PROCEEDINGS

OF THE 7TH INTERNATIONAL CONFERENCE ON COMPARATIVE BIOLOGY OF MONOCOTYLEDONS

(MONOCOTS VII)

BOTANICAL ESSAYS FROM
LANKESTER BOTANICAL GARDEN
2024

Chaubardiella pacuarensis Jenny

by Sylvia Strigari

PROCEEDINGS
OF THE 7TH INTERNATIONAL CONFERENCE ON COMPARATIVE BIOLOGY OF MONOCOTYLEDONS
(MONOCOTS VII)

INCLUDING THE

3RD WORLD CONGRESS OF BROMELIACEAE EVOLUTION
(BROMEVO III)

SAN JOSÉ, COSTA RICA, MARCH 11TH – 15TH, 2024
Costa Rica, where nature is always nearby

EDITED BY

Adam P. Karremans, Diego Bogarín and Franco Pupulin

LANKESTER BOTANICAL GARDEN
UNIVERSITY OF COSTA RICA

BOTANICAL ESSAYS FROM LANKESTER BOTANICAL GARDEN

Volume 1

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FOREWORD

The 7th edition of the International Conference on the Comparative Biology of Monocotyledons has been a milestone in the history of Lankester Botanical Garden, a research center of the University of Costa Rica. MONOCOTS VII, which celebrated the garden's 50th anniversary, is a prestigious botanical conference that is traditionally held every five years. Today, it covers a broad set of topics from anatomy, biogeography, biotechnology, conservation, ecology, genomics, history, and physiology to the more traditional themes of taxonomy, systematics, and evolution.

The conference has been held seven times: 1993, Monocotyledon Systematics and Evolution, Royal Botanic Gardens, Kew, England; 1998, International Conference on the Comparative Biology of Monocotyledons - Monocots II, Royal Botanic Gardens Sydney, Australia; 2003, Monocots III: The Third International Congress on the Comparative Biology of the Monocotyledons & The Fourth International Symposium on Grass Systematics and Evolution, Rancho Santa Ana Botanic Garden, California, U.S.A.; 2008, Monocots IV, University of Copenhagen, Denmark; 2013, Monocots V, Fordham University hosted by The New York Botanical Garden, U.S.A.; 2018, Monocots VI, Natal, Brazil; and most recently, 2024, Monocots VII, San José, Costa Rica.

Costa Rica, a country known for its breathtaking natural beauty, is a global hotspot for biodiversity. This tropical paradise boasts an incredible array of ecosystems, with a remarkable variety of flora and fauna, and it is not surprising that it was selected to host MONOCOTS VII under the motto "Costa Rica, where nature is always nearby". The 7th International Conference on the Comparative Biology of Monocotyledons was held at the Costa Rica Convention Center (CCCR), from the 11th to the 15th of March 2024. It is an important event for neotropical botany to have hosted the last two MONOCOTS conferences: Brazil in 2018 and Costa Rica in 2024. This speaks about the important efforts that local institutions are carrying out to study and conserve the extraordinary monocot diversity in neotropical ecosystems.

Costa Rica is well-known for being tiny in geographical area, but vast in biodiversity. With about 3500 species, Costa Rica hosts about 7% of the world's MONOCOTS, distributed among 39 plant families. Most representative of the country are, of course the orchids, a family of plants tightly woven into Costa Rican culture and society. Other species-rich monocot families include Araceae, Arecaceae, Bromeliaceae, Cyperaceae, and Poaceae many with species still awaiting to be discovered and studied. A whopping 30% of monocot species are endemic to the country, meaning studying and conserving them is our privilege and responsibility.

The MONOCOTS VII conference was organized by a multidisciplinary group of professors, technicians, and students from the University of Costa Rica. This event has been a collaborative effort between the Lankester Botanical Garden (JBL), the Biodiversity and Tropical Ecology Research Center (CIBET), and the School of Biology. The program featured 99 talks, including seven exhilarating keynotes, delivered by some of the world's most renowned plant scientists, as well as 20 symposia covering key topics such as anatomy and morphology, biotechnology, conservation, ecology, ethnobotany, genomics, physiology, taxonomy and systematics. Two poster sessions showcased 69 outstanding works, many contributed by talented students and young researchers. Additionally, the conference included a workshop on digital plant documentation and a visit to the National Orchid Show at the Lankester Botanical Garden.

For the first time, the talks from a MONOCOTS conference are available for free online viewing. Of the 99 talks presented, 68 are freely accessible on the MONOCOTS VII channel on YouTube (https://www.youtube.com/@MONOCOTSVII) and can also be accessed individually through the QR codes provided from page 227 onwards. At the time of the writing, the talks have been viewed online 671 times, which is a significant addition to the close to 200 participants who attended the event in person.

I thank my fellow editors and colleagues in the organizing committee, the scientific committee and all participants. I am deeply grateful to the directors, professors, administrators, technicians, students, volunteers, and everyone who supported this event. Finally, the organizing committee is thankful to the diverse other branches of the University of Costa Rica for supporting the MONOCOTS VII conference. Gustavo Gutiérrez Espeleta, Rector, Maria Laura Arias, Vice-rector of research, the directors and staff of OAICE, OCI, the postgraduate program and VIVE.

We commemorate this 50th anniversary of Lankester Botanical Garden by reflecting on the legacy of those who envisioned this botanical haven and the countless individuals who have contributed to its growth and dedication to advancing plant knowledge. Together with the other agricultural and botanical centers of the University of Costa Rica, these publicly funded institutions stand as a testament to our country's unwavering commitment to biodiversity education, research, and conservation.

Adam P. Karremans
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PROCEEDINGS

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(MONOCOTS VII)

KEYNOTE SPEECHES

HOW TO MAKE A SPECIES RICH FAMILY

James D. Ackerman* and Raymond L. Tremblay

Orchidaceae is not only old but also one of the most species rich of families of plants. Numerous attempts to explain the high species diversity in orchids have been suggested. The dominant explanation is that natural selection increases the probability of cross-pollination, which no doubt occurs but would also apply to all flowering plants so there must be other factors at play. A common approach is to identify correlates ("drivers") of high diversity such as adaptive radiation related to specialized pollinator services or mycorrhizal associations; stomatal density, genome, and guard cell size reductions; succulence; crassulacean acid metabolism; hard pollinia, dust seed produced in prodigious quantities; niche partitioning, and epiphytism. While these are traits and patterns associated with high diversity and often high diversification rates in orchids, they do not explain the evolutionary processes involved. Gentry and Dodson proposed a model for rapid evolutionary change in epiphytes based on Wright's Shifting Balance theory, which was elaborated upon by various authors culminating in a drift-selection model of Tremblay and collaborators that focused specifically on orchids. While subsequent research showed some support for the tenets of the model, other studies failed to do so, suggesting that it is not a universal process across the family. Recently, Pérez-Escobar and colleagues updated patterns of orchid species richness and have shown that regions of high diversity are indeed globally scattered, but only a few have high diversification rates. These are recent montane tropical areas dominated by epiphytes where environmental heterogeneity and instability abound across ecological to geological time frames. Such persistently stressful conditions may not only affect genome evolution but also require dispersal and repeated founder events generating unusual genotypes subjected to intense selection. While this model is most likely to occur among epiphytes in tropical regions of recent orogenesis, aspects of the model may be operational anywhere orchids exist.

