Rica. Puntarenas. La Gamba.	WU 0044557-8
<i>Psychotria platypoda</i> DC.	Nee, M.H. 34829	Brazil. Rondônia. Guajara Mirim.	W 2009-0012556
<i>Psychotria poeppigiana</i> Müll. Arg.	Berger, A. AB 16021012	Costa Rica. Puntarenas. La Gamba.	WU 0067459
<i>Psychotria poeppigiana</i> Müll. Arg.	Sedio, B. PYPO	Panama. Panamá. Barro Colorado Island.	
<i>Psychotria poeppigiana</i> Müll. Arg.	Bernhard, M. & Greger, H. HG 2601081	Costa Rica. Puntarenas. La Gamba.	WU 0044514-5
<i>Psychotria poeppigiana</i> Müll. Arg.	Bernhard, M. & Greger, H. HG 3007082	Costa Rica. Puntarenas. La Gamba.	WU 0044516
<i>Psychotria psychotriifolia</i> (Seem.) Standl.	Sedio, B. PYPS	Panama. Panamá. Barro Colorado Island.	
<i>Psychotria pubescens</i> Sw.	Sedio, B. PYPJ-1	Panama. Panamá. Barro Colorado Island.	
<i>Psychotria quinqueradiata</i> Pol.	Berger, A. AB 12021001	Costa Rica. Alajuela. Los Chiles.	WU 0067419-20, MO (3/3)
<i>Psychotria quinqueradiata</i> Pol.	Berger, A. AB 13031001	Costa Rica. Cartago. La Carpintera.	WU 0067418
<i>Psychotria racemosa</i> Rich.	Berger, A. AB 16021002	Costa Rica. Puntarenas. La Gamba.	WU 0067460
<i>Psychotria racemosa</i> Rich.	Sedio, B. PYRA	Panama. Panamá. Barro Colorado Island.	
<i>Psychotria racemosa</i> Rich.	Will, S. 04/08/99/80	Costa Rica. Puntarenas. La Gamba.	WU 0024780
<i>Psychotria serpens</i> L.	Greger, H. HG 1068	Thailand, Chanthaburi	WU 0067421-2
<i>Psychotria serpens</i> L.	Greger, H. HG 1069	Thailand, Chanthaburi	WU 0067423-4
<i>Psychotria solitudinum</i> Standl.	Berger, A. AB 15021002	Costa Rica. Puntarenas. La Gamba.	WU 0067461-2
<i>Psychotria stenostachya</i> Standl.	Boeke, J.D. & Ramirez, M. 1296	Peru. San Martín. Madre Mía.	W 2011-0002243
<i>Psychotria stenostachya</i> Standl.	Teixeira, L.O.A. et al. 456	Brazil. Rondônia. Ariqueemes.	W 2009-0001464
<i>Psychotria suerrensii</i> Donn. Sm.	Berger, A. AB 16021005	Costa Rica. Puntarenas. La Gamba.	WU 0067463-4
<i>Psychotria sylvivaga</i> Standl.	Berger, A. AB 01021002-B	Costa Rica. Cartago. Tapanti.	WU 0067427, MO (2/2)
<i>Psychotria sylvivaga</i> Standl.	Berger, A. AB 01021003	Costa Rica. Cartago. Tapanti.	WU 0067425, MO (1/2)
<i>Psychotria sylvivaga</i> Standl.	Berger, A. AB 01021008	Costa Rica. Cartago. Tapanti.	WU 0067428-9, MO (3/3)
<i>Psychotria tenuifolia</i> Sw.	Berger, A. AB 06021002	Costa Rica. Limón. Hitoy Cerere.	WU 0067385-9, MO (5/6)
<i>Psychotria tenuifolia</i> Sw.	Sedio, B. PYTE	Panama. Panamá. Barro Colorado Island.	
<i>Psychotria tsakiana</i> C. M. Taylor	Berger, A. AB 06021003	Costa Rica. Limón. Hitoy Cerere.	WU 0067465-7, MO (2/4)
<i>Psychotria tsakiana</i> C. M. Taylor	Bernhard, M. & Greger, H. HG 31070811	Costa Rica. Puntarenas. La Gamba.	WU 0044548
<i>Psychotria tsakiana</i> C. M. Taylor	Bernhard, M. & Greger, H. HG 31070812	Costa Rica. Puntarenas. La Gamba.	WU 0044547

taxon	collector	origin	herbarium nr.
<i>Psychotria verschuerenii</i> De Wild.	living collection	Cult. HBV	living collection
<i>Psychotria viridis</i> Ruiz & Pav.	living collection	Cult. HBV	living collection
<i>Psychotria zeylanica</i> Sohmer	Schinnerl, s.n.	Sri Lanka. Central. Hakgala Mountains.	WU-0066051
<i>Ronabea emetica</i> (L. f.) A. Rich.	Berger, A. AB 28021002	Costa Rica. Puntarenas. Corcovado.	WU 0046606-10
<i>Ronabea latifolia</i> Aubl.	Berger, A. AB 16021007	Costa Rica. Puntarenas. La Gamba.	WU 0060915-8
<i>Ronabea latifolia</i> Aubl.	Bernhard, M. & Greger, H. HG 19070810	Costa Rica. Puntarenas. La Gamba.	WU 0044585-6
<i>Rudgea cornifolia</i> (Kunth) Standl.	Berger, A. AB 21021001	Costa Rica. Puntarenas. La Gamba.	WU 0063824, MO (2/2)

13.3 Images of voucher specimens

Given the complexity of the *Psychotria* alliance in combination with difficulties in determination of species, misidentified voucher specimens are a critical issue. In most cases, voucher specimens are difficult to locate or obtain from herbaria and are therefore not readily available for confirmation or revision of species identity. To leave no doubt on the identity of the species of the present study, digitalised images of one voucher specimen per taxon are listed alphabetically in Fig. 32-103 of the appendix.

Psychotria ligustrifolia, *P. verschuereni* and *P. viridis* are grown at the Botanical Garden, University of Vienna (HBV) from whence leaf samples were obtained. These species could not be documented with vouchers because only small plants were available and should not be damaged or killed. Vouchers for the Ethiopian collections of *P. kirkii* and *P. orophila* are deposited at ETH, where no equipment for digitalisation is available. The sample of powdered *Carapichea ipecacuanha* was obtained from a drug collection and is not suitable for imaging.

