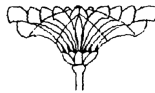


H. Ochoterena, T. Terrazas, P. De Block & S. Dessein
(editors)

**IV International Rubiaceae (Gentianales)
Conference**

Abstracts

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ABSTRACTS

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The abstracts are arranged in alphabetic order according to the first author's last name. The order is independent of whether the presentation is oral or a poster, which is indicated on the side. The presenting author is underlined and his/her e-mail is printed below the authors list.

Key note speakers for the conference are:

Thomas Borsch

What do we know about evolution and diversity of the Coffee family's most prominent member – *Coffea arabica*?

Robert H. Manson

Biocafé: developing sustainable management strategies that help balance biodiversity conservation and the socio-economic well-being of coffee farmers in central Veracruz

Charlotte M. Taylor

The status of the world floristic knowledge of Rubiaceae

The genus *Malanea* (Guettardeae, Rubiaceae) in Venezuela

Alix Amaya-Worm

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Fundación Instituto Botánico de Venezuela, Venezuela

Malanea comprises about 33 species distributed from South Central America to Bolivia; the genus is distinguished from other Rubiaceae genera by the climbing or vobule shrubby habit, valvate aestivation, corolla internally pubescent, yellow to orange, four petals, lineolate venation, fleshy fruits with a pyrene. This work reports preliminary results on the study of neotropical *Malanea* species. The infra-specific delimitation of this genus is not clear because it is mainly based on pubescence density. Comparative morphological analysis of specimens obtained from herbaria and collected materials were made. An identification key to species level, descriptions, and comments related to taxonomy and geographical distribution are included. The taxonomic revision of this genus showed that *Malanea* is represented by 17 species, twelve of these are endemic to Venezuela (*M. auyantepuiensis*, *M. chimantensis*, *M. fendleri*, *M. gabrielensis*, *M. guaiquinimensis*, *M. hirsuta*, *M. hypoleuca*, *M. jauaensis*, *M. macrophylla*, *M. microphylla*, *M. obovata*, *M. pariensis*, *M. ptariensis*, *M. sanluisensis*, *M. sarmentosa*, *M. setulosa*, *M. ursina*), distributed in the north of the country (Cordillera de la Costa) and south of the Orinoco River, but absent in the Andes region. These studies allowed us to conclude that *M. sipapoensis* and *M. ueiensis* are synonyms of *M. gabrielensis*, and that the varieties of *M. macrophylla* should not be recognized because the difference in pubescence density is continuous and this feature cannot be considered as taxonomically informative. This work represents the first report of the occurrence of distily in *Malanea* species.

Morphological, nomenclatural and palynological contributions for the taxonomic relocation of *Diodia assurgens*

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During the revision of American species of tribe Spermacoceae, species of the *Diodia s.lat.* complex were analyzed, and the characters that might contribute to their accurate identification were studied. The nomenclatural background of *Diodia assurgens* (Nees & Martius) K.Schum. was reviewed and the species description showed that it does not fit within the *Diodia* L. (*sensu* Bacigalupo & Cabral, 1999) concept. For this reason, it was transferred to another taxon within the Spermacoceae tribe. For the first time, its particular inflorescences are described, which distinguish it from related species. A new infraspecific classification is suggested. The taxonomic relocation of the species and the varieties proposed was supported by palynological studies. The species studied is described and illustrated, and the pollen grains of the infraspecific taxa are analyzed.

The taxonomy of *Rondeletia* and allied genera

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Linné described the genus *Rondeletia* based on *R. americana* of the Lesser Antilles. It has a set of flower and fruit characters which are in common with the other 160 species of the Caribbean, and which may be regarded as the character-combination of the genus in the Linnean sense. Since that time many authors described and included taxa with different character-combinations under the Linnean generic name, thereby amplifying the generic range of *Rondeletia*, but using the author name of Linné. A broad concept of *Rondeletia* has been created by the great world monographs by Bentham and Hooker and by Engler and Diels (Natürliche Pflanzenfamilien) and this trend culminated in Standley's Flora of North America where three genera and 98 species were united in one big genus *Rondeletia*. In this study the authors revised a large representative set of Mexican, Meso-American, West Indian and South-American species (*Rondeletia*-complex sensu Delprete 1999) from anatomical and morphological points of view. The study included more than 100 species and was supported by molecular studies by Rova and collaborators. Both approaches confirmed the polyphyletic nature of *Rondeletia sensu* Hooker 1873, Hemsley 1879, Schumann 1892, Standley 1918, and reinforced the monophyly of *Rondeletia* L. The splitting of *Rondeletia s.lat.* was started in 1849 by Planchon who, growing "*Rondeletia* species" of different origins in the Jardin des Serres, observed 3 different corolla types, and therefore segregated the genera *Arachnothryx* and *Rogiera*. These units were recognized and characterized by Steyermark and Borhidi but rejected by Williams and Lorence. Although chromosome-studies (different basic numbers) and several molecular studies supported the anatomical and morphological studies of the authors, ultimately Govaerts et al. re-established the monstrous-sized genus *Rondeletia*, creating several new combinations. Our question: what is taxonomy in the 21th century: opinionism or science?

Identity of three American species of *Borreria* (Rubiaceae): *B. latifolia*, *B. flavovirens* and *B. poaya*. New contributions to establish its delimitation: first chromosome counts, new synonyms, and expansion of geographical distribution

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The genus *Borreria* G.Mey. belongs to tribe Spermacoceae. Since its original description, some species of this genus went through changes regarding their taxonomic delimitation, or were segregated or included in other taxa. One of the reasons for this is the variety of exomorphological and palynological characters. Furthermore, many species have similar morphological aspects in the field and even more when they are herborized. That is why there are divergent views among specialists regarding the criteria for delimiting species, and the genus itself, with some authors merging *Borreria* within *Spermacoce*. Since molecular phylogenetic studies of the American species of the genus are pending, we consider that transferring all *Borreria* species to *Spermacoce* is at least precipitate. Erroneous names that increase confusion for the correct identification of three related species, *Borreria latifolia*, *B. flavovirens* and *B. poaya*, are commonly found in many herbaria. This work presents exomorphological, palynological, and cytological differences, as well as similarities among the three species. We also present the first chromosome counts for the three taxa. The geographical distribution of *B. latifolia* and *B. poaya* is expanded by recognizing new records from three South American countries. New synonyms for these last two species are given.

Rubiaceae diversity in the state of Veracruz, Mexico

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Rubiaceae is one of the largest Angiosperm families with approximately 620 genera and 13,000 species. Even though the largest diversity of the family is mainly concentrated in the tropical and subtropical regions of the world, it is possible to find species in temperate or even cold areas, such as the uplands and the cold regions of Northern Canada and Europe. In Mexico, Rubiaceae diversity is estimated at 103 genera and 500-585 species, from which Veracruz has approximately 54 genera and a little over 200 species. Even though the largest diversity is concentrated in tropical and subtropical forests, the species of this family are present in all vegetation types found in the state of Veracruz. They range from coastal dunes to pine forests in the central mountains of the state. Nevertheless, the greatest diversity can be found in the tropical rain forests (135 spp.), cloud forests (98 spp.), tropical oak forests (80 spp.), tropical deciduous forests (73 spp.), and tropical sub-deciduous forests (39 spp.). The region of Los Tuxtlas, forming part of a Biosphere reserve, includes about 50% of the species diversity for the family in Veracruz. In the state, most of the species are woody (trees or shrubs) and only about one fourth are herbaceous species. It is noticeable that most of the species are present in undisturbed forests, with only a limited number of species occurring in secondary forests. This is relevant considering that most of the original habitats in the state are highly threatened by human agricultural and farming activities, risking the family diversity.

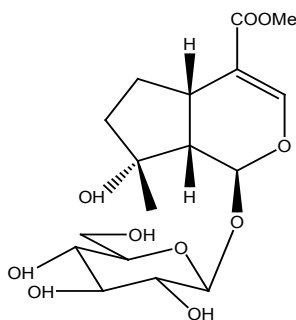
A preliminary study of secondary metabolites of *Guihailiothamnus acaulis* (Rubiaceae) and its chemotaxonomic significance

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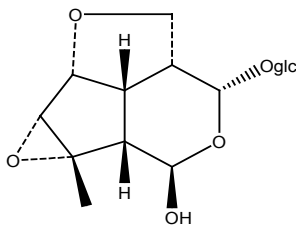
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Guihailiothamnus is a newly established monotypic genus whose systematic placement has not been satisfactorily determined. Some taxonomists believe it to be related to *Wendlandia* and include it in Rondeletieae, while others believe it to be closer to *Ixora* or *Gardenia* and included it in Gardenieae. We attempted to determine the relationship of *Guihailiothamnus* based on phytochemical evidence. Whole plants (1200 g) of *Guihailiothamnus acaulis* L. were extracted three times with 90% ethanol. The resultant pooled alcoholic extract, after concentration to c. 30 ml, was diluted to 100 ml with H₂O and successively fractionated with CHCl₃, EtOAc and finally n-BuOH to afford CHCl₃, EtOAc, and n-Bu-OH soluble fractions as well as a leftover liquor layer. The EtOAc soluble fraction was subjected to silica gel followed by Sephadex LH-20 chromatography to yield five pure compounds. The structures of two compounds were elucidated to be iridoid glucosides 8-epi-mussaenoside (**1**) and wendoside (**2**), while determination of the structure of other compounds is in progress.