ILLUMINATING THE WORLD'S BIODIVERSITY DARKSPOTS

Alexandre Antonelli

Delivering on the Kunming-Montreal Global Biodiversity Framework requires filling the most critical knowledge gaps. Here I will set the context for why understanding plant diversity and distribution is so critical to science and society, and then present a framework for prioritising regions around the world with highest chances of yielding new species to science as well as most new geographic records. I will discuss how new technological developments, from remote sensing to citizen science, environmental DNA and semi-automated inventories can (and should) be integrated with expert surveys and analysed under robust methods in order to properly identify the most important areas for biological conservation and restoration needed to halt biodiversity loss and advance scientific knowledge.

PROGRESS AND PROSPECTS IN MONOCOT PHYLOGENOMICS AND EVOLUTIONARY BIOLOGY

Thomas Givnish

Our insights into the phylogeny and evolution of the monocotyledons has grown geometrically since the first Monocot Conference in 1993. Here I will trace the progress we have made in identifying relationships among monocots, and developing insights in their adaptation and geographical spread, as we climbed the information ladder from sequences of single plastid genes to multiple plastid genes, whole plastomes, and few to hundreds of single-copy nuclear genes. I will also outline the new directions we might take over the next decade to increase further the power and scope of our analyses.

DIOECY AND SEX CHROMOSOMES IN THE GENUS *ASPARAGUS*

James Leebens-Mack

Approximately 6% of angiosperm species evolved separate sexes (dioecy) producing strictly pistillate or staminate flowers, functioning as either female or male individuals, respectively. In the genus *Asparagus*, approximately 27% of extant species are dioecious. We had previously identified the sex-determination genes in garden asparagus (*Asparagus officinalis*), but genomic analyses of another dioecious species, *A. horridus*, revealed a completely different sex-determination system. We infer distinct sex chromosomes evolved in separate origins of dioecy in the clades represented by *A. officinalis* and *A. horridus*. Both origins of dioecy occurred as each ancestral linage shifted their ranges out of Southern Africa to Asia and the Mediterranean Basin, respectively. Both of these dioecious *Asparagus* clades exhibit an XY sex determination system, but there is no overlap in the known or hypothesized sex-determination genes. Continued investigation of dioicous *Asparagus* species and their hermaphroditic relatives is elucidating the dynamic evolution of plant sex chromosomes.

ASSEMBLING THE MONOCOT TREES OF LIFE: EXAMPLES FROM RESEARCH AT KEW

Oscar Pérez-Escobar

With over 70,000 species, monocots are one of the largest natural groups within the Angiosperms. This group includes a vast array of useful plants to humankind, showcasing a remarkable range of adaptations that have enabled them to thrive in aquatic environments as well as nearly all terrestrial ecosystems. Understanding the spatio-temporal evolutionary dynamics of monocots and identifying the wild relatives of useful plants requires a solid phylogeny, but how far along are we from producing such a densely sampled and robust phylogenetic framework? In this talk, I will discuss recent advances made in constructing the monocot trees of life. Drawing on research conducted under the Plant and Fungal Trees of Life project at Kew, I will present findings related to the origin and speciation of orchids, family-level relationships across monocots, and the evolutionary history date palms (*Phoenix*, Arecaceae) through the lenses of ancient DNA and population genomics.

GENOMIC INSIGHTS INTO ROLE OF ADAPTATION IN THE EVOLUTION OF NOVELTY: TALES FROM *COSTUS* L. AND *CALOCHORTUS* PURSH.

Chelsea Specht

Fifty years since Dr. Paul J.M. Maas published his first monograph of the New World Costoideae, we continue to struggle with species boundaries and evolutionary relationships within this charasmatic lineage of tropical monocotyledonous plants. In fact, recents efforts to explore, collect, describe and discover have led to even more questions about the dynamics, patterns, and processes leading to speciation and diversification across the Neotropical Costaceae. In this talk, I will discuss the recent monographic revision and its critical role in establishing a framework for evolutionary and ecological studies of the Neotropical *Costus* lineage within a phylogenetic context. The tempo and mode of speciation events are correlated with morphological changes that influence organismal interactions, including pollination and herbivory. Ecologic, morphologic, and biogeographic conditions that appear to promote hybridization and result in the potential for hybrid speciation are discussed across the genus, and implications for developing a stable taxonomy – and whether or not that is even possible or desirable - is discussed.

Symposium

ADAPTATION AND TRAIT DIVERSIFICATION IN MONOCOTS: PERSPECTIVES FROM MACRO- AND MICROEVOLUTIONARY PROCESSES

ECOLOGICAL SPECIALIZATION IN THE FACE OF GENE FLOW: LEVERAGING CLINAL VARIATION TO INVESTIGATE LOCAL ADAPTATION

Adriana I. Hernández

A key goal of biology is to understand how biodiversity is driven and maintained across natural heterogeneous and changing landscapes. Here, we leverage parallel evolutionary histories, strong population structure, and climatic clines in *Calochortus venustus* (Liliaceae) to identify loci under selection for key plant functions linked to plant adaptation to California's Mediterranean climate. We identify targets of selection using genotype-environment associations to detect loci under selection from each of nine climatic variables. Despite gene flow between individuals of different floral phenotypes and between populations, we find evidence of ecological specialization at the molecular level, including genes associated with cold stress, heat, water deprivation, salt stress, and root development. Single nucleotide polymorphisms (SNPs) present in both lineages that evolved across independent transects show parallel trends in allelic similarity across latitudes indicating adaptation to northern climates, as well as divergent genetic evolution across longitude suggesting adaptation to either coastal or inland habitats. Spatial allelic patterns provide further evidence that loci under selection are responsible for the evolution of adaptive phenotypes, however, experimental evidence is necessary for validation.

INCORPORATING PRIOR INFORMATION OF DEVELOPMENTAL GENETICS IN TRAIT EVOLUTION WITH THE THRESHOLD MODEL: THE UNBELIEVABLE *AMARYLLIS* UMBEL AS A CASE STUDY

Jesús Martínez-Gómez

Development constraints morphological evolution, shaping patterns of trait distribution across a phylogeny. However, current models of trait evolution do not account for prior knowledge of developmental processes. We investigate whether incorporating prior knowledge of development allows for more adequate and realistic models of morphological evolution, using the flower branching architecture of the *Amaryllis* family (Amaryllidaceae) as a case study. The branching structure of Amaryllidaceae is characterized as an 'umbel' and multiple hypotheses based on comparative morphology have been proposed to explain its evolutionary origin, however these have not been explicitly tested in a phylogenetic framework. To test this hypothesis, we implement a multivariate Brownian motion version of the threshold model (MBMT) with apriori thresholds set by an independent morphospace analysis of branching architecture in a Bayesian framework. Simulation test show that this approach is at least as accurate as a multivariate Brownian motion model is in inferring ancestral states. We fit these two versions of this model to the inflorescence architecture data of Amaryllidaceae: One where umbellate inflorescences are derived from racemes and the other where umbellate inflorescences are derived from thyrsoids. Ancestral state inference under MBMT model unexpectedly infers a panicle-derived origin of the umbel in contrast to prior developmental morphology which states that the umbel being thyrsoid- derived. We emphasize the need to understand the developmental process that generates phenotypes and how explicitly modeling those processes is a necessary step for modeling morphological evolution.