Images of voucher specimens were obtained from the virtual herbarium database JACQ at <http://herbarium.univie.ac.at/database/search.php>. Images were cropped and processed using the Photoshop Levels Tool.



Fig. 32: *Carapichea affinis* (Standl.) L. Andersson: Bernhard, M. & Greger, H. HG 2007086, fig. ex WU 0044533.



Fig. 33: *Chassalia curviflora* (Wall.) Thwaites: Greger, H. HG 1074, fig. ex WU 0045614.



Fig. 34: *Geophila repens* (L.) I. M. Johnst.: Berger, A. AB 28021003, fig. ex WU 0067347.



Fig. 35: *Notopleura aggregata* (Standl.) C. M. Taylor: Berger, A. AB 07031001, fig. ex WU 0067348.



Fig. 36: *Notopleura anomothyrsa* (K. Schum. & Donn. Sm.) C. M. Taylor: Berger, A. AB 13021006, fig. ex WU 0067349.



Fig. 37: *Notopleura capacifolia* (Dwyer) C. M. Taylor: Berger, A. AB 05031003, fig. ex WU 0067352.

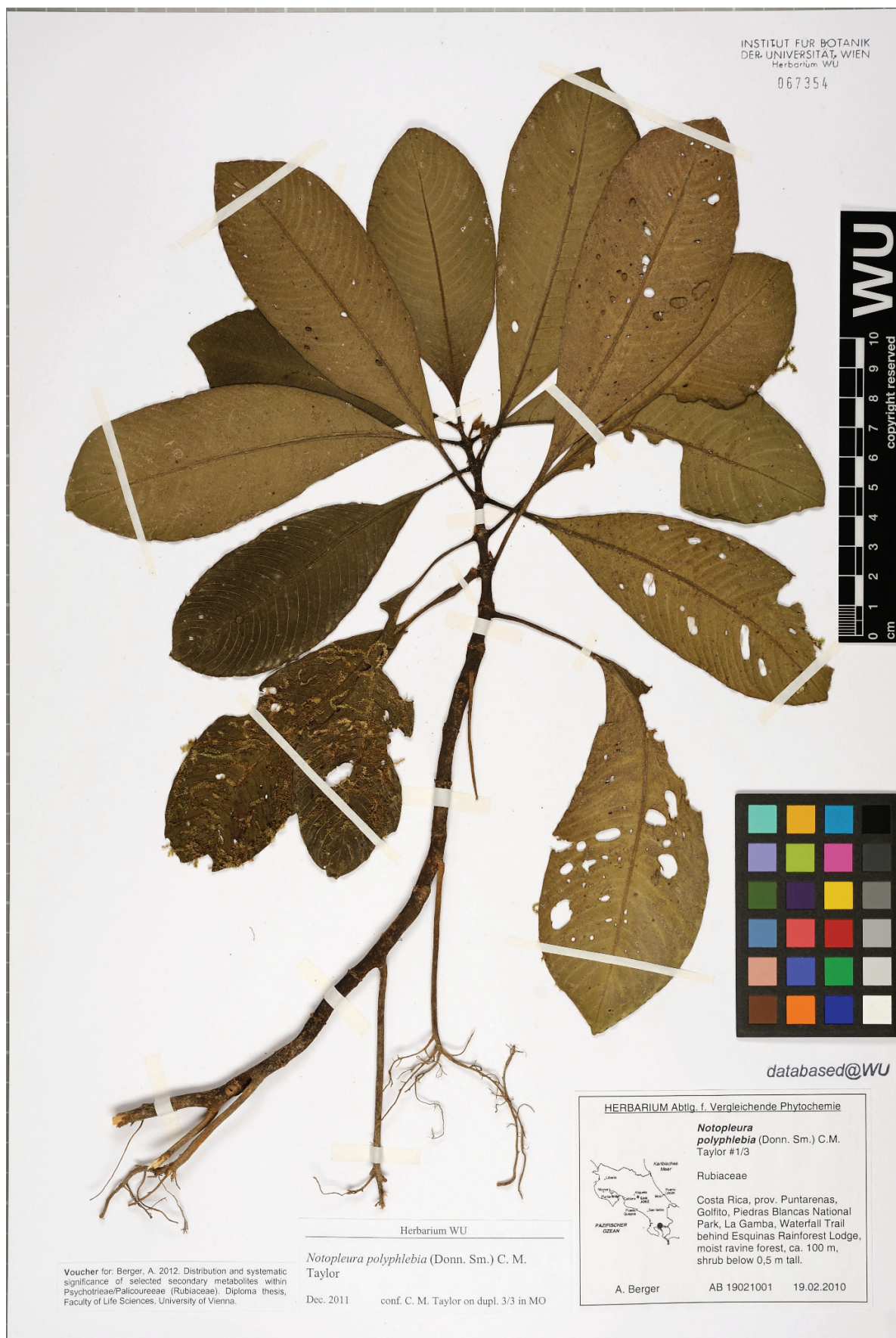


Fig. 38: *Notopleura polyphlebia* (Donn. Sm.) C. M. Taylor: Berger, A. AB 19021001, fig. ex WU 0067354.



Fig. 39: *Notopleura tolimensis* (Wernham) C. M. Taylor: Berger,A. AB 13031003, fig. ex WU 0067356.



Fig. 40: *Notopleura uliginosa* (Sw.) Bremek.: Berger, A. AB 05031002, fig. ex WU 0067357.



Fig. 41: *Palicourea acuminata* (Benth.) Borhidi: Bernhard, M. & Greger, H. HG 2407082, fig. ex WU 0044532.



Fig. 42: *Palicourea adusta* Standl.: Berger, A. AB 10021002, fig. ex WU 0067361.



Fig. 43: *Palicourea corymbifera* (Müll. Arg.) Standl.: Till, W. 3066, fig. ex WU 0067341.



Fig. 44: *Palicourea crocea* (Sw.) Roem. & Schult.: Berger, A. AB 12021002, fig. ex WU 0067366.



Fig. 45: *Palicourea discolor* K. Krause: Berger, A. AB 01021006, fig. ex WU 0067369.



Fig. 46: *Palicourea garciae* Standl.: Berger, A. AB 01021001, fig. ex WU 0067370.



Fig. 47: *Palicourea grandiflora* (Kunth) Standl.: Ehrendorfer, F. 74922-1-4, fig. ex WU 0067344.