8-epi-mussaenoside (**1**)



Wendoside (**2**)

It is noteworthy that iridoid glucosides are commonly present in species of *Wendlandia*, such as in *W. formosana* and *W. tinctoria*, which are the only two species of the genus so far chemically investigated. Furthermore, compounds **1** and **2** were also isolated from *W. tinctoria*, implying a relationship between *Guihaiothamnus* and *Wendlandia*, since wendoside is still the only iridoid glucoside in which the C-11 hydroxymethyl group is involved in the formation of a tetrahydrofuran ring with C-6 carbon. Our preliminary phytochemical evidence therefore supports the hypothesis that *Guihaiothamnus* is related to *Wendlandia*.

Ontogeny of inflorescences, flowers and fruits of *Canephora madagascariensis* (Octotropideae)

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Canephora Juss. is endemic to Madagascar, comprising less than ten species. The genus belongs to Octotropideae (Ixoroideae) and possesses the typical characters of this tribe: axillary and usually pedunculate inflorescences, campanulate flowers, contorted aestivation, bilocular ovaries, axile placentation with few ovules per placenta, superior radicle, wrinkled exotesta. Unique within the tribe and the family Rubiaceae are the flattened, green, photosynthetic phylloclade-like structures comprising the peduncle and branching of the inflorescence, including up to nine apically positioned floral buds. The genus name refers to this structure: *Canephora* (Greek) means basket bearer. An elaborate study of the ontogeny of inflorescences, flowers and fruits of *Canephora madagascariensis* based on SEM and LM observations is presented, with emphasis on inflorescence structure and gynoecium development. Different possible interpretations of the phylloclade-like inflorescence structures are explored. Rubiaceae usually have epigynous flowers with gynoecea of which the ovary wall cannot be distinguished from the surrounding tissue. However, in *Canephora*, the ovary wall of the inferior gynoecium can be distinguished from the surrounding tissue in young flowers. Therefore, the nature of the inferior position of the gynoecium is investigated. *Canephora madagascariensis* possesses U-shaped placentas with six to eight pendulous ovules, of which one to eight develop into mature seeds. The placentas are attached to the upper half of the septum. Such a type of placentation is considered primitive within the Rubiaceae family. The placenta grows considerably in size and partly covers the seeds in the fruit, which was hitherto unreported for the tribe Octotropideae.

Phylogeny of the Spermaceaceae *s.str.*: a quest for well defined genera hampered by morphological plasticity

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Molecular data have shown convincingly that the pantropical tribe Spermaceaceae as traditionally delimited, further on named Spermaceaceae *s.str.*, makes the tribe Hedyotideae paraphyletic. Therefore, a new tribal concept, Spermaceaceae *s.lat.*, has been proposed, including most of the former Hedyotideae genera and all genera of the Spermaceaceae *s.str.* This last group, easily characterized by its one-seeded locules and often pluri-aperturate pollen grains, still forms a monophyletic group if *Gomphocalyx* and *Phylohydrax* are excluded. It comprises more than 350 species distributed over at least 19 genera. Despite the problematic delimitation of the genera within the group, not a single phylogenetic study was devoted to study the relationships among Spermaceaceae *s.str.* In the present study, we reconstruct the phylogeny of the clade using five chloroplast (*atpB-rbcL*, *trnL-F*, *rpL32-trnL*, *petD*, *trnH-psbA*) and two nuclear markers (ITS and ETS). The phylogeny was inferred using both Bayesian and Parsimony analyses.

Towards a complete Rubiaceae instalment for the ‘Flore du Gabon’

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Gabon is situated along the west coast of Africa on the equator. About 80% of the country is covered by rainforest, the rest being savannas. It is part of the Central African rainforests, which form the second largest area of continuous tropical forest in the world. With an estimated 7500 plant species, Gabon is the richest rainforest area in Africa. A vast network of thirteen National Parks covers about 10% of the country’s surface. Started in 1961, the ‘Flore du Gabon’ is published irregularly by the *Museum National de l’Histoire Naturelle* in Paris. Until now 37 volumes have been published. Two of these, authored by N. Hallé, treat the Cinchonoideae as traditionally delimited, i.e. those Rubiaceae taxa characterized by multi-ovulate placentas. Rubiaceae with a single ovule per locule (= Coffeoidae sensu Schumann) were never dealt with. Recently, initiatives have been undertaken by the Libreville and Wageningen herbaria to revitalize ‘Flore du Gabon’. The Rubiaceae team of the National Botanic Garden of Belgium was invited to complete the Rubiaceae treatment. Recent field work in Gabon has revealed that many Rubiaceae species remain undescribed, even among the genera already treated in the Flora volumes. Therefore it was decided not only to treat the uni-ovulate Rubiaceae but also to rework the existing volumes already more than 40 years old. In total 95 Rubiaceae genera are reported in the flora area. These represent c. 600 species. The new flora volumes will more or less follow the new insights in the Rubiaceae phylogeny.

A new species of *Colletoecema* (Rubiaceae) from southern Cameroon with a discussion on the relationships among basal Rubioideae

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During an expedition to the Ngovayang massif (southern Cameroon), a new species of the enigmatic genus *Colletoecema* was collected. A comparative morphological study illustrates the similar placentation and fruit anatomy of the novelty and *Colletoecema dewevrei*, the only species so far described in the genus. The new species mainly differs from *C. dewevrei* in having larger leaves, more condensed inflorescences, possibly isostylous flowers and larger, sessile fruits. Its position within *Colletoecema* is confirmed by *atpB-rbcL* and *rbcL* chloroplast sequences. The relationships among the basal lineages of the subfamily Rubioideae, to which *Colletoecema* belongs, are briefly addressed. Based on our present knowledge, a paleotropical or tropical African origin of the Rubioideae is hypothesized.

A preliminary survey of hypotheses of homology assessment in the systematics of *Crusea* (Spermacoceae, Rubiaceae)

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A cladistic analysis begins with the assessment of character variation within a group of organisms and the subsequent representation of that variation as a data matrix. Conversion of observed character variation into a data matrix has been considered subjective, contentious, under-investigated, imprecise, unquantifiable, intuitive, as a black-box and, at the same time, the most influential phase of any cladistic analysis. Although it is considered as a reproducible homology assessment of the observed character variation, it has been suggested that different interpretations of characters and coding are possible, since systematists perceive and define characters in distinct ways (conventional or non-conventional sensu Hawkins, 2000). In comparative biology two main lines of work are mixed: identification of species (for example using keys) and evolutionary studies. In the first case typological, homologous, and homoplasious characters are all potentially useful. In the second one, only homologies must be used. In the last decades there have been important advances in the field of molecular systematics, which provide a conceptual frame to discover homologies in the analysis of DNA sequences composed by thousands of bases. But morphological characters are still used in systematic studies without being considered as hypothesis of homology. This preliminary review is aimed to know if the assessment of the homology hypothesis has been properly accomplished in evolutionary studies of *Crusea*. In such studies qualitative characters cover 41% (24 characters) of morphological variation in the taxa (conventional [7%] and non-conventional [34%]), while quantitative variation is considered in the rest (34 characters or 59 %). The use of similarity, conjunction and congruence criteria to evaluate the morphological variation in *Crusea* gives the opportunity to test new and old hypotheses of homology for the study of the phylogeny in this genus.

Introns and spacers as tools towards resolving the radiation of Rubiaceae

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Rapidly evolving and non-coding chloroplast DNA has recently been shown to contain high levels of phylogenetic structure that allows inferring well resolved and statistically supported phylogenetic trees for relationships as deep as the major lineages of angiosperms. These non-coding regions therefore appear to be more effective markers than conserved genes such as *rbcL* or *atpB*. Several phylogenetic analyses of introns and spacers have been published for basal angiosperms and eudicots. This suggests that the use of rapidly evolving and non-coding DNA regions will help to better understand the evolution of species rich radiations such as the Rubiaceae. For this project, we focused particularly on the molecular evolution and phylogenetic potential of group II introns in Rubiaceae. So far, only few group II introns have been used (*rps16* intron) for phylogenetic reconstruction of Rubiaceae. We therefore generated a dataset of four group II introns (those located in the *atpF*, *petD*, *trnG* and *rps16* genes) as well as a comparative dataset of more commonly used spacers (*atpB-rbcL*, *trnL-trnF*) and the *trnL* group I intron. We selected about 90 genera covering all tribes within Rubiaceae, largely based on material from the living collections of the National Botanic Garden of Belgium (NBGB) and the Botanical Garden of Berlin (BGBM). Aims are to compare phylogenetic structure *R* in the respective character partitions, to examine molecular evolutionary patterns that could explain different performance in phylogenetic reconstruction, and to find optimal markers for analysing species-rich radiations. All character partitions agree on placing *Luculia* as sister to all remaining Rubiaceae and on Rubioideae being a clade. Monophyly of Cinchonoideae is indicated with moderate statistical support by spacer partitions, whereas introns depict three major clades of not fully clear relationships to each other: Rubioideae, Cinchonidinae I and II (= supertribe Cinchonidinae), and Ixoridinae.