REGIONS UNDER SELECTION IN THE CRYPTIC RADIATION OF THE *GEONOMA UNDATA* SPECIES COMPLEX

María José Sanín*, Carmen Webster, Margot Paris
and Ingrid Olivares

The Tropical Andes are home to spectacular plant radiations entailing astonishing morphological divergence between recently diverged, or even speciating, taxa. However, some radiations are far from apparent, leading to a mismatch between genetic divergence and phenotypically based circumscriptions. Genus *Geonoma* Willd. (Arecaceae: Arecoideae: Geonomateae) has had a complicated history of species delimitation, with 68 accepted species and over 140 taxa (subspecies, morphotypes). In particular, the *Geonoma undata* Klotzsch species complex of predominantly cloud forest palms, is an amalgam of isolated populations undergoing rapid genetic divergence and exhibiting a widely variable level of morphological displacement between genetic groups. In particular, the Northern Andes of Colombia concentrate various genetic groups possibly reflecting a particular connecting point between isolated areas of divergence in Central America and the Central Andes. In this portion of the Andes, several of the most displaced as well as the most intermediate morphologies are found within short distances. These forms are also related to 5 genetically distinct groups. Through Hybrid capture, we sequenced 4000 nuclear coding regions and geographically intensive sampling to understand which regions are seemingly involved in species divergence by 1) teasing out regions of the genome with outstanding F statistics and 2) outstanding selection (dx/dy and Tajima's D). Altogether, this study is a first attempt at teasing out regions (in the genome, and in geography) where genetic groups are diverging, and at tying outstanding genomic regions to potential ecological functions implicated in the speciation process.

Symposium

ADAPTIVE GROWTH AND FUNCTIONAL MORPHOLOGY OF MONOCOTYLEDONS

* * *

CAN THE FUNCTIONAL MORPHOLOGY AND ADAPTIVE GROWTH IN MONOCOTS INSPIRE TECHNOLOGY?

Linnea Hesse

In monocotyledons, the loss of the vascular cambium led to morphological and histological changes that determine the arrangement of the isolated, closed collateral vascular bundles of the atactostele. On the basis of this fiber-reinforced bauplan, different growth forms evolved with high structural diversity, but unable to adapt by means of a vascular cambium. The question arises whether the different growth forms share common biomechanical features, how monocotyledons adapt mechanically to their environment despite the absence of a vascular cambium, and whether a deeper understanding of the form-structure-material-function relationships of monocots can inspire engineering fiber-reinforced design.

We perform biomechanical experiments (bending and torsion) together with light microscopy, micro-computed tomography and magnetic resonance imaging in order to understand and compare the biomechanical properties and structural pattering of aerial stems of different monocots with varying growth form. In addition, we investigate the adaptive responses and growth of monocots at different structural levels to gain a better understanding of plastic adaptations to environmental changes.

The results serve as input for biomimetic projects aimed at transferring the structural and adaptive principles of monocots into bio-inspired, fiber-reinforced composites with responsive and tailored mechanical properties and their manufacturing.

WOUND CLOSURE IN MONOCOT STEM WITH SECONDARY GROWTH

Joanna Jura-Morawiec

In some monocotyledonous plants, the stem can grow in thickness due to the activity of the monocot cambium. This meristem gives rise to a three-dimensional network of secondary vascular bundles embedded in parenchyma cells. Monocotyledons with secondary growth in the stem can live for hundreds of years, and during this time, they can suffer traumatic injuries caused by various biotic and/or abiotic agents. The question is how plants with a monocot cambium and secondary vascular tissues organized in bundles cope with injury. This talk will discuss the results of the morpho-anatomical studies of wound closure in the stem of the Macaronesian dragon tree *Dracaena draco* L. Particular attention will be paid to the formation of vascular tissue after wounding. The results indicate that (a) wound closure varies depending on the size and depth of the wound, and the age of the plant, and that (b) the vascular bundles formed by the monocot cambium after wounding have a different course from the vascular bundles before the injury.

VARIATIONS OF MONOCOT VASCULAR BUNDLE SYSTEMS ABOVE AND BELOW GROUND

Hansjörg Krähmer

Vascular bundles of monocot stems are not arranged randomly nor scattered. They form patterns which can be traced back to several sites of origin. Most bundles are formed at stem apices. Additional bundles arise as connections between axillary buds and the stem. Nodes are matrices which influence the bundle course and the shape of vascular bundles. Within nodes, specific bundles often function as horizontal interfaces between stem, leaf bundles and buds in the form of a plexus. Plexus bundles are often amphivasal and not collateral as internode bundles. Monocot nodal bundles are usually modified. So-called glomeruli are found in a few monocots such as bamboos, *Dioscorea* species and in *Phragmites* communis. Stem-borne roots arise close to stem bundles, and root bundles form characteristic stem connections. Vascular bundle cylinders clearly separated from a cortex by borders do often not exist in above ground monocot stems. We find, however, typical border structures within rhizomes which allow us to define a separate central bundle cylinder different though from eudicots. Most rhizomes carry scales or reduced leaf structures from which only minor traces enter the stem. Rhizome nodes are usually not as spectacular as above ground stem nodes. At the transition of above ground into below ground stems, collateral bundles often turn into amphivasal bundles. Roots arise along the whole rhizome in contrast to above ground stems where they arise primarily at the stem base, at sites which touch ground or at pre-formed sites only. The characteristic vasculature of monocots is specific and not directly comparable to that of eudicots and ANA clade representatives. These findings will be demonstrated with a few selected examples and compared with results published by different authors in the past.

UNFOLDING LEAVES – PARTICULARITIES IN THE LAMINA OF *SYNGONIUM PODOPHYLLUM*

Michelle Modert*, Tom Masselter and Thomas Speck

In natural habitats, adult leaves as well as leaf buds come in many forms. Accordingly, different unfolding patterns have developed. Among those are densely packed leaves that unfold via creases and leaves with a furled lamina prior to unfolding. The latter can be found in many Araceae species, e.g. *Syngonium podophyllum*. Here, the two leaf halves open successively as a part of the developmental process. In our study, we mainly investigate the leaves' structural and biomechanical characteristics during the unfolding process and *Syngonium podophyllum* serves as a model species. More specifically, we measured forces during leaf unfolding and applied a tensile load to different lamina regions for different sample orientations, i.e. parallel (1) and perpendicular to secondary venation (2), as well as parallel to direction of unfolding (3). The experiments were performed for both juvenile (one leaf half open) and adult (unfolding completed) leaves. *Syngonium podophyllum* leaves (ca. 200 µm thick) reach forces in the range of ten to hundred millinewton while unfurling. Upon tensile stress, juvenile leaves show significantly lower tensile moduli than adult leaves. In secondary veins, the vascular bundles are already lignified when juvenile, but are significantly stiffer when adult. Thus, after having completed unfolding, both the parenchymatous tissue and the veins undergo structural stiffening. Moreover, independent of leaf region, tensile modulus depends on orientation, with highest values parallel and lowest values perpendicular to secondary venation. Presumably, the lamina thickness together with the ratio of vascular bundles to parenchyma causes these anisotropic directional properties, as no directional differences were visible in a species with thicker leaves and less prominent veins (i.e. *Pilea pereomioides*).

Symposium

Advances in the taxonomy and phylogeny of Panicoideae (Poaceae)

CURRENT STATUS OF PHYLOGENETIC STUDIES IN THE GENUS *PASPALUM* L.