Fig. 48: *Palicourea guianensis* Aubl.: Berger, A. AB 16021010, fig. ex WU 0067374.



Fig. 49: *Palicourea lasiorrhachis* Oerst.: Berger, A. AB 01021005, fig. ex WU 0067378.



Fig. 50: *Palicourea padifolia* (Willd. ex Roem. & Schult.) C. M. Taylor & Lorence: Berger, A. AB 02021001, fig. ex WU 0067381.



Fig. 51: *Palicourea tetragona* (Donn. Sm.) C. M. Taylor & Lorence: Berger, A. AB 16021001, fig. ex WU 0067384.



Fig. 52: *Psychotria adenophylla* Wall.: Greger, H. HG 1067, fig. ex WU 0067393.



Fig. 53: *Psychotria aubletiana* Steyerl.: Berger, A. AB 13031002, fig. ex WU 0067431.



Fig. 54: *Psychotria bahiensis* DC.: Irwin, H.S. et al. 30770, fig. ex W 1978-0010335.



Fig. 55: *Psychotria barbiflora* DC.: Amaral, I.L. 112489, fig. ex W 2006-0017004.

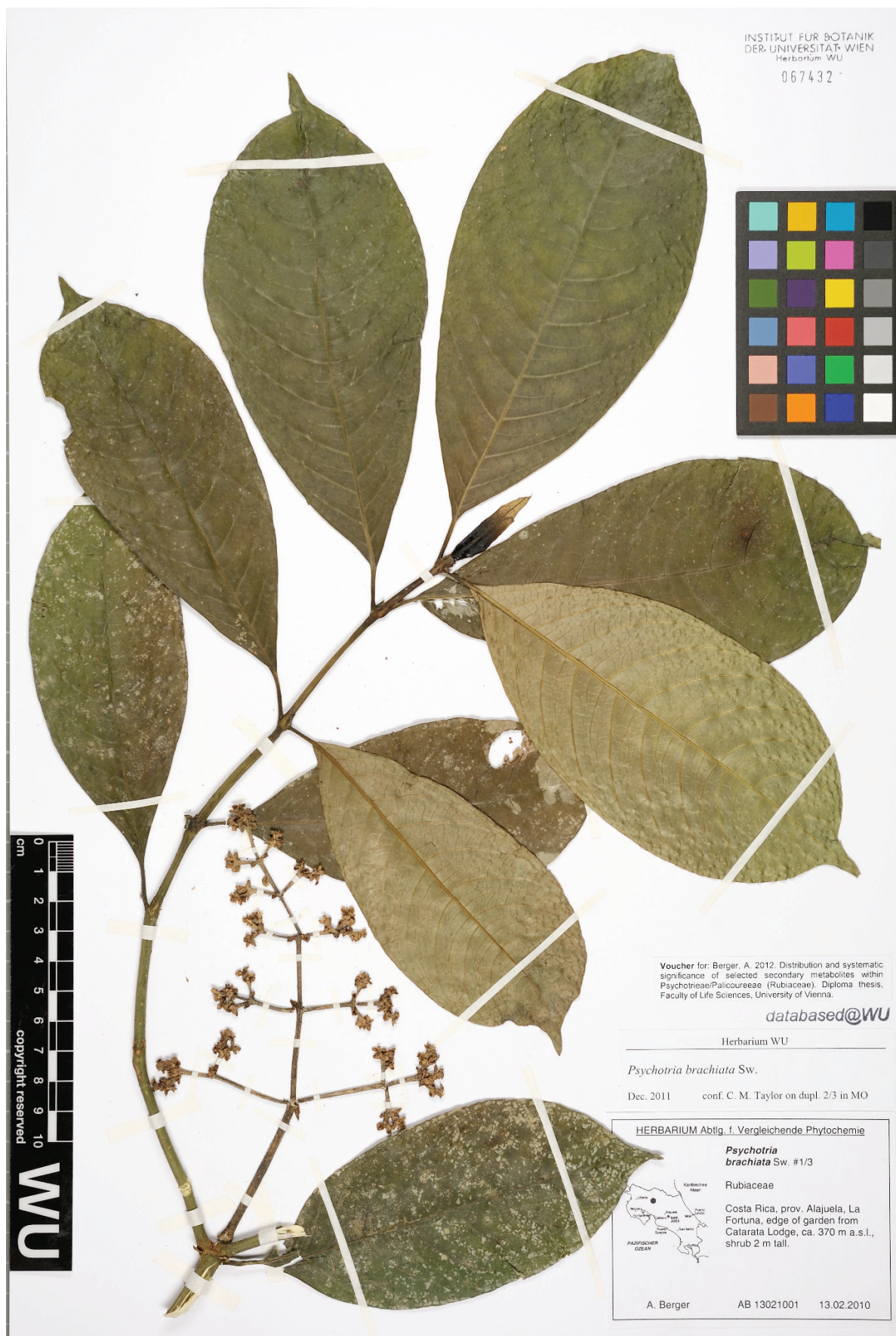


Fig. 56: *Psychotria brachiata* Sw.: Berger, A. AB 13021001, fig. ex WU 0067432.



Fig. 57: *Psychotria buchtienii* (H. Winkl.) Standl.: Berger, A. AB 21021002, fig. ex WU 0067436.



Fig. 58: *Psychotria campylopoda* Standl.: Boom, B.M. 9428, fig. ex W 2007-0021889.



Fig. 59: *Psychotria capensis* (Eckl.) Vatke: Greger,H. HG 1066, fig. ex WU 0044541.



Fig. 60: *Psychotria capitata* Ruiz & Pav.: Berger, A. AB 22021003, fig. ex WU 0067438.



Fig. 61: *Psychotria carthagenensis* Jacq.: Greger, H. HG 1063, fig. ex WU-HBV 0044545



Fig. 62: *Psychotria chagrensis* Standl.: Berger, A. AB 16021006, fig. ex WU 0067395.



Fig. 63: *Psychotria chiriquiensis* (Standl.) C. M. Taylor: Berger, A. AB 02021008, fig. ex WU 0067442.



Fig. 64: *Psychotria convergens* C. M. Taylor: Berger, A. AB 10021001, fig. ex WU 0067398.