Multidisciplinary approach traces the biogeographic origin and evolution of Madagascar's Spermaceae

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Madagascar has a unique and spectacularly rich flora with a very high percentage of endemism. On this hotspot island, Rubiaceae are the second largest family of flowering plants. Until now, the geographic origin of the various Rubiaceae taxa in Madagascar has never been studied in detail. Based on phylogenies inferred from plastid and nuclear DNA we investigated the origin and evolution of the Malagasy members of Spermaceae. In its current delimitation, the herbaceous tribe Spermaceae unites the former tribes Spermaceae *s.str.* and Manettieae, and the *Hedyotis-Oldenlandia* group. The tribe is represented in Madagascar by c. 30 species. They are present in all terrestrial niches, from the coastal dunes in the south(west) to the high plateaus in the centre of the island. Some taxa are local endemics, others are pantropical weeds. The present study focused on the species of the *Hedyotis-Oldenlandia* group endemic to Madagascar (c. 15 species). The phylogeny of Spermaceae was inferred based on *atpB-rbcL*, *rps16*, *trnL-trnF* and *petD* sequences in order to reveal the origin of these Madagascan endemics. Results support the hypothesis that at least two colonisation events gave rise to the current diversity of the *Hedyotis-Oldenlandia* group on the island. One group of species is sister to the neotropical genus *Arcytophyllum* and the North and Central American species presently classified in the genera *Houstonia*, *Oldenlandia*, and *Stenaria*. The second clade contains the endemic genera *Lathraeocarpa* and *Gomphocalyx*, and is closely related to the Afro-Madagascan genus *Phylohydrax*. To improve resolution, a second, more focused analysis was performed with ETS and ITS included as extra nuclear markers using a smaller set of taxa. To date the two colonisation and radiation events, we expanded our Spermaceae phylogeny to comprise representatives of all Rubiaceae tribes. This allowed incorporation of multiple fossil-based calibration points from the Rubiaceae pollen record. A morphological investigation of flowers, fruits, seeds and pollen was carried out to find character support for the new phylogenetic relationships detected.

Distribution and evolution of circular mini-proteins in Rubiaceae

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Cyclotides are disulfide-rich mini-proteins with the unique structural features of a circular backbone and knotted arrangement of three conserved disulfide bonds. These features make them exceptionally stable and they have potential applications as insecticidal agents and stable drug frameworks. So far, they have been found only in two plant families: in every species of the violet family (Violaceae), and in few species of the coffee family (Rubiaceae). In this study we analyzed over 200 Rubiaceae species, distributed in many tribes, for the occurrence of cyclotides. Their presence was confirmed in 21 species, all belonging to the subfamily Rubioideae. Additionally we analyzed over 140 species in plant families outside the Rubiaceae and Violaceae and report for the first time the occurrence of cyclotides in the Apocynaceae. On the basis of the phylogeny of cyclotide-bearing plants and the analysis of cyclotide precursor gene sequences, we hypothesize that the evolution of circular proteins occurred as independent events after the divergence of Asterids and Rosids in various plant families. This is further supported by recent findings on the biosynthesis of cyclotides, which involves ubiquitously present enzymes for folding and processing. We further predict that the number of cyclotides within the Rubiaceae may exceed 10,000 and could be as great as 50,000, potentially making cyclotides one of the largest protein families in the plant kingdom.

PalRub, an online database for palynological data in Rubiaceae

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The Rubiaceae or coffee family exhibit a remarkable variation in pollen morphology. Pollen grains differ among representatives in dispersal unit, size and shape, number, position and shape of apertures, and wall ornamentation. That might not come as a surprise for an angiosperm family of c. 13,000 species. However, published accounts convincingly illustrate the high systematic value of several major pollen characters at different taxonomic levels, i.e. tribe, genus and (sub)species. The compound apertures in Rubiaceae received considerable attention and particularly the structure of the endoapertures proved to be an exciting ‘new’ character delimiting monophyletic groups. The Laboratory of Plant Systematics (K.U.Leuven, Belgium) has been and still is very active in documenting the morphology of pollen and orbicules in Rubiaceae. In order to make our palynological data at generic level available for the entire (Rubiaceae) research community, we provide an online searchable database including general information on the genus (incl. phylogenetic position, distribution, number of species, photograph) and detailed descriptions of pollen morphological features and orbicule data if available. To date 488 genera (approx. 75%) are documented palynologically. The database has been implemented in MySQL over Linux server platform. PHP links the database to the web interface designed with Drupal. It can be accessed freely at <http://bio.kuleuven.be/sys/palrub>. We are positive that the tool will stimulate the application of palynological data in full evidence phylogenetic analyses. Moreover, the database can be useful for palaeopalynologists who encounter rubiaceous pollen in their samples and we hope it will stimulate palynological research within the family.

Phylogenetic studies in *Faramea* (Rubiaceae, Coussareeae) using morphologic and molecular data from *rps16* and ITS

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Faramea Aubl. is a neotropical Rubiaceae genus comprising approximately 200 species. Its main centres of diversity are found in Central America, especially Costa Rica and Panama, north-western South America, including Colombia, Ecuador, Peru, Venezuela and the Guianas and the Brazilian and Bolivian Amazon, and the Atlantic Forest of eastern Brazil. This work aims to test the monophyly of *Faramea* and *Coussarea*, evaluate the infrageneric classification of *Faramea* from the phylogenetic viewpoint, and to clarify the placement of the genus *Standleya* within Rubiaceae, subfamily Rubioideae, through molecular analysis of nuclear (ITS) and plastid (*rps16*) DNA, as well as morphologic data. It is concluded that: tribe Coussareeae forms a monophyletic group (BP 100%) including *Standleya*; *Faramea* is a monophyletic group with high support (BP 97% ITS, BP 98% *rps16*) both in individual and combined analyses; it is possible to recognize two heterotypic sections within *Faramea*: section *Hypochoasma* with high support (BP 100%), and section *Homalocladus*, with lower support level (BP 57%), while sections *Faramea* and *Tetramerium* did not form monophyletic groups. Further studies will be needed to provide a clearer understanding of phylogenetic and morphologic relationships between the genera of tribe Coussareeae.

Molecular phylogenetic analysis of the tribe Alberteae (Ixoroideae, Rubiaceae)

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The small tribe Alberteae has a complex taxonomic history, and has long been an artificial grouping of genera. In this study, phylogenetic analyses of five chloroplast DNA regions and the ITS regions of the nuclear ribosomal DNA, were done to assess the delimitation of the tribe. In the resulting phylogenetic hypothesis, several genera previously associated with Alberteae, are found nested within other tribes or form separate clades within Ixoroideae. Furthermore the genus *Alberta* is found to be paraphyletic as presently circumscribed, with the Malagasy species forming a sister group to *Nematostylis*. The taxonomic implications and the morphology of the tribe are discussed.

Taxonomical and ecological studies on the family Rubiaceae in Sudan

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Fifty six genera and 152 species within the family Rubiaceae are found in Sudan. Taxonomical and ecological studies were carried out on the family, including a checklist of the species, their distribution and economic importance. The study revealed that the diversity of the family is enormous with all sorts of habits from small cushion plants to large forest trees. Most of the species were found to be endemic to the equatorial region in Sudan. Coffee is by far the most economically important species in Sudan and there are two main species that are used in the production of coffee, *C. arabica*, generally known as Arabica coffee, and *C. canephora*. Also, four Rubiaceae species were reported for their folk uses as they are medicinal plants, *Gardenia ternifolia*, *Mitracarpus villosus*, *Nauclea latifolia*, and *Xeromphis nilotica*. There are also a number of ornamental species found within this family in Sudan, notably species from the genera *Gardenia* and *Ixora*.

Out of Africa: the tribe Sabiceae s.lat. (Rubiaceae)

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Based on ITS and *trnT-F* sequence data of 78 accessions, the tribe Sabiceae is circumscribed to comprise only four genera, *Hekistokarpa* (1 sp.), *Tamridaea* (1 sp.), *Sabicea* (c. 145 spp.), and *Virectaria* (8 spp.). In this circumscription, Sabiceae clearly falls within subfamily Ixoroideae, while the genera *Acranthera*, *Amphidasya*, *Pentaloncha*, and *Temnopteryx*, formerly associated with Sabiceae, fall within Rubioideae. *Sabicea* is monophyletic only if *Ecpoma*, *Pseudosabicea*, *Schistostigma*, and *Stipularia* are included. *Sabicea* could be shown to be one of the very few (15 out of 660) Rubiaceae genera with a true paleotropical-neotropical disjunction. In the Old World, *Sabicea* is centred in tropical Africa, with disjunctions in Madagascar (6 spp.), São Tomé and Príncipe (3 spp.) and Sri Lanka (1 sp.). In the New World, *Sabicea* is widespread from Central Mexico to Southern Brazil, and in the Caribbean. New World *Sabicea* species form a monophyletic group nested within the African species. Further research is needed (1) to test phylogenetic relationships between the hitherto poorly resolved clades of Old World *Sabicea*; (2) to identify the species most closely related to the New World clade; (3) to identify the New World species which are sister to all other New World species in order to understand the radiation of the genus in the New World; (4) to rigorously test the monophyly of the Madagascan and of the New World radiation; (5) to date the major nodes of the phylogeny of *Sabicea* and the Sabiceae; (6) to reconstruct the patterns of radiation in *Sabicea*; (7) to analyze the distribution of characters in *Sabicea*, and (8) to circumscribe monophyletic subgeneric partitions of this large genus.