G.H. Rua, M. Vaio, M.G. Bonasora, P.R. Speranza and C. Silva*

With about 350 species, the genus *Paspalum* is one of the most numerous in Poaceae. Distributed across the American continent, it includes valuable forage and turfgrass species. Phylogenetic relationships are somewhat obscured by frequent hybridization, polyploidy, and apomixis. Current phylogenies based on cpDNA and ETS markers are so far fragmentary and poorly resolved, although some major clades could be recognized and parental relationships of some known allopolyploid species could be inferred. Ongoing analyses based on plastid whole genomes throw well resolved trees mostly consistent with previous ones, but the species sampling is still limited. On the other hand, separate analyses of some species complexes involving several ploidy levels and reproductive systems, such as *P. dilatatum*- and *P. stellatum*-complexes, reveal allopolyploid formation between distantly related species belonging to different clades and recurrent polyploidization events resulting in intricate polyploid series where each clone is maintained in nature through apomixis. In groups like *Paspalum*, with complex evolutionary histories, it is only possible to understand such histories by applying a multiscale analysis, which combines the phylogenetic analysis of the whole group using different markers with the detailed study of each of the clades in which hybridization and polyploidization phenomena occur.

IMPACTS OF MOLECULAR PHYLOGENETICS ON GENERIC CLASSIFICATION IN PANICEAE AND PASPALEAE (POACEAE, PANICOIDEAE)

Christian Silva* and Fernando Omar Zuloaga

Panicoideae is the second largest among the 12 subfamilies in the grass family (Poaceae), with over 3,320 species. It has traditionally been divided into tribes and subtribes, recognized based on macro and micromorphological characters. However, since the 1990s, when molecular phylogenetic studies became essential to systematics, many taxonomic groups have been reassessed to fulfill the monophyly criterion. The panicoid tribe Paniceae, historically recognized as a natural group, was one of the grass taxa that underwent significant changes in its internal circumscription, especially at the generic level. In this talk, we will summarize the changes made over the last 30 years in Paniceae using molecular phylogenetics. This will be based on a literature review, using the classification of Clayton and Renvoize (1986) as a starting point. Generic realignments based on molecular studies primarily began in the early 21st century. Paniceae itself was diagnosed as not monophyletic and divided into Paniceae s.s. and Paspaleae, a reinstated tribe. Later, another tribe, the monotypic Lecomtelleae, was reinstated. This posed a practical problem as no morphological synapomorphies were found to distinguish these tribes. There are high levels of homoplasy in most morphological characters of taxonomic significance. Within Paniceae and Paspaleae, four new genera were initially proposed based on morphology and later confirmed by molecular data. Additionally, 30 new genera were created to accommodate species from para- or polyphyletic genera. Sixteen genera had to be recircumscribed. Some of these were splitted into smaller genera (e.g. *Echinolaena*), while others were expanded by lumping two or more genera (e.g. *Cenchrus*). *Panicum* is the genus that has undergone the most changes, mainly resulting in new genera. Seven genera were reestablished from synonymy, while 21 were synonymized. The circumscription of 68 genera remained unaltered. A few genera were never included in molecular phylogenetic studies (e.g. *Baptorhachis*). Changes will continue to be made until each genus become monophyletic. There are several challenges to achieve this goal. Even with the enormous amount of data provided by genomic sequencing techniques, unraveling phylogenetic relationships can be challenging due to evolutionary processes such as hybridization or incomplete lineage sorting, especially in rapid radiations.

UPDATES ON THE PHYLOGENY AND CLASSIFICATION OF ANDROPOGONEAE (POACEAE: PANICOIDEAE)

Cassiano Aimberê Dorneles Welker

The tribe Andropogoneae (Poaceae: Panicoideae) comprises about 1,230 species, including some of the most important crops in the world, such as sugarcane (*Saccharum officinarum*), maize (*Zea mays*), and sorghum (*Sorghum bicolor*). Great advances have been made in the phylogeny and classification of Andropogoneae in recent years, based mainly on plastome sequences and low-copy nuclear genes. Recent dated phylogenies suggest that Andropogoneae diverged from Arundinelleae in the Early Miocene and originated in East Asia. Intercontinental dispersal has been common since then, with many independent dispersal events to Africa and the New World. The most recent classification of the tribe, based on plastome data, includes 14 monophyletic subtribes, assigning to a subtribe about 90% of the Andropogoneae species. The remaining taxa are placed incertae sedis pending additional molecular data. Several Andropogoneae genera are para- or polyphyletic and some of them have already been investigated to define their circumscriptions. The genus *Tripidium* was confirmed as a distinct genus from *Saccharum*, based on plastome and nuclear data. Similarly, the genus *Anatherum* was recently reestablished and expanded to incorporate about a third of the *Andropogon* species. Species delimitation has also been investigated in many Andropogoneae genera, such as *Saccharum*, *Eriochrysis* and *Schizachyrium*, based mainly on low-copy nuclear genes. Several changes in the circumscriptions of genera and species in Andropogoneae are expected to occur in the coming years. (Funding: CNPq, FAPEMIG).

Symposium

BromEvo-III: BromeliOmics

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THE MITOCHONDRIAL GENOME IN TILLANDSIOIDEAE (BROMELIACEAE): ASSEMBLY AND COMPARATIVE ANALYSIS

David Cruz Plancarte*, Matthias Jost,
and Carolina Granados Mendoza

The angiosperm mitochondrial genome (mtDNA) is characterized by its high and complex structural variability, high repeat content, and low mutation rates. Despite the small number of protein-coding genes (PCGs) encoded in the mtDNA, the length of these genomes varies widely, from 60 kbp to 11 Mbp. The bioinformatic challenges posed by their assembly have led to the under-exploration of this source of phylogenetic information, leaving the vast majority of these genomes poorly understood. As of now, only around 500 land plant mtDNAs have been assembled and reported, a notably small number when compared to the documented number of chloroplast genomes (~8,000). In the monocot family Bromeliaceae, no mitochondrial genomes have been characterized. In this study, we aimed to assamble and annotate the mtDNA of species within the subfamily Tillandsioideae. For this, we used publicly available data including short reads (Illumina) and long reads (PacBio). The mtDNA of Tillandsioideae species is characterized by its composition of multiple structures, totaling ~1.2 Mbp in length, with an approximate GC content of 44%. The annotation revealed that the mtDNA encodes 68 unique genes and two pseudogenes, comprising 39 PCGs, 25 tRNAs, and three rRNAs. The results of this study contribute significantly to the growing knowledge of angiosperm mtDNA, as this comparative analysis represents the first of its kind for Bromeliaceae.

NEW PLASTOME STRUCTURAL REARRANGEMENTS DISCOVERED IN CORE TILLANDSIOIDEAE (BROMELIACEAE) SUPPORT RECENTLY ADOPTED TAXONOMY

Carolina Granados Mendoza*, Sandra I. Vera-Paz,
Daniel D. Díaz Contreras Díaz, Matthias Jost, Stefan Wanke,
Andrés J. Rossado, Rebeca Hernández-Gutiérrez,
Gerardo A. Salazar, Susana Magallón, Eric J. Gouda,
Ivón M. Ramírez-Morillo, and Sabina Donadío

Despite the amount of genomic data that has greatly contributed to a better understanding of the plant tree of life and accelerated the study of plant plastome diversity and evolution, some lineages remain understudied. Full plastome sequences from the highly diverse (>1500 spp.) subfamily Tillandsioideae (Bromeliaceae, Poales) have been published for only three (i.e. *Guzmania, Tillandsia,* and *Vriesea*) out of 22 currently recognized genera. Here we focus on core Tillandsioideae, a clade within subfamily Tillandsioideae, and explore the utility of full plastomes for resolving deep and shallow divergences within this group. We generated 37 high quality plastome assemblies and performed a comparative analysis in terms of plastome structure, size, gene content and order, as well as CG content. Using the obtained phylogenetic context, we reconstructed the evolution of these plastome attributes and assessed if significant shifts on their evolutionary rates have occurred in the evolution of the core Tillandsioideae. Our results agree with previously published phylogenetic hypotheses based on plastid data, providing stronger statistical support for previously recovered but weakly supported clades. However, phylogenetic discordance to previously published nuclear marker-based phylogenetic hypotheses was found. Our analyses confirmed the phylogenetic utility of several plastid markers that have been consistently used to ad-

dress phylogenetic relationships of the Tillandsioideae and further loci are here identified as promising additional markers for future Sanger sequencing-based studies. Novel lineage-specific plastome rearrangements were found to support traditionally defined taxonomic groups, including large inversions and expansions and contractions of the inverted repeats. Evolutionary trait rate shifts associated with changes in size and GC content of the plastome regions were found across the phylogeny of core Tillandsioideae. Our study significantly enriches Tillandsioideae genomic resources and improves resolution and statistical support for the phylogeneny of the group. Furthermore, we provide insights on the Tillandsioideae plastome diversity and evolution.