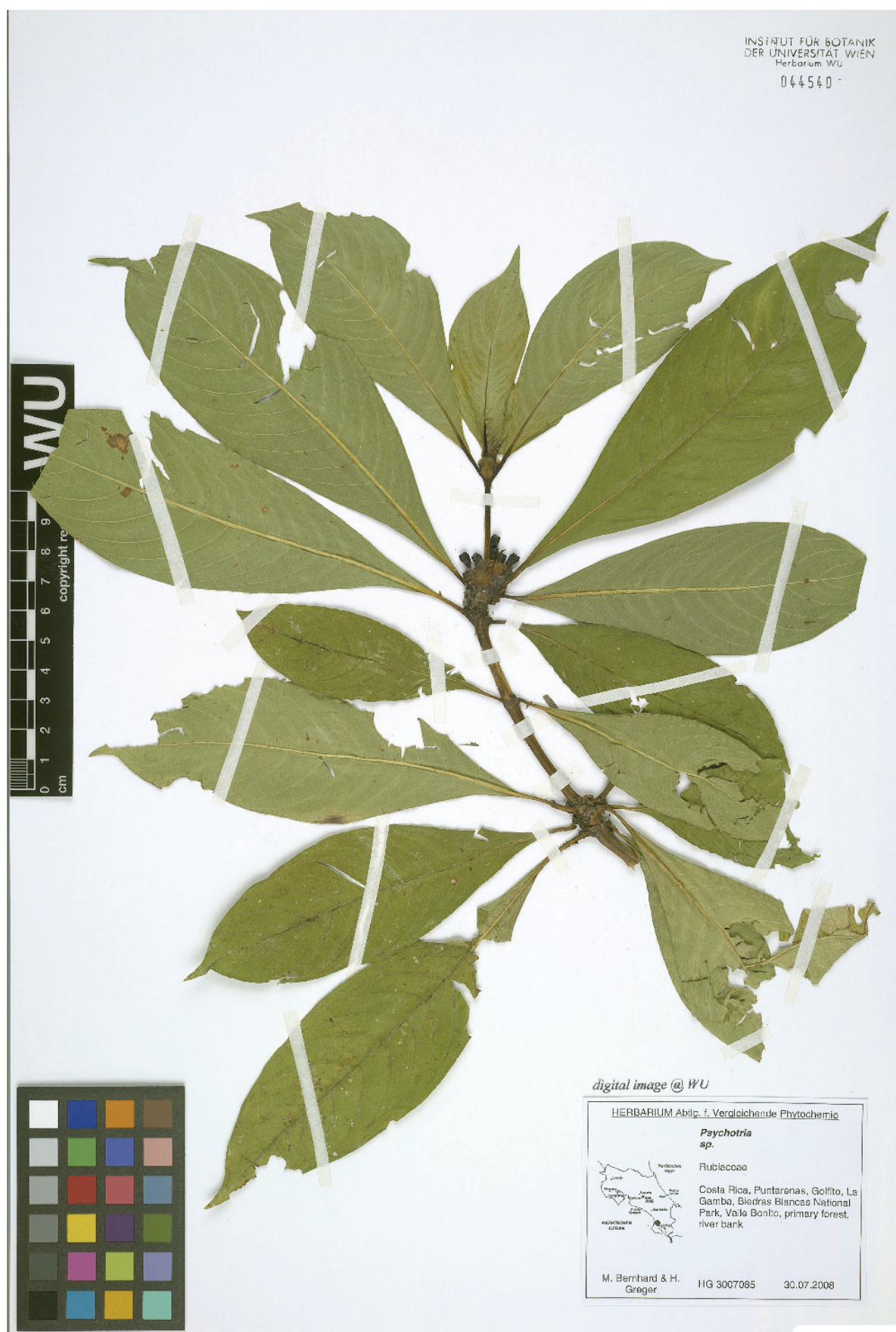


Fig. 65: *Psychotria cooperi* Standl.: Bernhard, M. & Greger, H. HG 3007085, fig. ex WU 0044540.



Fig. 66: *Psychotria cornigera* Benth.: Zimmermann, G. 36 131/71/36, fig. ex WU 0063826.



Fig. 67: *Psychotria cuspidulata* (K. Krause) Standl.: Palacios, W. & Neill, D. 739, fig. ex WU 0063825.



Fig. 68: *Psychotria cyanococca* Seem. ex Dombrain: Berger, A. AB 06021001, fig. ex WU 0067445