Bacterial leaf symbiosis in Rubiaceae: distribution, characterization, and evolution

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Bacterial leaf symbiosis is an intimate interaction between bacteria and plants in which bacteria are harboured in healthy leaves. Two forms of symbiosis exist: bacteria occur free in the leaf mesophyll or they are restricted to leaf nodules. Leaf nodulation is rare and has only been reported in Dioscoreaceae, Myrsinaceae and Rubiaceae. The latter family contains the largest number of nodulating bacteriophilous species despite the fact that this type of bacterial leaf symbiosis is limited to three, not closely related genera, i.e. *Pavetta* (c. 250 nodulated species), *Sericanthe* (11 nodulated species), and *Psychotria* (73 nodulated species). Bacteria occurring free in the leaf mesophyll have hitherto only been reported from two genera of the Rubiaceae, i.e. *Vangueria* and *Fadogia*. Our research focuses on the Rubiaceae species producing bacterial nodules. Earlier research reports that bacterial leaf nodulation is both a cyclic and obligate interaction. Bacteria are transferred from one plant generation to the next through the seeds. In addition, there is strong evidence that the presence of the bacterial partnership is absolutely required for the host's survival. These two characteristics of bacterial leaf nodulation provide evidence to postulate strict coevolution between bacteria and host species. In a molecular study the bacterial endosymbionts of selected *Pavetta*, *Sericanthe*, and *Psychotria* species have been sequenced and identified as *Burkholderia*. Based on 16S rDNA and *recA* DNA regions, it is shown that the endosymbionts form a strongly supported monophyletic group. In order to test the presumed coevolution between bacteria and hosts, the phylogenetic relationships of continental African nodulated *Psychotria* species and their bacterial endosymbionts have been reconstructed based on respectively six (ITS, ETS, *rps16*, *trnLF*, *trnG*, *rps16-trnK*) and two (16S rDNA, *recA*) DNA regions. Parsimony and Bayesian analyses clearly prove the monophyly of continental African nodulated *Psychotria* species and their endosymbionts. It is also shown that a given plant species is inhabited by only a single species-specific *Burkholderia* species. However, comparison between the phylogenies of host species and endosymbionts does not give conclusive evidence for strict coevolution between plants and bacteria.

Woodiness within the Spermacoceae-Knoxieae alliance (Rubioideae, Rubiaceae): a case of reversal to the basal woody condition?

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The tribe Spermacoceae is a mainly herbaceous Rubioideae lineage, except for some species that can be called “woody” herbs or even small shrubs. The occurrence of herbaceous and woody species within the same group raises the question whether the herbaceous taxa are derived from woody taxa, as is the case in the basal Rubioideae, or vice-versa. Earlier publications favour the second option, which would mean that at least some woody Spermacoceae are secondarily woody, i.e. derived from herbaceous ancestors. We want to assess the origin of woodiness in Spermacoceae using two independent methods: (1) reconstructing a molecular phylogeny of the tribe, including a wide sampling of herbaceous and woody species, and (2) studying the microscopic wood anatomy of 22 Spermacoceae species. Furthermore, we aim to compare the wood structure of Spermacoceae with that of some members of its sister tribe Knoxieae. Our preliminary wood anatomical data in Spermacoceae support the available molecular sequence data, indicating that all woody species observed are secondarily derived. Well-known wood anatomical characters that demonstrate this shift from the herbaceous to the woody habit are the typical decreasing length-on-age curves for vessel element length, the presence of exclusively upright ray cells, or even the (near) absence of rays. These so-called paedomorphic wood features are not observed in the Knoxieae genera *Triainolepis* and *Placopoda* (now included in *Dirichletia*), which demonstrate that they are primarily woody.

Towards a wood anatomical overview of the Apocynaceae: searching for meaningful non-DNA characters within subfamily Rauvolfioideae

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Wood anatomical studies in the dogbane family (Apocynaceae) are fragmentary. This study represents a first attempt to unravel the phylogenetic significance and major evolutionary trends in the wood of the family, using the large subfamily Rauvolfioideae as a case study. Phylogenetic analyses of molecular sequence data have demonstrated that Rauvolfioideae are paraphyletic, and morphological characters previously used to define the tribes appear to be strongly homoplasious. Consequently, our major aim is contributing to the search for meaningful non-DNA characters at the tribal level, using LM and SEM observations of 91 species belonging to all ten tribes. We found that most of the current tribes are characterized by a unique combination of wood characters, such as vessel grouping, vessel element length, fiber type, abundance of uniseriate rays, and fused multiseriate rays, despite the co-occurrence of erect as well as climbing taxa in five tribes. Climbing rauvolfioid taxa can generally be distinguished from erect species by their wider vessels, tendency to form paratracheal axial parenchyma, and the presence of tracheids and laticifers. With respect to the entire family, there is a general phylogenetic tendency towards shorter vessel elements, more pronounced vessel grouping, higher tracheid abundance, more paratracheal parenchyma, and fewer axial parenchyma cells per strand in the more derived Apocynaceae. Most of these evolutionary patterns are likely to be triggered by drier environmental conditions and/or shifts from the erect to the climbing habit.

A revision of *Randia* (Rubiaceae) in Brazil

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Randia L. was contemporarily circumscribed as a neotropical genus with about 90 species. Gustafsson and Persson recognized three groups within *Randia*: one including species from Mexico, Central America and the Antilles, and two others from South America, one including mainly the lowland species and the other with strictly the Andean species. However, there are many morphological variations in species characters and the taxonomy of the genus is not stable. *Randia* comprises trees, shrubs or dioecious lianas characterized by thorns, conspicuous short branches, congested leaves and stipules, pollen in tetrads, unilocular ovaries with two parietal placentas and fruits with many seeds embedded in a sweet pulp that becomes dark when dry. Schumann in Flora Brasiliensis considered only one species with three varieties with occurrence in Brazil: *Randia formosa* var. *densiflora* K.Schum., *R. formosa* var. *nitida* K.Schum. and *R. formosa* var. *longiflora* (Ruiz & Pav.) K.Schum. In the same monograph he considered four species of *Basanacantha*, three described as new, that later were synonymized to *Randia*. Since then 25 names of *Randia* with probable occurrence in the country have been published. A review of Brazilian herbarium specimens of *Randia* indicates that at least 14 species and 1 subspecies are readily distinguished. The Amazonian region presents the highest diversity of species. *Randia armata* (Sw.) DC. and *R. calycina* Cham. have a widespread distribution in Brazil.

Pollen morphology of the Rubiaceae from Beijing

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Pollen morphology of twelve species (*Rubia chinensis* Regel, *R. cordifolia* L., *Galium pauciflorum* Bge., *G. aparine* L., *G. spurium* L., *G. boreale* L., *G. paradoxum* Maxim., *G. bungei* Steud., *G. linearifolium* Turcz., *G. verum* L., *Leptodermis oblonga* Bge., *Gardenia jasminoides* Ellis) belonging to four genera of Rubiaceae from Beijing was examined using light and scanning electron microscopy. The results show that the pollen grains are small to medium in size and spheroidal or subprolate in shape, with a few being prolate. Pollen morphology is remarkably diverse, particularly in aperture and exine ornamentation. The exine is usually perforate, finely reticulate or coarsely reticulate. Pollen data appear to be morphologically informative and useful for distinguishing species.

**Intertribal relationships within subfamily Cinchonoideae s.str.
(Rubiaceae)**

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Subfamily Cinchonoideae s.str. of Rubiaceae is characterized as small trees or shrubs with imbricate or valvate corolla aestivation and, often, dry fruits. The subfamily includes c. 1500 species in approx. 100 genera, predominantly from the Neotropics but also from the Old World tropics. Cinchonoideae s.str. is a monophyletic group and so far nine tribes are recognized (Cinchoneae, Chiococceae, Guettardeae, Hamelieae, Hillieae, Hymenodictyeae, Isertieae, Naucleaeae s.lat., and Rondeletieae). Despite several molecular studies of Rubiaceae, no study has yet focused on the entire Cinchonoideae s.str. However, sister group relations between some tribes have been shown, but further intertribal relationships are largely unknown, or resolutions are in conflict. In this study we aim to reconstruct the phylogeny of Cinchonoideae s.str. using five chloroplast markers (*atpB-rbcL* spacer, *ndhF*, *rbcL*, *rps16* intron, and *trnT-F*) to rigorously assess the tribal delimitations and relationships within the subfamily. Our intention is to include at least one representative of the genera traditionally and/or presently recognized. The results of the phylogenetic analyses will be presented and discussed.

Phylogenetic analysis of Hamelieae tribe (Rubiaceae) based on structural data

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The Hamelieae tribe has a controversial position within Rubiaceae and its circumscription varies from two to 11 genera depending on the author. A parsimony analysis based on structural characters was performed in order to test the monophyly of Hamelieae sensu Robbrecht and to evaluate the relationships within the tribe, as well as to evaluate the potential inclusion of *Syringantha* within Hamelieae. Seventy-five structural characters of 21 species representing *Deppea*, *Hamelia*, *Hoffmannia*, *Omiltemia*, *Pinarophyllon*, and *Plocaniophyllon* as potential members of the tribe, as well as *Bouvardia*, *Exostema*, *Hintonia*, *Psychotria* and *Randia* representing other tribes, were included in the analysis. Our results support the monophyly of Hamelieae only if *Syringantha* is included. The synapomorphies of the tribe are: petiole leaf trace type I, midvein having an arc of parenchyma cells, contorted corolla aestivation, yellow corolla, stamens adnate to the corolla tube base, and more than ten orthotropous ovules per locule with tannins in their integument. *Syringantha* and *Hamelia* are sister taxa, as suggested by previous authors. *Omiltemia* and *Deppea* were not recovered as sister. Instead, *Hoffmannia*, *Plocaniophyllon*, and *Pinarophyllon* are sister to *Omiltemia*, the relationship supported by the intervascular vestured type III, leaf trace type III, flowers with imbricate aestivation and triangular stamen filaments. The monophyly of all genera is recovered based on morphological characters suggested by previous authors as well as by new structural characters, such as: type of wood rays and intervascular vestured types in the stem; ramification pattern of the quaternary vein leaves, as well as leaf trace types; type of flower aestivation, and filaments and stigma shapes; fruit type and dehiscence. This study suggests that structural characters are phylogenetically informative within Hamelieae. An ongoing molecular study of these taxa will allow us to further test the relationships within Hamelieae and whether to include Hillieae within Hamelieae.