BROMELIACEAE PHYLOGENOMIC ANALYSES ELUCIDATE GENOME EVOLUTION ACROSS DIVERSIFICATION BURSTS

Jim Leebens-Mack

Phylogenetic analyses of Bromeliaceae have implicated bursts of diversification both recent and ancient diversification. More recent phylogenomic analyses of whole plastomes and large sets of nuclear loci have also recovered species tree estimates with rapid diversification on the spine of the Bromeliaceae phylogeny and strong support for Brocchinioideae sister to a clade including all other bromeliad subfamilies. This talk will introduce a haplotype resolved chromosomal genome assembly for *Brocchinia micrantha* and discuss the utility of whole-genome comparisons for elucidating the evolutionary history of Bromeliaceae. Changes in gene content and genome structure will be presented and discussed in the context of evolutionary innovations.

THE DIVERSIFICATION IN HECHTIOIDEAE (BROMELIACEAE) IN THE DRY FORESTS OF NORTH AND CENTRAL AMERICA

Katya Jeanneth Romero Soler

Hechtioideae is a bromeliad lineage that includes about 92 species, and it is morphologically characterized by being succulent, usually strongly armed, and dioecious plants. Its species grow as terrestrial or rupicolous, mainly in dry areas from southern USA to northern Nicaragua. Phylogenetics in Hechtioideae have been based on a few plastid (rpl32-trnL and ycf1) and one nuclear (PRK) loci. These loci have retrieved trees with low resolution and support, unable to resolve the relationships among the three currently recognized genera (i.e. *Bakerantha, Mesoamerantha,* and *Hechtia*), as well as within the diverse clade of *Hechtia*. This lack of resolution and support at different phylogenetic depths is a common phenomenon in the Bromeliaceae family. Here we used a complete plastome approach to generate a more robust phylogenetic hypothesis for Hechtioideae. We newly sequenced and annotated 67 species of Hechtioideae and added data from other Bromeliad lineages available in public databases. The phylogenetic inference was performed using Maximum Likelihood. The tree reconstructed with the complete plastome strongly support the monophyly of Hechtioideae and its three genera. Based on this evidence Mesoamerantha is sister of a clade formed by *Bakerantha* and *Hechtia*. Within *Hechtia*, the relationships are highly supported, except for a few nodes within Hechtia. The use of complete plastomes helped solving several recalcitrant relationships in Hechtioideae. Our phylogenetic hypothesis will allow us to establish a clearer spatio-temporal framework for the evolution of this subfamily and identify key traits that allowed its diversification within the dry ecosystems of Mesoamerica.

PLASTOME PHYLOGENOMICS REVEALS AN EARLY PLIOCENE NORTH- AND CENTRAL AMERICA COLONIZATION BY LONG-DISTANCE DISPERSAL FROM SOUTH AMERICA OF A HIGHLY DIVERSE BROMELIAD LINEAGE

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Andrés J. Rossado, Claudia A. Montes-Azcué,
Rebeca Hernández-Gutiérrez, Susana Magallón,
Luis A. Sánchez-González, Eric J. Gouda, Lidia I. Cabrera,
Ivón M. Ramírez-Morillo, María Flores-Cruz,
Xochitl Granados-Aguilar, Ana L. Martínez-García,
Claudia T. Hornung-Leoni, Michael H.J. Barfuss
and Stefan Wanke

Bromeliads are known to have undergone one of the most remarkable plant adaptive radiations, however, the study of historical biogeography of some lineages inside has been hindered by the lack of dated, resolved, and strongly supported phylogenetic frameworks. The clade K, a highly diverse *Tillandsia* subgenus *Tillandsia* (Bromeliaceae, Poales) lineage, is hypothesized to have undergone a rapid radiation across the Neotropical region also Central America and Mexico has been proposed like centers of diversification. Previous studies using few Sanger-sequenced markers has recover a lack of phylogenetic resolution hinder the reconstruction of its historical biogeography. In this work, we assess the spatial and temporal frameworks for the origin and dispersal history of the expanded clade K. We assembled and annotated 162 full plastomes from Hyb-Seq and shallow sequencing data of a dense taxon sampling of the expanded clade K plus a careful selection of outgroup species and used them to generate a time-calibrated phylogenetic framework. Then we used the dated phylogenetic hypothesis to test sev-

eral biogeographic models and perform ancestral area reconstructions based on an extensive compilation of geographic information. The expanded clade K colonized North and Central America by long-distance dispersal from South America ca. 4.86 Mya in where its internal clades diversified. After that, several dispersal events took place to the southern Nearctic region, the Caribbean, and the Pacific dominion during the last 2.8 Mya a period characterized by pronounced climate fluctuations, derived from glacial–interglacial climate oscillations during the Pleistocene, and substantial volcanic activity mainly in the Trans-Mexican Volcanic Belt. The taxon sampling herein employed let us to generate a significant amount of genomic data information and to calibrate for the first-time nodes for several bromeliad, that could facilitate future macroevolutionary studies.

Symposium

BromEvo-III: Reproductive biology, ecophysiology, and ecology of the Bromeliaceae

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EXPLORING REPRODUCTIVE INVESTMENT AND BREEDING SYSTEM EVOLUTION IN BROMELIACEAE

Josh Felton* and Rachel Jabaily

As a family harboring a great deal of taxonomic, morphologic, and ecologic diversity, Bromeliaceae is an ideal lineage for elucidating evolutionary patterns in reproductive biology. In particular, we are interested in understanding differences in reproductive investment across the family. Here we present an experimental greenhouse study that uses pollination manipulation to explore the morphological mechanisms associated with effective pollination in two epiphytic Bromeliaceae species, *Vriesea rafaelii* Leme and *Billbergia brasiliensis* L.B.Sm. We found that these species exhibit both selfing and outcrossing modes of fertilization, and that they develop distinct fitness traits depending on reproductive mode. Notably, *B. brasiliensis* displays higher carpel and total seed mass in outcrossed flowers, while *V. rafaelii* demonstrates a similar, albeit statistically insignificant, trend. These findings imply the presence of inbreeding depression in both species, substantiated by reduced seed mass in flowers that are self-pollinated. Finally, we discuss the broader implications the loss of self-incompatibility through highlighting the diverse mechanisms maintaining mating systems in the family while outlining future research directions aimed at unraveling the intricate tale of breeding system evolution within Bromeliaceae.