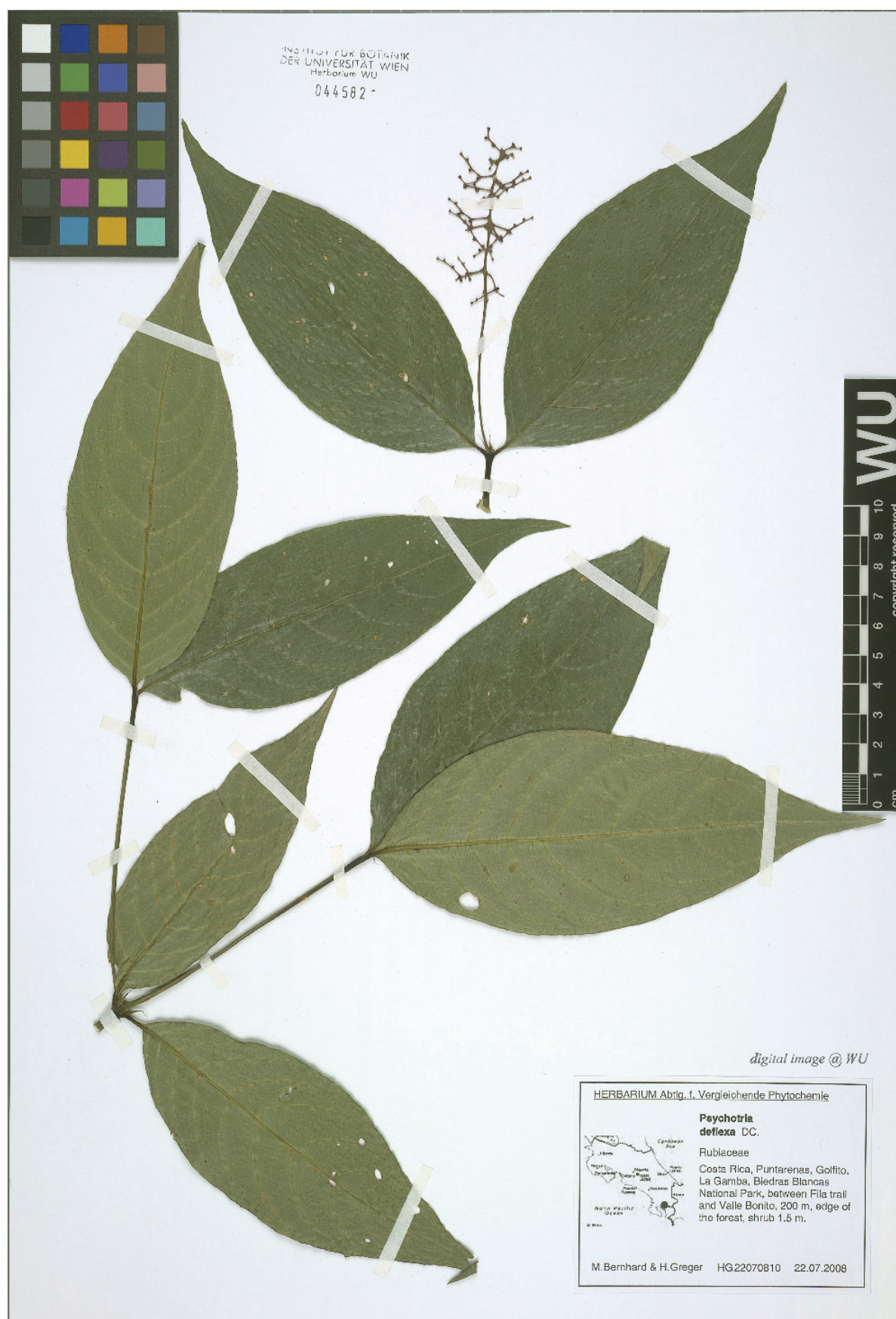


Fig. 69: *Psychotria deflexa* DC.: Bernhard, M. & Greger, H. HG 22070810, fig. ex WU 0044582.



Fig. 70: *Psychotria elata* (Sw.) Hammel: Bernhard, M. & Greger, H. HG 2407081, fig. ex WU 0044517.



Fig. 71: *Psychotria goldmanii* Standl.: Berger, A. AB 02021009, fig. ex WU 0067452.



Fig. 72: *Psychotria gracilentia* Müll. Arg.: Cid Ferreira, C.A. 4083, fig. ex W 2008-0022287.



Fig. 73: *Psychotria graciliflora* Benth.: Berger, A. AB 13021003, fig. ex WU 0067401.



Fig. 74: *Psychotria hawaiiensis* (A. Gray) Fosberg: Kiehn, M. & Kiehn, M. MK-890731-2/2b, fig. ex WU 0066071.



Fig. 75: *Psychotria hexandra* H. Mann: Kiehn, M. 920805-1/2, fig. ex WU 0066070.

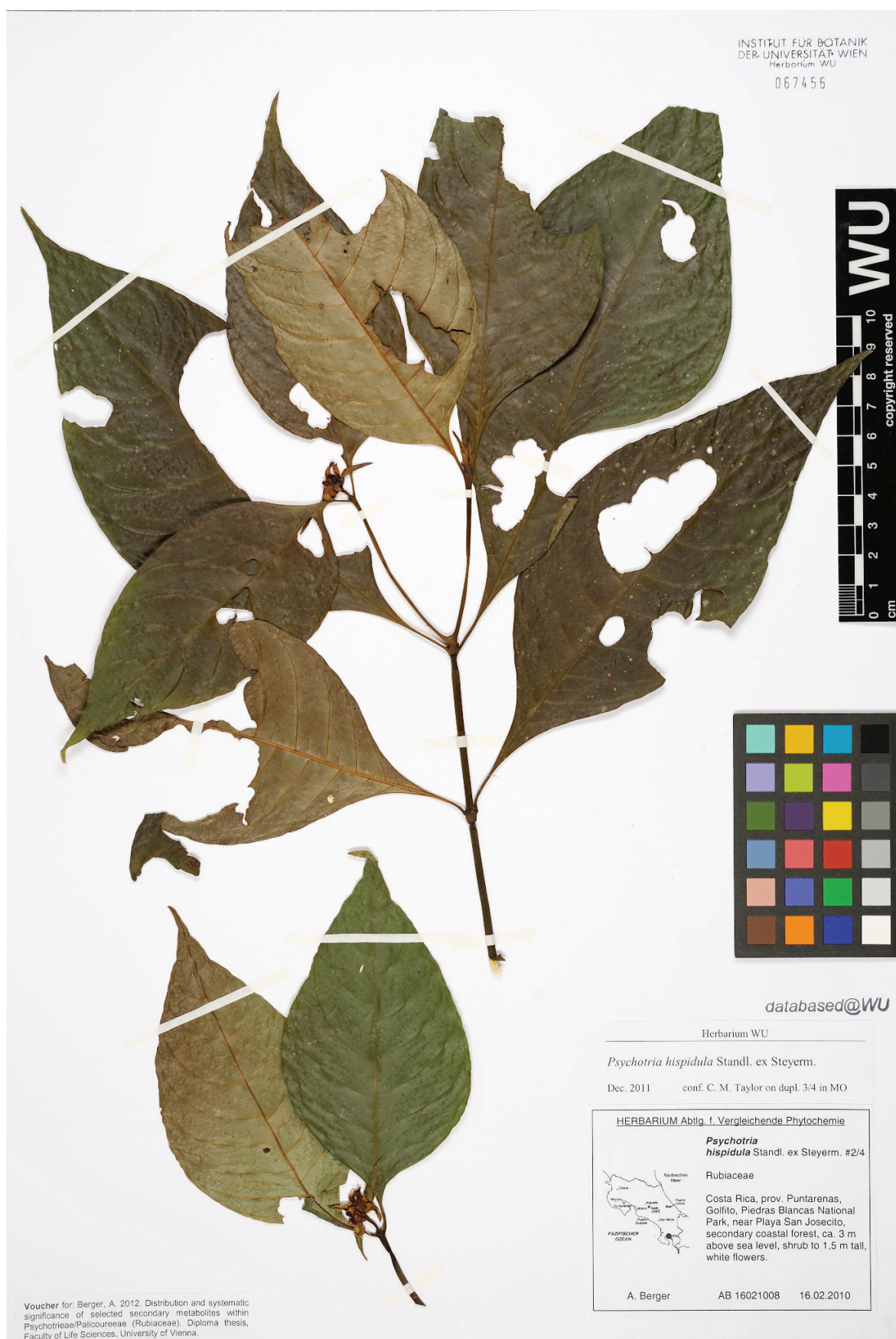


Fig. 76: *Psychotria hispidula* Standl. ex Steyerm.: Berger, A. AB 16021008, fig. ex WU 0067456.