***Borreria* was inspiration for Darwin's Theory of Evolution**

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One hundred and fifty years after the publication of the *Origin of Species*, it is revealed that the Rubiaceae provided the stimulus for Darwin's Theory of Evolution and Natural Selection. The finches and tortoises of the Galapagos Islands are often credited with inspiring Charles Darwin on the Voyage of the H.M.S. Beagle to start thinking that species are not static, but are mutable. However, this was not really the case. In fact, it was Darwin's training in, and passion for botany that first led him to contemplate his evolutionary ideas while exploring the Galapagos. The birds he assigned to be the same as those on the mainland of South America. It was the plants, in particular the genus *Borreria*, which caught his attention when he first started noticing species variation among islands and habitats. It was not until later that he became aware of the variation among the birds and tortoises. This presentation will discuss the phylogeny of Spermaceae and in particular the evolution and derivation of the lineage on the Pacific Islands and new findings on the evolution of *Borreria* in the Galapagos Islands.

Out of Malesia: biogeography and molecular dating of the showy genus *Ixora* (Rubiaceae)

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Biogeographical and temporal histories within Rubiaceae subfamily Ixoroideae remain poorly investigated, despite the recent development of methods based on phylogenetic data. The large genus *Ixora* (Rubiaceae) has recently been re-circumscribed in a broad sense. *Ixora* has a pantropical distribution, with about 60 species in tropical America, 35 spp. in Africa, 40 spp. in Madagascar, 100 spp. in Continental Asia, 180 spp. in Malesia, and 100 spp. in Oceania. A recent phylogenetic study based on c. 80 *Ixora* species showed that the main lineages within *Ixora* form geographical units. In order to study the historic biogeography of the genus, we conducted a phylogenetic analysis based on an extended sampling of *Ixora* (c. 150 taxa) for nuclear ETS and ITS, and chloroplast *trnT-F* and *rps16* markers. A biogeographical analysis was performed based on a restricted phylogeny (c. 120 taxa), according to DIVA parameters. There seems to be two main lineages of *Ixora*, which both appear to have originated from the Malesian region: (Malesia((Malesia-Asia)(Mascarene(Neotropics(Madagascar-Africa-Madagascar)))))) and (Malesia(Fiji((New Caledonia)(Fiji-New Caledonia-Vanuatu-Polynesia))). Considering the sister group relationships between *Ixora* and the Malesian *Aleisanthia* lineage, the centre of origin of *Ixora* is more likely resolved in Malesia. Based on the same restricted sampling of *Ixora*, molecular dating analysis was performed using a relaxed molecular clock constrained by pollen fossil and geological event ages. The age of *Ixora* is about 15 Myrs and the diversification within the genus started c. 10 Myrs ago. Individualization of early *Ixora* lineages in Malesia and Asia occurred during late Miocene. Colonization of the other tropical areas (Mascarenes, Madagascar, Africa, America, Pacific Islands) occurred during early Pliocene.

Gardenieae, extravagant Rubiaceae with problematic relationships

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The pantropical tribe Gardenieae *s.lat.* comprises c. 70 genera of various sizes in terms of species number. With more than 1000 species, Gardenieae forms a species rich and very diverse group. Among these species, interesting morphological and ecological features suggest an important plasticity and a high rate of evolution, as the occurrence of the largest flowers and fruits of Rubiaceae and several reproduction types. The members of the tribe have left contorted corolla aestivation and generally berry-like fruits. The circumscription of Gardenieae has been discussed and investigated for a long time based on morphological and molecular data. Since it was described, many new genera have been included, mainly from the tropics. A high number of monotypic genera or genera with few species have been recognized during the last century, most of these are segregates from the genera *Gardenia* and *Randia*. In contrast to the morphological diversity, recent molecular studies have shown rather low levels of nucleotide substitutions within the chloroplast markers investigated. The low amount of information has resulted in poorly resolved phylogenies, although tree topologies have always indicated Gardenieae as polyphyletic, but with all clades included in the core Ixoroideae. In order to clarify the phylogeny and classification of the Gardenieae *s.lat.*, we investigated representatives of Bertiereae, Coffeae, Cremasporeae, Gardenieae, Octotropideae, and Pavetteae, using five plastid gene regions (*rbcL*, *trnT-F*, *rps16*, *ndhF*, and *atpB-rbcL*). We have included an extensive sampling as we investigate one representative species per genus (preferably the type species), out of the c. 160 genera recognized in the core Ixoroideae complex. Special attention was paid to the numerous small African, American, Continental Asian, and Malesian genera, poorly known and never included in phylogenies so far. According to the newly inferred relationships, adjusted tribal limits within core Ixoroideae are proposed. Problematic positions of several genera such as *Burchellia*, *Didymosalpinx*, and *Schumanniophyton* are discussed in the light of their morphology.

The *Hedyotis-Oldenlandia-Kohautia* complex (Rubiaceae) in Nepal: Fruit, seed, and pollen characters and their taxonomic significance

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Pollen of eleven species and seed morphology of twelve species of Nepalese representatives of the *Hedyotis-Oldenlandia-Kohautia* complex (Hedyotideae; Rubiaceae) have been investigated by scanning electron and light microscopy. Members of the complex are found to show remarkable variation in fruit, seed and pollen features. Pollen grains are all colpate with the aperture number varying from 3-4 or occasionally 5. The ectoaperture is a colpus, whereas three types of endoapertures have been observed: endocinguli, lalongate endocolpi and costa endopori. Sexine reticulation is variable, being perforate, reticulate, or microreticulate. Three species have a double reticulum. Supratectal elements are generally absent; however, in some species granules are present on the inside of the muri. Seeds are non-crateriform, small and numerous per capsule. Three types of seed are distinguished based on shape: (1) lenticular with a narrow wing-like margin, (2) trigonous, and (3) globose/subglobose. Trigonous seeds further exhibit marked variation in color, size, and shape. The data from pollen and seed morphology were combined with information on fruit dehiscence types. Based on this information five natural groups could be identified within the complex. Generic status for all three genera (*Hedyotis*, *Oldenlandia*, and *Kohautia*) is maintained in this study. Morphology of fruit dehiscence, seed and pollen proved to have a great taxonomic value in the complex in delimiting supra- and infrageneric groups. Future studies will incorporate these data into a phylogenetic framework. This project will focus on the Asian species of *Hedyotis* and their close relatives and be used to help define generic and species delimitations in the complex using both molecular and morphological data.

Phytogeography of Central Africa based on distribution patterns of Rubiaceae

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The “Flore d’Afrique centrale” area (DR Congo, Rwanda and Burundi) is characterized by a unique and very diverse flora. These three countries harbour a large part of the Central African rainforest, which is the second largest area of tropical forest in the world. These forests form the natural habitat for notable conservation worthy species such as chimpanzee, bonobo, mountain gorilla, okapi and white rhino. However, the region includes much more than rainforests. In total 14 of the 119 ecoregions in Africa are represented, of which six are almost restricted to this area. Better understanding of the vegetation of the “Flore d’Afrique centrale” area, including better chorological knowledge, is therefore very important from a conservation point of view. Several, partially conflicting, subdivisions of the “Flore d’Afrique centrale” area in phytogeographic territories have been proposed in the past. These subdivisions, however, were not the result of a formal analysis of distribution patterns of taxa. In the present poster, we outline our study which will analyse distribution patterns of taxa belonging to the subfamily Rubioideae (Rubiaceae) in view of a chorological subdivision of the area. In total more than 15000 herbarium records have been databased. These plant voucher specimens are permanent, reliable and verifiable records of the presence of a species at a given time and place. Additionally, label data often provide detailed ecological information about the collections and hence about the species they represent. Consequently, the entire set of specimens is a witness of the past and present vegetation and plant diversity of this region. The subfamily Rubioideae was chosen for our study as the group is well represented throughout the study area and members of the subfamily can be found in a large number of ecological niches. The distribution data will be analysed using ArcView 3.3, Maxent, Worldclim 1.4 and MVSP 3.1.

Phylogenetic position of the monotypic genus *Carterella* (Rubiaceae)

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Carterella Terrell is a monotypic genus endemic to a restricted area in the Baja California Peninsula, Mexico. It has been placed in tribe Hedyotidae, together with *Houstonia*, *Hedyotis* and *Oldenlandia*, among other genera. However, its phylogenetic position has not yet been established and moreover its taxonomic status remains controversial. The only species classified within the genus was transferred from *Bouvardia* and it has also been transferred to *Hedyotis*. Furthermore, *Carterella alexandrae* (A.M.Carter) Terrell was retrieved in a clade with two Mexican species of *Stenotis*, nevertheless, the floral morphology is similar to *Bouvardia*. To establish the taxonomic status and phylogenetic position of *C. alexandrae*, results from a parsimony analysis using DNA sequences from *trnL-F*, *matK*, and *rbcL*, and including representative taxa of Hedyotidae as well as some *Bouvardia* species are presented.