LIFE HISTORY AND TRADEOFFS IN THE BROMELIACEAE

Rachel S. Jabaily

Life history variation is an exciting aspect of bromeliad biology that helps to explain the family's success in many biomes throughout the Neotropics. Traits shaping the varying life history strategies in Bromeliaceae have been neglected by our scientific community. I will overview life history strategies across Bromeliaceae, explaining the development of morphological features associated with each category. I will present a multi-year greenhouse observational experiment utilizing three exemplar species spanning the variation within the multi-ramet iteroparous life history category (*Aechmea recurvata, Billbergia brasiliensis, Vriesea rafaelii*). We tracked vegetative growth, biomass accumulation and BRIX sucrose concentration over 3+ years of development. We found differences in relative timing of axillary ramet and inflorescence production in each species. The biomass of reproductive tissues relative to vegetative mass, or reproductive effort values, also differs between species, indicating differential tradeoffs for sexual and asexual reproduction. I end by clarifying the types of data our community of bromeliad biologists could be taking in the field and in the greenhouse to better understand life history diversity in the Bromeliaceae.

WHAT WE CAN LEARN FROM AQUAPORINS OF EPIPHYTIC TANK-FORMING BROMELIADS AND HOW THEY CAN BE USEFUL FOR PLANT BIOTECHNOLOGY

Helenice Mercier

Adult leaves of the epiphytic tank-forming bromeliads are considered the main organs of water and nutrient uptake. The presence of absorbing trichomes on leaf surface, mainly in the basal portion, increases the leaf permeability to take up water and, in special, nitrogen (N). Both resources are known to be scarce or, at least, intermittent in the canopy. In general, the epiphytic habitat of the tank-forming bromeliads is characterized by ammonium (NH_4^+) as the main type of inorganic N found into the tank. Recent data revealed an upregulation of the high-affinity N-transporter genes in the leaf base, indicating efficient N uptake even in low concentrations by the trichomes. Previous work with leaf of Vriesea gigantea has shown a high capacity to absorb NH_4^+ and urea and it was found that different types of aquaporins, like plasma membrane intrinsic proteins, PIP, and tonoplast intrinsic protein, TIP, were involved in this process. Two aquaporins, VgPIP1;2 and VgTIP2;1, were cloned and functionally characterized as transporter of water, besides NH_4^+ and urea, respectively. Based on these data, new possibilities of bioengineering crops through the insertion of transporters and/or aquaporin genes were envisaged. The improvement in N and water use efficiency in crops is being recognized as an important research topic and a crucial factor that contributes to sustainable agriculture and environmental protection.

REPRODUCTIVE COEXISTENCE IN EPIPHYTIC BROMELIADS OF THE GENUS *WERAUHIA* J.R.GRANT (BROMELIACEAE) IN A MONTANE FOREST, COSTA RICA

Stephanie Núñez-Hidalgo* and Alfredo Cascante-Marín

Reproductive isolation mechanisms in plants enable the coexistence of species with a shared evolutionary history within the same habitat. These mechanisms also contribute to maintaining the evolutionary independence of species. Objective: To comprehend the mechanisms facilitating such coexistence and the preservation of local diversity in Neotropical plants, particularly in the Bromeliaceae family, we explore the strength and contribution of different reproductive isolation mechanisms (pre- and post-pollination) in four sympatric species of bromeliads from the genus *Werauhia* (*W. ampla, W. nephrolepis, W. pedicellata, W. subsecunda*) within a montane forest in Costa Rica. Methodology: The contribution of different mechanisms was estimated using Reproductive Isolation Indices (RI), considering reproductive phenology, floral morphology, interspecific compatibility, and the production and viability of hybrid seeds. Main results: Temporal isolation through floral phenology exhibited high general RI values, signifying a significant contribution to reproductive isolation. Disparities in flower size and the position of reproductive structures lead to mechanical isolation, as pollen is deposited on different parts of the pollinator's body. Interspecific manual crosses demonstrated complete incompatibility between species, except for the combination *W. subsecunda* (pollen recipient) × *W. ampla* (pollen donor), resulting in a 47.1% success rate, albeit with low seed production and germination capacity. Conclusions: Prepollination mechanisms (phenology and floral design) play a crucial role in reproductive isolation among the studied *Werauhia* species. Accurate estimation of reproductive isolation

mechanisms in other plant groups will enhance our understanding of how species coexist, and diversity is maintained in Neotropical ecosystems.

Symposium

BromEvo-III: Systematics and evolution of the Bromeliaceae

BROMELIAD EVOLUTION AND CONSERVATION IN THE HIGH ELEVATION ANDES

Julián Aguirre-Santoro

The Puna and Páramo biomes in the Andean Cordillera harbor the greatest diversity of high elevation plants in the world. Nevertheless, the biodiversity of the region is in danger and requires urgent efforts to understand the diversity of species, their origin, dynamics, and trajectories. This presentation discusses the diversity patterns of the Bromeliaceae family in the high elevation biomes of the tropical Andes, as well as the perspectives that have emerged on the systematic study and conservation biology of this family in South America. The presentation will focus on the latest advances in the evolution, systematics, and conservation of the main lineages of bromeliads that diversified in the high elevation tropical Andes, with a special emphasis on the genera *Puya* and *Greigia*. The evidence obtained from these studies shows how phylogenetic approaches at the species level, combined with ecological, geographical, and morphological data, can help understand the processes that shaped biodiversity in the high elevation biomes of the Andes and make informed decisions for their conservation.

RECENT ADVANCES IN THE TAXONOMY AND EVOLUTIONARY HISTORY OF *VRIESEA* LINDL. (BROMELIACEAE)

Igor M. Kessous*, Dayvid Rodrigues Couto, Beatriz Neves
and Andrea Ferreira da Costa

The generic boundaries in Tillandsioideae (Bromeliaceae) are recognized as confusing and complex. *Vriesea* Lindl., one of most diverse genera in this group, has been divided into nine genera since the last monograph. Despite previous efforts and recent advances, several taxa were still imprecisely positioned in this genus, and several nomenclatural questions still needed elucidation. Thus, our work focused on understanding the boundaries of the genus as well as its evolutionary history. For that, we collected samples in natural populations, visited herbaria worldwide, elaborated phylogenies based on Sanger and RAD-seq data, and performed robust nomenclatural and taxonomic descriptions. A phylogenetic reconstruction based on plastid and nuclear markers, in addition to morphological and anatomical traits, led us to combine eight species previously assigned to *Vriesea* into *Stigmatodon* Leme, G.K.Br. & Barfuss. This study revealed exclusive anatomical traits in *Stigmatodon* compared to the whole lineage. Also, through a ddRAD-seq data phylogeny, despite their different floral morphologies, we reinforced the non-monophyly of the two sections of *Vriesea*. These results suggest that the different pollination syndromes associated with the flower morphologies evolved repeatedly in the lineage. Furthermore, through extensive herbaria and literature work, we observed that morphological differences suggest that nine species previously placed in *Vriesea* should be combined into the genera *Cipuropsis* Ule, *Tillandsia* L., and *Werauhia* Grant. All of these species occur outside the center of species richness of the genus (in the Atlantic Forest), mainly in countries of northern South America and the Caribbean. These species differ morphologically from known species of *Vriesea* s.s., primarily by the floral characteristics. Our work over the past few years has provided new morphological and evolutionary information about species within the genus *Vriesea*. However, it is important that further studies, utilizing more data and new analyses, be conducted to confirm our results and elucidate new issues.