Fig. 77: *Psychotria hoffmannseggiana* (Willd. ex Roem. & Schult.) Müll. Arg.: Berger, A. AB 28021001, fig. ex WU 0067458.



Fig. 78: *Psychotria horizontalis* Sw.: Berger, A. AB 28021004, fig. ex WU 0067402.



Fig. 79: *Psychotria limonensis* K. Krause: Berger, A. AB 05021002, fig. ex WU 0067404.

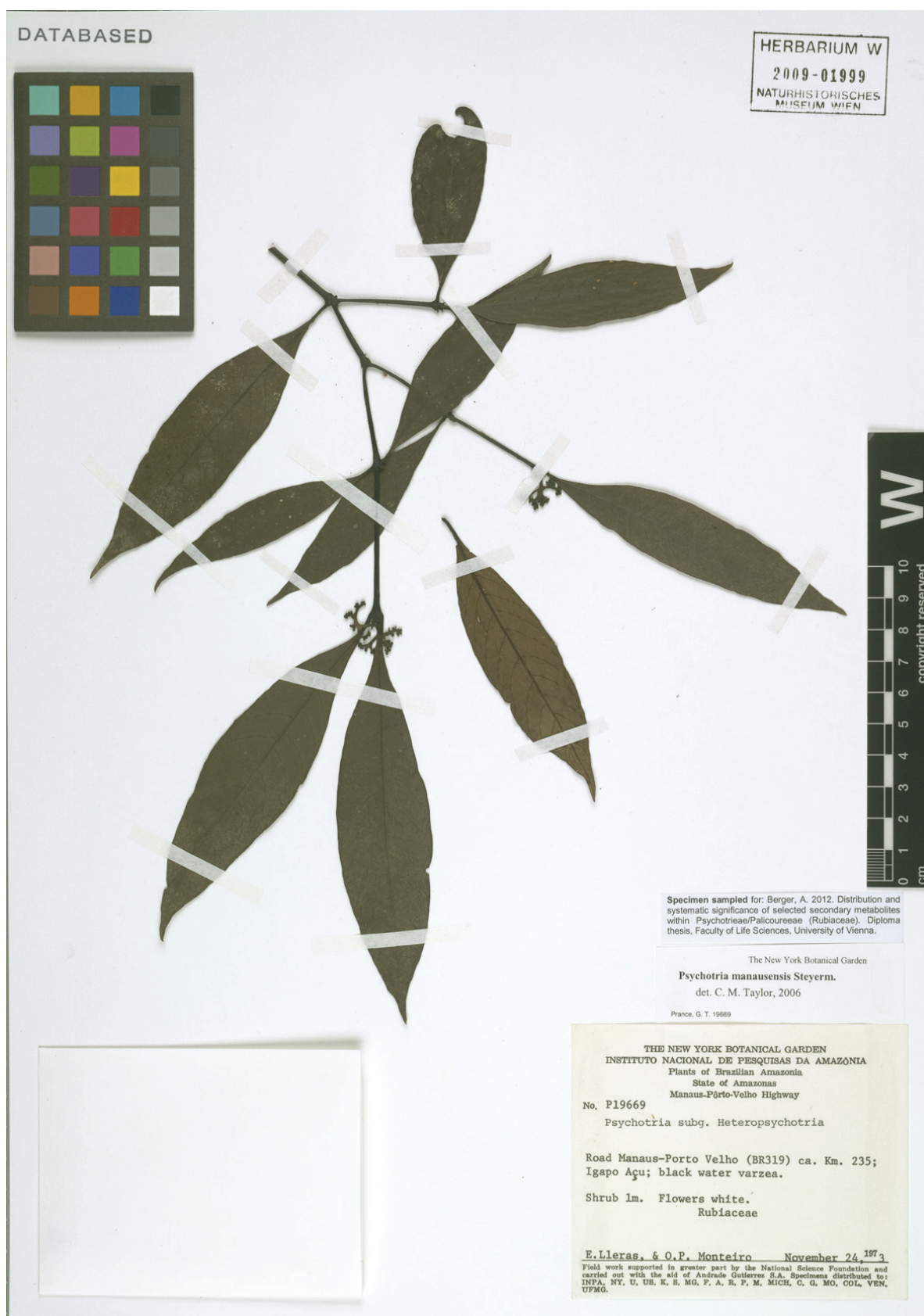


Fig. 80: *Psychotria manausensis* Steyererm.: Lleras, E. & Monteiro, O.P. P19669, fig. ex W 2009-0001999.



Fig. 81: *Psychotria mariniana* (Cham. & Schltdl.) Fosberg: Kiehn, M. 920907-1/5, fig. ex WU 0066072.



Fig. 82: *Psychotria marginata* Sw.: Berger, A. AB 06021005, fig. ex WU 0067407.



Fig. 83: *Psychotria micrantha* Kunth.: Berger, A. AB 15021005, fig. ex WU 0067409



Fig. 84: *Psychotria mortoniana* Standl.: Bernhard, M. & Greger, H. HG 2407087, fig. ex WU 0044555.



Fig. 85: *Psychotria nervosa* Sw.: Berger, A. AB 05021001, fig. ex WU 0067412.



Fig. 86: *Psychotria officinalis* (Aubl.) Raeusch. ex Sandwith: Zerny, H. s.n., fig. ex W 1959-0010741.



Fig. 87: *Psychotria orosiana* Standl.: Berger, A. AB 01021004, fig. ex WU 0067413.



Fig. 88: *Psychotria parvifolia* (K. Schum.) De Wild.: Berger, A. AB 02021005, fig. ex WU 0067417.



Fig. 89: *Psychotria pilosa* Ruiz & Pav.: Bernhard, M. & Greger, H. HG 2607086, fig. ex WU 0044558.



Fig. 90: *Psychotria platypoda* DC.: Nee, M.H. 34829, fig. ex W 2009-0012556.



Fig. 91: *Psychotria poeppigiana* Müll. Arg.: Bernhard, M. & Greger, H. HG 2607081, fig. ex WU 0044515.