Genetic variation of *Palicourea padifolia*, a shrub pollinated and dispersed by birds

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Heterostyly is a genetic polymorphism characterized by the reciprocal placement of stigmas and anthers in flowers of animal-pollinated plants. Two classes of heterostyly can occur depending on whether there are two (distyly) or three (tristyly) floral morphs within plant populations. In distylous species, populations typically contain plants that produce either all long-styled (LS) flowers with the stigmas positioned above the anthers or all short-styled (SS) flowers with the anthers positioned above the stigma. Distyly has been classically viewed as a polymorphism that promotes disassortative pollen flow between anthers and stigmas of floral morphs. However, distylous morphs often vary in a number of other floral characters promoting differential pollinator visitation or efficiency, pollen donation and receipt, and therefore, male and female plant reproduction. In this study we used two chloroplast markers to investigate the consequences of seed dispersal on the genetic variation and population structure of *Palicourea padifolia*, a self-incompatible, distylous, hummingbird-pollinated shrub. We found low levels of population structure and high levels of genetic diversity within populations, suggesting gene flow among populations. Most genetic variation was explained by differences among individuals within populations and by differences between groups that corresponded to populations in different geographic areas. Our preliminary results suggest high levels of seed dispersal and that habitat fragmentation does not seem to affect the genetic diversity and gene flow of the species.

A chronicle of a death foretold: androecium development in distylous, hummingbird-pollinated *Palicourea padifolia* (Rubiaceae)

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Programmed cell death (PCD) occurs early in sexual development in the selective abortion of primordia for one or the other sexual organ in some unisexual plants and during development of the male and female reproductive organs and gametes. Although genetically PCD occurs in some specific plant cells (i.e., formation of unisexual flowers), its occurrence has been rarely documented in hermaphrodites where PCD during meiosis will cause cell degeneration and sterility. Understanding mechanisms involved in the maintenance of floral dimorphism among distylous natural populations leading to gender specialization requires evidence concerning differential phenotypic expression between floral morphs and/or male sterility. Within this context, we describe the development of anthers and pollen grains in short- (SS) and long-styled (LS) flowers of *Palicourea padifolia*, and combine embryological and ecological evidence to explore the developmental basis and the mechanism that could maintain male sterility. Our developmental study showed manifestations of male sterility in *P. padifolia*. Stamens are well formed but pollen grains are deformed or absent because microsporogenous cell abortion occurred before or after meiosis. Most abnormalities occurred in the anthers of SS flowers, in the microspore mother cells, and during tetrad formation and young pollen grain stages. Furthermore, the presence of sterile pollen grains in locules with apparently normal development of anther wall layers is evidence of failure in the later stages of pollen development. In normal pollen development, shortly after microspore release from the tetrad and before mitosis, tapetal cells begin to degenerate to death. Presumably, this PCD occurs before microspores are released from the tetrad for proper microspore development and/or for their differentiation into functional pollen grains. Alternatively, abnormal microsporogenesis found early in development, at the premeiotic stage in the microspore mother cells, indicate that the effects of male sterility are expressed early in the development of SS pollen grains.

Leaf architecture of the genus *Didymaea* (Rubiaceae)

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We examined 532 specimens representing eight species of *Didymaea* (Rubiaceae) to describe their leaf architecture. Characters observed in the herbarium specimens and cleared leaves were leaf base, apex, and margin; trichome type and distribution; vein orders from primary to higher order veins, veinlets, and ultimate marginal vein. Six morphometric variables were analyzed through variance and principal component analyses to identify which characters are useful to distinguish species. Most of the species have a brochidodromous venation pattern, except *D. hispidula* that has an eucamptodromous venation pattern; marginal ultimate venation is looped or incomplete and veinlets are scarce. *Didymaea floribunda* is unique in having cylindrical and bicellular trichomes. Principal component analyses revealed that 78% of the total variation is mainly related to leaf maximum width. Variance analysis showed significant differences between *D. microflosculosa* and the remaining species. The species with largest lamina have the lowest areolar density (*D. microflosculosa* and *D. hispidula*), while one of the species with smallest lamina (*D. crassifolia*) has one of the highest areolar densities. Architectural features were useful for differentiating all the species in the genus and so, an identification key for the species of *Didymaea* based only on leaf characters is presented.

New findings in the Catesbaeeae Chiococceae Complex based on preliminary ITS data of Cuban taxa

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The classification of the Catesbaeeae and Chiococceae tribes was recently studied and many issues debated in the past now seem settled. The Catesbaeeae-Chiococceae Complex (CCC) includes approximately 28 genera and 190 species primarily concentrated in the Greater Antilles, Central and South America, and in the western Pacific. Earlier molecular studies have shown the CCC to be monophyletic although several genera (*Bikkia*, *Chiococca*, *Exostema* and *Solenandra*) within the complex have been shown to be poly- or paraphyletic as presently treated. Additionally, the monophyly of some genera still could be described as untested since sampling in some genera was limited. Spurred by an extensive collecting trip in Cuba, phylogenetic sampling has been expanded to include the yet unplaced genus *Ceuthocarpus* and increased sampling in the large genera *Catesbaea*, *Exostema*, *Phialanthus*, *Schmidtottia*, and *Scolosanthus*. One genus and 22 species from several genera not included in earlier phylogenetic trees have been included in the present study. The present results are part of an ongoing research project to further increase our understanding of the relationships within the CCC and its unusual biogeographic patterns.

Diversity of Rubiaceae in the dry interior of Paraíba, northeast Brazil

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The dry interior (Sertão) of Paraíba encloses an area of 22,720 km², in the arid Caatinga, a heterogeneous vegetation found in areas of low water availability for most of the year. The Caatinga has a high diversity of xeric species, some of them endemic, that lose their leaves during dry periods. Studies of the Caatinga flora in the state Paraíba have been underway for some years, with the aim of protecting, preserving, and maintaining the biodiversity of this environment. The family Rubiaceae is distinguished for the rich number of species in the region. To elaborate a checklist of these species, we surveyed the specimens deposited in the Herbarium of the Federal University of Paraíba (JPB). The identifications were carried out through detailed analyses of specimens, consulting diagnoses and descriptions in the literature, types or phototypes, by means of analytical keys in the literature, and revision of historical collections in other herbaria. Currently, the Rubiaceae are represented in the Caatinga of Paraíba by 13 genera and 22 species, one probably new to science: *Borreria latifolia* (Aubl.) K.Schum., *B. ocyimifolia* (Willd. ex Roem. & Schult.) Bacigalupo & E.L.Cabral, *B. scabiosoides* Cham. & Schltld., *B. verticillata* (L.) G.Mey., *Borreria* sp., *Chomelia intercedens* Müll. Arg., *Cordia* sp., *Coutarea hexandra* (Jacq.) K.Schum., *Diodia apiculata* (Willd. ex Roem. & Schult.) K.Schum., *D. teres* Walter, *Guettarda platyphylla* Müll. Arg., *G. sericea* Müll. Arg., *Machaonia spinosa* Cham. & Schltld., *Manettia cordifolia* Mart., *Mitracarpus frigidus* (Willd. ex Roem. & Schult.) K.Schum., *M. hirtus* (L.) DC., *Randia armata* (Sw.) DC., *R. nitida* (Kunth.) DC., *Richardia grandiflora* (Cham. & Schltld.) Steud., *Staelia virgata* (Link ex Roem. & Schult.) K.Schum., *Tocoyena formosa* (Cham. & Schltld.) K.Schum., and *T. sellowiana* (Cham. & Schltld.) K.Schum. An analytical key, comments and geographic distribution of taxa are presented.

Paraphyly of the pantropical genus *Morinda* and its taxonomic implications

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A recent molecular phylogenetic study on the subfamily Rubioideae (Rubiaceae) has led to establishment of new tribal limits for the largely tropical Psychotrieae alliance. Morindeae has been recircumscribed in a narrow sense to include only six genera (*Appunia* Hook.f., *Coelospermum* Blume, *Gynochthodes* Blume, *Morinda* L., *Pogonolobus* F.Muell., and *Syphonandrium* K.Schum.). The two genera of the *Mitchella* group, the East Asian genus *Damnacanthus* Gaertn.f., and North American and East Asian genus *Mitchella* L., are currently classified in the newly described tribe Mitchelleae Razafim. & B.Bremer. We conduct Bayesian and parsimony phylogenetic analyses of 72 Morindeae taxa using sequence data from two chloroplast gene (*rps16* intron and *trnT-F*) and *nrITS* regions to test the monophyly of the pantropical genus *Morinda*. The results of this study are presented and discussed.

Phylogeny and tribal limits of the Psychotrieae alliance (Rubiaceae)

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The Psychotrieae alliance is a predominantly woody group consisting of c. 3000 species (c. ¼ of Rubiaceae) belonging to c. 54 genera, which have been previously classified in four to six tribes. We perform Bayesian and parsimony analyses of sequence data of 58 Rubioideae (Rubiaceae) taxa from five plastid gene and nrITS regions to rigorously assess the phylogenetic relationships and tribal limits in the Psychotrieae alliance. The results of this study are presented and discussed.