BIOGEOGRAPHY OF CARIBBEAN *PITCAIRNIA* (BROMELIACEAE)

Natalia Ruíz-Vargas

Pitcairnia is the second largest genus in the family Bromeliaceae. It is distributed from Mexico to northern Argentina and is the only one with presence in Africa. There are approximately 15 species reported for the Caribbean islands, most of which are island endemics. Based on chloroplast data, Shubert (2017) placed them all in a clade along with other South and Central American species, but relationships within remained unresolved. Using a target Sequence Capture approach with the Bromeliad 1776 baits set (Yardeni *et al.* 2021) we analyze the relationships within the Caribbean group and discuss biogeographic implication. Our analysis indicate that 1) the colonization of the Caribbean is the result of one event and subsequent spread and diversification throughout the islands and 2) species diversity has been overestimated likely because of high phenotypic plasticity in the group.

Symposium

CURRENT TOPICS IN THE STUDY OF NEOTROPICAL SUBTRIBE LAELIINAE (ORCHIDACEAE)

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TOWARDS A FORMAL INFRAGENERIC CLASSIFICATION OF *EPIDENDRUM*, THE LARGEST NEOTROPICAL GENUS OF ORCHIDACEAE

Eric Hágsater*, Adam P. Karremans, Diego Bogarín,
Mónica A. Flores-Iniestres, Rubi D. Meza, Lidia I. Cabrera,
Liza M. Linares, Jessy P. Arista, Carolina Granados Mendoza,
Susana Magallón and Gerardo A. Salazar

Neotropical *Epidendrum* (Orchidaceae, Laeliinae) is one of the largest genera of flowering plants, comprising about 2400 species and representing a likely "explosive" evolutionary diversification. A comprehensive, natural classification of *Epidendrum* (i.e. one that formally recognizes only monophyletic groups) has so far been elusive due to the sheer number of species concerned and to the inconsistent variation exhibited by every single morphological character that traditionally has been considered for generic and/or infrageneric delimitation. Such characters have been subjected to convergent or parallel evolution in distantly related clades, as revealed by molecular phylogenetic analyses of a few Sanger-sequenced loci.

Advances in molecular phylogenetics have started to provide insights towards a sound infrageneric classification resulting in more manageable units within the genus, either at subgeneric or lower levels. For instance, phylogenetic analysis of nuclear ribosomal ITS DNA sequences (~840 bp) for a sample of 625 accessions of *Epidendrum* (plus suitable outgroups) provided independent corroboration for many informal groups within *Epidendrum* delimited intuitively by combinations of vegetative and reproductive characters. However, internal support for many such clades is at most marginal, and the relationships among such clades (i.e the "spine" of *Epidendrum*) is largely unresolved.

Rapid, affordable access to datasets of genomic magnitude promise to boost both phylogenetic resolution and support as a basis for, among others, a stable, predictive classification. Preliminary results of phylogenetic analysis of full plastomes for a subsample of 96 *Epidendrum* species, selected to represent clades previously recovered by ITS, is enabling to test support and relationships among groups that align with previously proposed classifications based on morphological information. Based on the molecular trees, advances and challenges involved in making taxonomic sense of this huge lineage are discussed, focused specifically on three clades: *Epidendrum* subg. *Amphiglottium*, the "Mexican clade", and the *Takulumena* group.

PLASTOMICS OF *BARKERIA* (ORCHIDACEAE): INSIGHTS INTO THE EVOLUTION OF A GENUS FROM TROPICAL DRY FORESTS

Cekouat León Peralta*, Gerardo A. Salazar, Eduardo Pérez García,
Lidia I. Cabrera, Carolina Granados Mendoza, Susana Magallón
and Rubí Meza

The genus *Barkeria* (Orchidaceae: Laeliinae) comprises about 17 species found mainly along the Pacific coast of Mexico through Panama. Although it is one of the most emblematic orchid groups from Mesoamerican seasonal dry forests and is common in cultivation, it has been little studied beyond the species' taxonomy, and its intrageneric relationships have not been fully resolved by the few nuclear and plastid markers analyzed in previous works. In this study, we used multilocus data obtained by means of a "hyb-seq" approach to de novo assemble the plastomes of 14 *Barkeria* species and analyzing them to infer phylogenetic relationships within the genus. Plastomes in *Barkeria* have the typical quadripartite structure found in plants, and their average size is 147,659 bp, with the largest found in *B. uniflora* (149,419 bp) and the smallest in *B. naevosa* (144,046 bp). The differences in plastome size are mainly due to the total or partial loss of ndh genes. Maximum likelihood analysis of the aligned plastomes recovered *Barkeria* as a monophyletic group with strong support, with *Myrmecophila galeottiana* as its sister group. Within *Barkeria*, were recovered three main clades that agree with previous morphological and molecular phylogenetic analyzes of the genus, namely, the groups of *B. uniflora, B. obovata* and *B. lindleyana*. Our data indicate a close relation between the *B. obovata* and *B. lindleyana* groups, which agrees with chromosome number and flow cytometry-based genome size estimations. However, the relationships recovered within the *B. lindleyana* group differ from those obtained in other studies. On the other

hand, although the loss of ndh genes did not define a particular clade within *Barkeria*, species more closely related share a similar set of ndh genes, either functional or pseudogenized. The analysis of plastid genomes recovered a robust phylogenetic framework useful to investigate the evolution of various biological attributes of the genus *Barkeria*.

GENOME SIZE VARIATION AND EVOLUTION IN THE ORCHID SUBTRIBE LAELIINAE

Rubi N. Meza-Lázaro*, Aleida Núñez Ruiz,
Miguel Ángel Alcalá Gaxiola, Ricardo Alcocer Vázquez,
Cekouat E. León-Peralta, Mónica A. Flores-Iniestres,
Lidia I. Cabrera, Gloria Soldevilla, Pedro Mercado-Ruaro,
Eduardo A. Pérez García, Eric Hágsater, Carolina Granados Mendoza,
Susana Magallón and Gerardo A. Salazar

Genome size (GS) exhibits significant variation across species and bears implications for the evolution of the phenotype. Larger genomes entail higher energetic costs for an organism to maintain and replicate, impacting cell division rates and developmental processes, with consequences on an organism's growth rate, reproduction, life history traits, and responsiveness to environmental demands. Genome size can be influenced by phenomena such as whole genome duplications (polyploidization) and re-diploidization, as well as the dynamics of repetitive DNA sequences like satellites and transposable elements. Comparative studies spanning species with varying genome sizes can illuminate the intricate interplay between genome size and phenotype evolution. This study aims to characterize genome size variation within the orchid subtribe Laeliinae and provide insights into the biological significance of GS diversity. By examining flow cytometry-based genome size estimates for over 100 Laeliinae species in an explicit phylogenetic context, we found that genome size in Laeliinae varies nearly 7-fold, ranging from 2C = 1.37 in *Meiracyllium gemma* to 9.18 in *Barkeria barkeriola*. Notably, within the genera *Epidendrum* and *Barkeria*, genome size exhibit a 6-fold and 5-fold difference, respectively, whereas within *Laelia* GS varies 2-fold. Moreover, the densely sampled genus *Barkeria* consists of a major clade comprising species with small genomes, contrasting with its sister clade made up of species with large genomes. Various terrestrial orchid lineages, including representatives of subfamily Cypripedioideae and Orchidoideae, as well as terrestrial Epidendroideae (Sobralieae, Calypsoeae), have notably larger genomes than predominantly epiphytic Laeliinae, in agreement with prior findings that terrestrial species, overall, have larger genomes than epiphytes. Our data also suggest a negative relation between GS and Crassulacean Acid Metabolism.