Fig. 92: *Psychotria quinquerradiata* Pol.: Berger, A. AB 13031001, fig. ex WU 0067418.



Fig. 93: *Psychotria racemosa* Rich.: Berger, A. AB 16021002, fig. ex WU 0067460.



Fig. 94: *Psychotria serpens* L.: Greger, H. HG 1068, fig. ex WU 0067421.



Fig. 95: *Psychotria solitudinum* Standl.: Berger,A. AB 15021002, fig. ex WU 0067462.



Fig. 96: *Psychotria stenostachya* Standl.: Teixeira, L.O.A. et al. 456, fig. ex W 2009-0001464.



Fig. 97: *Psychotria suerrensis* Donn. Sm.: Berger, A. AB 16021005, fig. ex WU0067464.



Fig. 98: *Psychotria sylvivaga* Standl.: Berger, A. AB 01021008, fig. ex WU 0067428.



Fig. 99: *Psychotria tenuifolia* Sw.: Berger, A. AB 06021002, fig. ex WU 0067388.



Fig. 100: *Psychotria tsakiana* C. M. Taylor: Berger, A. AB 06021003, fig. ex WU 0067465.



Fig. 101: *Psychotria zeylanica* Sohmer: Schinnerl, J. s.n., fig. ex WU 0066051.



Fig. 101: *Ronabea emetica* (L. f.) A. Rich.: Berger, A. AB 28021002, fig. ex WU 0046606.



Fig. 102: *Ronabea latifolia* Aubl.: Berger, A. AB 16021007, fig. ex WU 0060916.

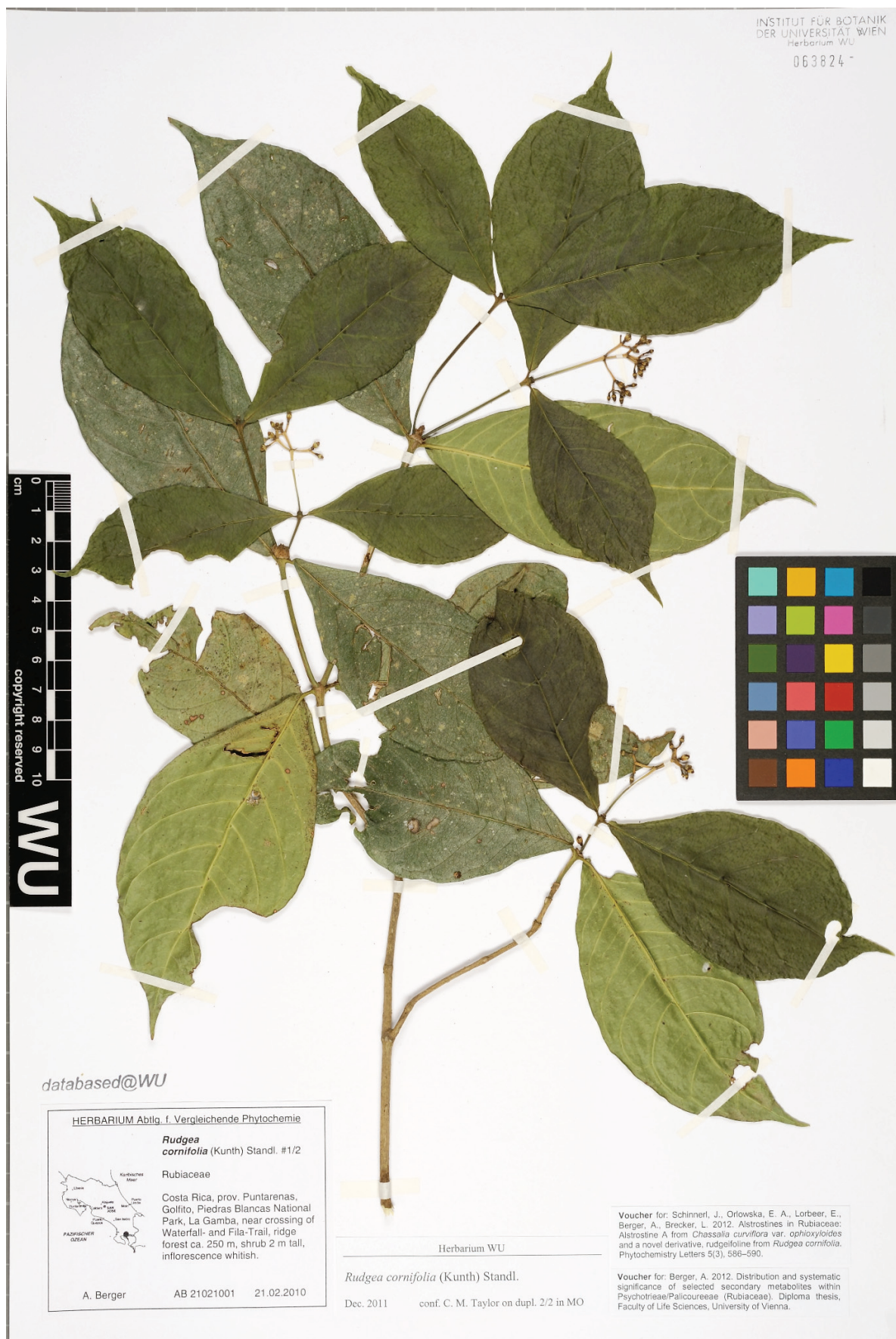


Fig. 103: *Rudgea cornifolia* (Kunth) Standl.: Berger, A. AB 21021001, fig. ex WU 0063824.

13.4 Curriculum Vitae

Andreas Berger

Born: 23.05.1986 in Vienna, Austria

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Education

- 1992 - 1996 Primary School, Katholische Privatschule der Erzdiözese Wien, Maurer-Lange-Gasse 115, 1230 Vienna, Austria.
- 1996 - 2003 Secondary School, Realgymnasium GRG 13 Wenzgasse, Wenzgasse 7, A-1130 Vienna, Austria.
- 2003 - 2004 Secondary School, Realgymnasium GRG 23 Alt Erlaa, Anton Baumgartner Straße 123, A-1230 Vienna, Austria. Graduation: Matura, passed with honour.
- 2005 - Studies of Biology, University of Vienna, Austria.
- 2007 - Studies of Botany, University of Vienna, Austria.
- 2009 - Diploma thesis: „Distribution and systematic significance of selected secondary metabolites within Psychotrieae/Palicooureeae (Rubiaceae)“, Chemodiversity Research Group, Department of Systematic and Evolutionary Botany, University of Vienna, Austria.