Woody Rubiaceae diversity in the tropical montane rain forest from La Chinantla, Oaxaca, Mexico

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Floristic information and altitudinal gradient analysis is presented for c. 70 species (13 genera) of woody Rubiaceae occurring in the tropical montane rain forest from the La Chinantla region, Northern Oaxaca Range. This area, ranging in elevation from 600 to 2,600 m a.s.l., is one of the most humid in the country. The floristic and structural data (abundance and richness per unit area for 39 species) confirm the biological importance of the region, which has a high degree of endemism and from where several new taxa have recently been described. Within this region, the area of Santa Cruz Tepetotutla community has a considerable proportion (c. 80%) of its area (11,250 ha) occupied by tropical montane rain forest. These forests, connected through a vegetation gradient, are very well preserved. A total of 1,645 collections from this area produced a list of 657 species of vascular plants; within Magnoliopsida, 74 families and 322 species of trees and shrubs are present. Rubiaceae is one of the most diverse families with 33 species, among which only seven are herbaceous. Only Lauraceae and Melastomataceae have a greater richness of woody species (30 and 28, respectively). Asteraceae is the only family with woody representatives that outnumber the Rubiaceae diversity, with 39 species (17 woody) while Piperaceae (30 species, 19 woody) follows it. In terms of herbaceous species, Orchidaceae is by far the most diverse family with 54 species. Despite the floristic importance of the family for the area's diversity, Rubiaceae only moderately contributed to the forest structure in Santa Cruz Tepetotutla. Only nine woody species had individuals with DBH \geq 2.5 cm. In all cases, the individuals belong to low abundance species, only contributing 0.2-5% of total density; in no site did Rubiaceae contribute more than 1.3% of the total basal area. Comparisons of Rubiaceae diversity with other tropical forests in Mexico and worldwide are presented. These comparisons demonstrate the importance of Rubiaceae in the tropical montane rain forest of La Chinantla in terms of woody diversity. Our results suggest that the proportion of woody Rubiaceae in these forests can serve as an indicator of the ecosystem's degree of conservation.

Diversity of Rubiaceae in the Cariri region of Paraíba, northeast Brazil

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The Rubiaceae known from Brazil comprise 130 genera with about 1500 species present in all natural regions with the highest diversity in the Atlantic Coastal forest. In the northeast region of the country, with most of its area in a semi-arid climate, Rubiaceae are also numerous with about 70 genera and 310 species. Despite its botanical and economic importance floristic and taxonomic studies within the family in Brazil and particularly in the northeast are still very few. The Cariri Paraibano encloses an area of 1689 km² located in a depression of the Borborema plateau. The climate is semi-arid, with concentrated and scarce rain fall and occurrence of long drought periods. The average annual rainfall is 350 mm, the lowest in Brazil. It is considered one of the high priority areas for the conservation of caatinga. A study of the flora and vegetation of the Cariri region was carried out in order to support strategies for conservation and sustainable use of the region's caatinga. In the floristic survey 14 genera and 21 species of Rubiaceae were observed: *Chiococca*, *Cordia*, *Coutarea*, *Leptoscella*, *Manettia*, *Oldenlandia*, *Richardia*, *Staelia*, *Randia* (1 species), *Diodia*, *Guettarda*, *Tocoyena* (2 species), *Borreria* and *Mitracarpus* (3 species). Keys for determining the species, descriptions, distributions, specimens studied, illustrations and synonymies for all species are provided.

Spermacoceae tribe *s.str.*: delimitation of the genus *Staelia*, rehabilitation of *Tessiera* and description of a new genus from the central Planalto of Brazil (Rubiaceae)Roberto M. Salas & Elsa L. Cabral

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During the study of the American genus *Staelia* Cham. & Schldtl. we noticed that some species classified in this genus do not exhibit its diagnostic characters, perhaps reflecting the paraphyletic nature of *Staelia*. Therefore, we studied all species included in *Staelia* to determine which of them fit in *Staelia s.str.* and to relocate the remaining taxa in other genera of Spermacoceae. The most problematic species are the ones endemic to Mexico. In order to determine the relationships between the species we studied the type specimens and found that *Tessiera* and *Diphragmus* are homotypic synonyms. In spite of the close historical relationship between *Tessiera*, *Diphragmus* and *Staelia*, contemporary authors treated *Tessiera* and *Diphragmus* as synonyms of *Spermacoce* L. and not of *Staelia*. In order to contribute to the clear separation of these taxa, we studied pollen morphological, carpological and seed morphological characters. We noticed that the Mexican species display many differences that we consider sufficient to separate them from *Staelia*. Therefore, we propose to rehabilitate the genus *Tessiera* with the election of *T. lithospermoides* DC. as type species. A new combination is made too and a new species is described from Acapulco, Mexico. Finally, another group of species, which includes two endemic taxa of the Central Brazilian high plateau, is excluded from *Staelia*; we propose to include them in a new genus. Consequently, we present the description of this new genus and we discuss its differences from the potentially related Spermacoceae genera. Two new combinations are made and a new species is described, endemic to the state of Goiás, Brazil.

The phylogeny of Urophyllaeae

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We used cpDNA (*rps16* and *trnTF*) and nrDNA (ITS and ETS) to produce a robust phylogeny of the pantropical tribe Urophyllaeae in order to address questions about its evolutionary history, biogeography, and classification. The overall topologies of trees based on cpDNA and nrDNA data are congruent and strongly supported. *Temnopteryx* is the sister of the remaining Urophyllaeae, which is shown to consist of three subclades; one American, one African, and one South East Asian clade. The large African genus *Pauridiantha* is monophyletic only if several small genera, such as *Commitheca*, *Poecilocalyx*, and *Stelechantha*, are included. The Asian *Urophyllum* is also paraphyletic and includes the genera *Pravinaria*, *Praravinia*, *Maschalocorymbus*, and *Pleiocarpidia*. There are, however, some areas where cpDNA and nrDNA trees are incongruent. The strength of these conflicts, as well as possible explanations for them, will be discussed. Results from a biogeographic analysis, testing the hypothesis that Urophyllaeae originated in Africa and later dispersed to Asia and the New World, will also be presented.

Synopsis of the genus *Mitriostigma* (Rubiaceae) with a new monocaulous species from southern Cameroon

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Mitriostigma is a continental African genus comprising four species. Both morphological and molecular data place the genus in the tribe Gardenieae of the subfamily Ixoroideae. The genus is closely related to *Oxyanthus* from which it mainly differs in the shorter corolla tubes, the apiculate anthers, and the often winged stigmatic clubs. During field work in South Cameroon, a new *Mitriostigma* species was collected. Its floral characters are in agreement with what is observed in the other *Mitriostigma* species, but the novelty is easily separated by its monocaulous growth form, the other species being shrubs or subshrubs. Another unique character for the species is its supra-axillary inflorescences paired at the nodes. The new species is related to *Mitriostigma barteri*, from which it further differs in the larger leaves with a more pronounced acumen.

Molecular phylogeny of tribe Rubieae, subfamily Rubioideae

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Tribe Rubieae is a group of temperate herbs in subfamily Rubioideae comprised of 11 genera and approximately 675 species that are characterized with whorls of leaves and leaf-like stipules. Previous research using the chloroplast *atpB-rbcL* intergenic region discovered 5 major clades within the tribe and the paraphyly of 2 genera, *Galium* and *Asperula*. We have increased sampling within the tribe to 130 taxa among 9 genera utilizing four chloroplast regions (*trnL-trnF*, *trnF-ndhJ*, *rpoB-trnC*, and *trnC-psbM*). Our results correspond with previous molecular analyses. Five strongly-supported clades within Rubieae are the *Rubia*, *Sherardia*, *Asperula* sect. *Glabella*, *Asperula* sect. *Asperula*, *Cruciata*, and *Galium* sect. *Galium* clades. The genus *Rubia* is monophyletic and the only member of the *Rubia* clade. The *Sherardia* clade includes the genera *Sherardia*, *Callipeltis*, *Crucianella*, *Phuopsis*, and members of *Asperula* sect. *Cruciana*, *Cynanchica*, *Hexaphylla* and *Thliphthisa*. The *Asperula* sect. *Glabella* clade includes *Asperula* sect. *Glabella* and *Galium* sect. *Aparinoides*. The *Asperula* sect. *Asperula* clade includes members of *Asperula* sect. *Glabella* in addition to *Asperula* sect. *Asperula*. The *Cruciata* clade includes the genera *Cruciata*, *Valantia*, and *Galium* sect. *Baccogalium*, *Bataprine*, *Depauperata*, *Lophogalium*, *Platygalium* and *Relbunium*. The *Galium* sect. *Galium* clade includes *Galium* sect. *Galium*, *Hylaea*, *Leiogalium*, *Leptogalium*, *Kolgyda*, and *Orientigalium*.

***Craterispermum*, an enigmatic Afro-Madagascan Rubiaceae genus**

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Craterispermum Benth. (subfamily Rubioideae) is a tropical Afro-Madagascan genus comprising less than twenty species. It is easily recognized by the, often compact, (supra-)axillary inflorescences and by the typical yellow colour of the dried plants, which is the result of aluminium accumulation. The genus is badly known taxonomically. No recent monograph of *Craterispermum* exists and identification of plant material to species level is difficult. The taxonomic position of *Craterispermum* also remains unclear: recent molecular studies place it in the monogeneric tribe Craterispermeae close to Morindeae. Testament to this isolated position, *Craterispermum* possesses a number of highly derived characters, such as its unique discontinuous exotesta. The exotesta cells are thin-walled except for ring-like thickenings extending around the entire cell (inner and outer tangential and radial walls). We have started a multidisciplinary study to unravel the secrets of this enigmatic genus. Our aims are: to produce a monograph of *Craterispermum*; to study its biogeography; to investigate its morphological, anatomical, palynological, ecological and biological characters; to reveal the phylogenetic relationships between its species and to produce an evolutionary hypothesis for the genus. Furthermore, we wish to understand the underlying processes and function of aluminium accumulation. The revision will follow normal practice of herbarium taxonomy. In order to produce a well-founded phylogenetic hypothesis for the genus we will integrate data from different sources: sequencing of chloroplast and nuclear markers, genome comparison, chromosome studies, character research, and biogeography. Aluminium accumulation will be charted using “the aluminon” test on both living and dried leaves and the “Chrome Azuro1-S Solution” test on wood tissues. The molecular aspects of aluminium accumulation will be investigated using two approaches: study of the biochemical components implicated in the chelation of aluminium, and identification of genes involved in this process.

Cladistic and phenetic analysis of relationships in *Galium* species (Rubiaceae) by morphology and PCR-RAPD

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Variation of 65 multistate morphological characters and PCR- RAPD data among thirteen *Galium* species in comparison with *Rubia tinctorum* and *Asperula odorata* is described and analyzed with cladistic parsimony and phenetic neighbour-joining methods by using two different ways of coding. Morphological analyses showed that *Galium* is monophyletic and revealed subgroups in Flora Iranica. Parsimony analysis of PCR-RAPD as presence/absence characters revealed three monophyletic clades: 1) *G. elegans*, 2) *G. odoratum*, and 3) species belonging to section *Orientalium*. N-J analysis of PCR-RAPD data revealed the same three basic groups differing only in the relative position of some species in subclusters. Phylogenetic congruence and discordance among morphological and PCR-RAPD analyses, coding ways, homoplasy and weighting of characters are discussed.

The neotropical Palicoureeae-Psychotriaceae today, a significant part of the world Rubiaceae flora

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In 1996 the Psychotriaceae was surveyed for the Neotropics, and comprised eleven genera with nine of them wholly neotropical. Today's overview of this group incorporates recent work by a number of Rubiaceologists in a more detailed and rather different classification. Of the previous survey's genera, *Pagamea* is now included in Gaertnereae, *Declieuxia* is included in Coussareae, three former species of *Psychotria* now constitute *Ronabea* in Lasiantheae, and *Pagameopsis* has been excluded from the tribe. The small Guyana Highlands genera *Aphanocarpus* and *Coryphothamnus* are still poorly known and only doubtfully included in Psychotriaceae. This group in the Neotropics now is treated in two tribes, the Psychotriaceae including *Psychotria* (i.e., *Psychotria* subg. *Psychotria*) with c. 200 species out of a larger pantropical group, and the Palicoureeae. Palicoureeae includes *Geophila* with c. 5 species out of a larger pantropical group; *Rudgea* with c. 140 neotropical species; *Palicourea s.lat.* (including *Psychotria* subg. *Heteropsychotria*) with c. 600 neotropical species; *Notopleura* with c. 100 neotropical species; *Margaritopsis* with c. 30 species out of a larger pantropical group; and *Carapichea* with c. 20 neotropical species of a group that is probably also represented in southeastern Asia. The monotypic Amazonian genus *Stachyococcus* belongs with either *Palicourea (s.lat.)* or *Carapichea*. The greatest diversity of genera is in northeastern and Amazonian South America; the greatest number of species is in the wet middle elevations of the northern Andes and Central America. With lineages throughout the tribe more clearly delimited, some intriguing morphological convergences are evident. Understanding and classification of this group are complicated by the particular considerations that accompany large genera and species-rich lineages.

Genetic diversity of wild *Coffea arabica* in Ethiopia: molecular analyses as a basis for conservation and sustainable use

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The CoCE project (“Conservation and use of the wild populations of *Coffea arabica* in the montane rainforests in Ethiopia”) aims to find solutions for the conservation and sustainable use of wild coffee populations in Ethiopia. Wild *Coffea arabica* is highly endangered by increasing settlement and land-use pressure on the montane rain forests. Therefore, the importance of conservation of these montane rain forests can be viewed against the background of man-made destruction or change in about 60% of the Ethiopian forests during the last thirty years. As the wild populations of *Coffea arabica* are part of the forests, their conservation can only be guaranteed if these montane rainforests themselves are protected. Based on a sampling of about 300 individuals from well documented forest populations, genetic diversity of *Coffea arabica* in Ethiopia was assessed. Populations of *C. arabica* were compared throughout forests of Ethiopia using ISSRs. This interregional analysis reveals complex geographical patterns of genetic diversity, with most regions possessing their own genotypes. Adding Ethiopian landraces (farmer’s varieties) showed that wild populations of *C. arabica* are genetically different and can be distinguished from semi-domesticated plants. In previous studies, different Ethiopian individuals of *C. arabica* were subsumed under “spontaneous material”. Interregional patterns of genetic diversity in wild *C. arabica* indicate that a hierarchical-geographical structure is obscured by naturally occurring gene flow. Moreover, dense sampling of *C. arabica* with ISSRs in forests of Berhane Kontir and Yayu (Geba Dogi) shows a fine-scale spatial patterning of genotypes within wild populations. Currently, work is focusing on nuclear microsatellites with the aim to establish a co-dominant marker system that allows assessing heterozygosity and gene flow in wild *Coffea*. Extensive screening of chloroplast microsatellites and other rapidly evolving spacers and introns did, however, not encounter any infraspecific variation within *C. arabica*. Results of the CoCE project provide a first assessment of genetic diversity of *C. arabica* in forest and semi-forest systems throughout Ethiopia. Molecular data support the existence of truly wild populations of *C. arabica* that differ from semi-domesticated plants, and underscore the need of conserving forest coffee. Effective strategies that include different *C. arabica* genotypes require multiple sites for in situ conservation complemented with ex situ measures.

Assessing the phylogenetic utility of Glutamine Synthetase and Malate Synthase in the genus *Tricalysia* (Rubiaceae)

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Low copy nuclear genes have been shown to provide valuable sequence data for phylogenetic analyses, particularly at lower taxonomic levels. The widespread use of low copy nuclear genes has been hampered by difficulties in isolating and characterising these regions, as compared with plastid and nuclear ribosomal DNA. However, there are several advantages to the use of low copy nuclear genes, including a higher rate of molecular evolution than is present in plastid regions. In studies where plastid data or nrDNA are unable to generate sufficient resolution to formulate robust phylogenetic hypotheses, low copy nuclear genes may provide an essential additional source of phylogenetic information. The Afro-Madagascan genus *Tricalysia* A.Rich is one such example, where plastid and ITS data are insufficient to reconstruct a well-resolved phylogeny. Here we present provisional data for the phylogenetic utility of two low copy nuclear genes; plastid expressed Glutamine Synthetase and Malate Synthase. Their potential use in phylogenetic studies of *Tricalysia* is evaluated in comparison with a combined plastid dataset, comprised of five regions (*accD-psa1*, *petD*, *rpl16*, *trnL-F*, *trnS-fm*), and with nuclear ribosomal ITS.

The tribal placement of the monospecific tropical African genus *Petitiocodon* (Rubiaceae) based on molecular data and morphology

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A first phylogenetic placement of *Petitiocodon* based on molecular sequence data from three plastid regions (*accD-psa1*, *rpl16* and *trnL-F*) is presented, in conjunction with a reassessment of morphology for the genus. Our results do not support an evolutionary affinity between *Petitiocodon* and *Tricalysia* (Coffeae), as suggested by previous studies, but they confirm other research that *Petitiocodon* and *Didymosalpinx* are distinct genera. Placement of *Petitiocodon* in tribe Octotropideae is well-supported on the basis of molecular data, which is concomitant with the possession of key floral and carpological characters for this genus.

**Pollen flow and female reproductive success in the distylous plant
Palicourea demissa (Rubiaceae)**

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Distyly is a polymorphism in which plant populations are composed of two floral morphs (long-styled and short-styled) that differ reciprocally in the heights of anthers and stigmas in flowers (reciprocal herkogamy). Distyly is suggested as one of the pathways leading to the evolution of separate sexes in flowering plants. This transition involves increasing gender specialization of the long-styled and short-styled morphs, in some cases converting them into female and male plants. Little is known of the selective mechanisms that are involved in this transition. In this work, we assess if differences in receptivity of pollen promotes variations in fruit and seed production between long-styled and short-styled plants of *Palicourea demissa* (Rubiaceae), a hummingbird-pollinated shrub in Venezuelan Andean cloud forests. Our results indicate asymmetric pollen flow between morphs, since the long-styled morph was more efficient in pollen donation, while the short-styled morph was more efficient in pollen reception. Therefore, short-styled plants produce more fruits and seeds than long-styled plants. This suggests that long-styled plants transmit more genes to offspring through pollen donation while short-styled plants transmit it more through seeds. This also supports the hypothesis of an evolutionary pathway from distyly to dioecy.

Survey of Rubiaceae of the Golfo Dulce area, Costa Rica

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The Golfo Dulce area in southern Costa Rica is one of the most species-rich regions of Central America and worldwide. Taxonomic publications and additional collections made since the last publication of the Rubiaceae diversity for this region require several additions and changes. The new combinations, name changes and additional species are presented and discussed, and a new checklist for the 156 species and subspecies of Rubiaceae documented for the region is provided.