Employments

- 2004 - 2005 Civil service at the “Schulzentrum Ungargasse”, 1030 Vienna
- 2005 - 2009 Employment at Outdoor Dreams Handels GmbH
- 2007 & 2008 Tutorship „Diversität und Systematik der höheren Pflanzen“, University of Vienna, Austria
- 2009/01-03 Project collaborator in the FWF-project “Artbildung auf dem Balkan - Das Beispiel *Veronica*“: morphometric measurements in the *Veronica chamaedrys* group
- 2010, 2011 & 2012: Tutorship „Vegetations- und Landschaftsökologie – Monitoring in Großschutzgebieten“, University of Vienna, Austria
- 2010 & 2012: Tutorship „Ökologische Übung – Insel Krk“, University of Vienna, Austria
- 2011/06 - Employment at the herbarium of the Natural History Museum Vienna, Austria.

Grants and merit scholarships

- Leistungsstipendium aus Stiftungen und Sondervermögen der Universität Wien, 2008.
- Leistungsstipendium nach dem Studienförderungsgesetz der Universität Wien, academic year 2007/08.
- Kurzfristiges Auslandsstipendium (KWA), for field work in Costa Rica, February 2010.
- Förderungsstipendium nach dem Studienförderungsgesetz, for field work in Costa Rica, February 2010.

Languages spoken

German (first language)

English (fluent)

Italian and Spanish (basics)

Excursions and fieldwork

February 2008: Ethnobotanical - phytochemical field course in Thailand, University of Vienna, supervision a.o. Univ.-Prof. i.R. Dr. Harald Greger.

August 2008: Fieldwork for the project “Mapping the Flora of Austria”, supervision: Univ.-Prof. i.R. Dr. Harald Niklfeld.

April 2009: Botanical excursion to southern Spain, University of Vienna, supervision Mag. Dr. Peter Schönswetter & Ass.-Prof. Mag. Dr. Gerald Schneeweiß.

February-March 2010: Field work in the context of my diploma thesis, Costa Rica.

August 2010: Fieldwork for the project “Mapping the Flora of Austria”, supervision: Univ.-Prof. i.R. Dr. Harald Niklfeld.

February 2011: Botanical excursion to Ethiopia. Universität Wien, supervision: a.o. Univ.-Prof. Dr. Christian Puff.

April 2011: Fieldwork for the “Flora Ionica”, botanical excursion to the Ionian Islands, Greece. supervision: Dr. Walter Gutermann.

August-September 2011: Fieldwork for the project “Mapping the Flora of Austria”, supervision: Univ.-Prof. i.R. Dr. Harald Niklfeld.

April 2012: Fieldwork for the “Flora Ionica”, botanical excursion to the Ionian Islands, Greece. supervision: Dr. Walter Gutermann.

August 2012: August 2010: Fieldwork for the project “Mapping the Flora of Austria”, supervision: Univ.-Prof. i.R. Dr. Harald Niklfeld.

Research interests

- Chemodiversity, chemosystematic and floristic studies in *Psychotria* and related genera,
- tropical biology, especially botany and animal-plant interactions,
- flora of Austria, flora of the Ionian Islands,
- ethnobotany,
- lichenology

Publications and Presentations

Berger, A., Gilli, C. 2011. *Lathyrus sphaericus*. In: Fischer, W., Niklfeld, H.: Floristische Neufunde (99-123). – *Neilreichia* 6, 365-396.

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Berger, A., Dellinger, A. 2009 Vergesellschaftung, Habitatspezifität und pflanzensoziologische Bewertung der Vorkommen von *Trifolium saxatile* im Schalfthal, Ötztaler Alpen, Tirol. Verhandlungen der zoologisch- botanischen Gesellschaft in Österreich 146, 125-138.

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Berger, A., Fasshuber, H., Schinnerl, J., Robien, W., Brecker, L., Valant-Vetschera, K. 2011. Iridoids as chemical markers of false ipecac (*Ronabea emetica*), a previously confused medicinal plant. *Journal of Ethnopharmacology* 138, 756-761.

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Fasshuber, H., Bernhard, M., **Berger, A.**, Schinnerl, J., Robien, W., Greger, H., Brecker, L. 2010. NMR based structure determination of alkaloids isolated from plants of the genus *Psychotria* L. (Rubiaceae). Talk at the “5th Central European NMR Meeting”.

Hülber, K., **Berger, A.**, Gilli, C., Hofbauer, M., Patek, M., Schneeweiss, G. M. 2011. No evidence for a role of competitive capabilities of adults in causing habitat segregation of diploid and hexaploid *Senecio carniolicus* (Asteraceae). *Alpine Botany* 121, 123-127.

Hülber, K., Sonnleitner, M., Flatscher, R., **Berger, A.**, Dobrovsky, R., Niesser, S., Nigl, T., Schneeweiss, G. M., Kubešová, M., Rauchová, J., Suda, J., Schönswetter, P. 2009. Ecological segregation drives fine-scale cytotype distribution of *Senecio carniolicus* in the Eastern Alps. *Preslia* 81, 309-319.

Koehbach, J., Attah, A. F., **Berger, A.**, Hellinger, R., Kutchan, T.M., Rolf, M., Wong, G.K.-S., Dessein, S., Greger, G., Gruber, C.W. 2012. Cyclotide discovery in Gentianales revisited - Identification and characterization of cyclic cystine-knot peptides and their phylogenetic distribution in Rubiaceae plants. *Biopolymers: Peptide Science*, submitted.

Reich, D., **Berger, A.**, Flatscher, R., Gilli, C., Hofbauer, M., Lachmayer, M., Prehsler, D., Sonnleitner, M., Gutermann, W. 2012. A Greek Odyssey: Developing an online Flora of the Ionian Islands. Poster presentation at the „15. Treffen der Österreichischen Botanikerinnen und Botaniker“, Innsbruck, 27.-29.09.2012.

Schinnerl, J., Orlowska, E. A., Lorbeer, E., **Berger, A.**, Brecker, L. 2012. Alstrostines in Rubiaceae: Alstrostine A from *Chassalia curviflora* var. *ophioxyloides* and a novel derivative, rudgeifoline from *Rudgea cornifolia*. *Phytochemistry Letters* 5(3), 586-590.