MULTIDISCIPLINARY APPROACH TO SPECIES DELIMITATION: IMPACTS ON CONSERVATION AND BIOGEOGRAPHY OF THE MEGA-GENUS *EPIDENDRUM*

Edlley Pessoa

The taxonomic category "species" is a central unit for systematics, and it is used in all biological sciences. However, despite of its theoretical importance and long history of debates on its definition, the application of names for new or old taxa often is still lacking scientific rigor. First of all, the systematics community should reflect if taxonomy is a matter of opinion or if it is an objective discipline. In this talk I will bring some reflections on this topic using *Epidendrum* as an example. I will argue that taxonomy can and should be practiced testing hypothesis using current available tools. The focus will be on species complexes and species delimitation, showing how recognizing different numbers of species impacts estimates on conservation and biogeography. Distinct tools combined can be used to search for congruence of results in favor or against the acceptance of certain species names, aiming to satisfy as many as possible species concepts. Moreover, multidisciplinary studies are also able to found evidence that some species are paleohybrids. Nowadays many taxonomists are mainly interested on the discovery of undescribed species, devoting much less effort to providing taxonomic revisions and multidisciplinary studies to test previous species hypotheses. These aspects should be taken into consideration, since for some regions of the Neotropics, a good portion of the purported orchid species are known only from their type specimen that have never been subject of subsequent critical analysis, inflating estimates of diversity.

EPIDENDRUM PHYLOGENOMICS: INSIGHTS INTO A NEOTROPICAL MEGA-RADIATION

Gerardo A. Salazar*, Rubi N. Meza-Lázaro, Miguel Á. Alcalá-Gaxiola,
Ricardo Alcocer-Vásquez, Lidia I. Cabrera, Mónica A. Flores-Iniestres,
Cekouat E. León-Peralta, Carolina Granados Mendoza,
Susana Magallón and Eric Hágsater

With ~2400 documented species, *Epidendrum* is perhaps the most species-rich plant genus of the Neotropics. Its taxic diversity is mirrored by noticeable vegetative and ecological disparities, with different growth forms exploiting almost every niche available, in contrast with a conserved floral structure characterized by the fusion of the column to the base of the labellum. Phylogenetic analyses of a few Sanger-sequenced nuclear and plastid markers have been inconclusive regarding the monophyly of *Epidendrum*, identifying its sister group, resolving the backbone relationships of the genus and placing confidently many species. Here we present preliminary results from a Hyb-Seq approach in which we are generating plastid, mitochondrial, and nuclear datasets with the aims of carrying out a comparative analysis of the plastomes and establishing a solid phylogenomic framework of the genus as a basis for a revised classification and macroevolutionary studies. We also estimated nuclear genome size by means of flow cytometry. Analysis of 163 plastomes, including 137 species of *Epidendrum* and representatives of other genera of Laeliinae, support the monophyly of the genus and identify several strongly supported clades. The earliest diverging clade comprises a primarily Central American and Mexican montane group, a connection that was not identified in previous studies. Plastome structure is largely conserved, with the most salient variation involving loss or pseudogenization of ndh genes. Analyses of nuclear, plastid, and mitochondrial data for a subsample of taxa

show overall congruence among the phylogenetic patterns recovered independently by the separate genome partitions, with few "rogue" taxa of conflicting position. Our cytometric estimations reveal noticeable interspecific differences in nuclear genome size. In silico analyses suggest that such differences result from varying quantities of repetitive DNA.

PHYLOGENOMICS OF *CATTLEYA*

Cássio van den Berg

The neotropical genus *Cattleya* comprises 120 species including many of high horticultural value. Previous phylogenetic studies based on the Internal Transcribed Spacer (ITS) or nrDNA and plastid regions allowed a generic classification with 4 subgenera, 3 sections and 5 series. Here, we provide a phylogenomic reassessment of *Cattleya* and related genera based on ddRAD loci. We included 258 samples that covered all the species of *Cattleya, Brassavola, Rhyncholaelia* and *Guarianthe*, and one species of *Myrmecophila* used as outgroup. For library construction we standardized all samples to 30 ng/uL, followed by digestion with the enzymes pstI and mseI. Then, we followed the protocols of Poland et al. (2012), including ligation with adapters compatible with the enzyme overhangs, pooling and amplification by PCR and purification. The libraries were sequenced in the NextSeq 1000/2000 with 400M single-end 100bp reads. Trimming and demultiplexing clustering and locus identification were performed de novo using IPyrad, including clustering threshold and missing data optimization for each analysis. For better use of the data, we performed several analyses in a hierarchical manner. At first, a single analysis with all samples was performed to study the relationships of the major clades in *Cattleya*, that corroborated the main groups previously found with Sanger sequencing, resembling more closely the ITS phylogenies than plastid. After this overall analysis, we reanalized the samples of several subgroups corresponding to the infrageneric taxonomy from the raw data, that, due to increased taxonomic similarity, provided 100-1000 times more loci than the overall analysis. Withing the subgroups the progress in clarifying the phylogenetic relationships was much more pronounced due to the large number of loci obtained. The phylogenomic analysis based on ddRAD corroborated previous results but with higher support values, but provided significant advance in clarifying species relationships with high support in closely related species groups.

PHYLOGENY OF *PROSTHECHEA* (ORCHIDACEAE, LAELIINAE): REASSESSING THE LUMPER-SPLITTER DEBATE AND SHEDDING LIGHT ON ITS EVOLUTION

Tiago L. Vieira

Prosthechea (Orchidaceae) is a Neotropical genus of Orchidaceae comprising ca. 120 spp. Its current, and broader, circumscription represents a reconsideration of an old name to accommodate, overall, species previously placed in *Encyclia* subgen. *Osmophytum*. However, after the reconsideration, other classifications have been proposed splitting the group into smaller genera. This scenario brings taxonomic confusion, and there is still some debate concerning what would be the more appropriate way to circumscribe that group of species. In this work, we present a phylogeny based on nrITS and plastid DNA sequences, including 80 spp. of the genus and 12 spp. of related genera, using Bayesian inference. Our results corroborate the monophyly of the lineage corresponding to a broad circumscription of *Prosthechea*. The small lineages corresponding to *Euchile, Panarica,* and *Hormidium* are in turn monophyletic, whereas the larger *Anacheilium, Pollardia,* and *Pseudencyclia* are not. Splitting *Prosthechea* into previously proposed smaller genera is not supported by our phylogeny, and non-monophyly also hinders the proposal of a comprehensive infrageneric classification. Ancestral state reconstructions point to independent evolution of non-resupination and other morphological traits previously considered of taxonomic value in different lineages within *Prosthechea*. The phylogenetic relationships recovered reflect better the biogeographic patterns rather than the previously proposed morphological affinities, due to homoplasy in floral traits within the genus. Conflicts between nuclear and plastid data were detected, although our results were not conclusive between incomplete lineage sorting or reticulate evolution as the reason.

Symposium

IV Symposium on Neotropical Araceae

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MOLECULAR PHYLOGENIC RELATIONSHIPS AND MORPHOLOGICAL ASSESSMENT OF *PHILODENDRON* SPECIES IN CENTRAL AMERICA

Marco Vinicio Cedeño Fonseca*, Orlando Oriel Ortiz Castillo,
Nils Köster, Astrid de Mestier and Thomas Borschr

The genus *Philodendron* Schott colonized Central America relatively late from the Pliocene onwards. Despite this, in all available molecular phylogenetic studies in *Philodendron*, the representation of Mexican and Central American species has been relatively poor, consequently the evolutionary origin of species diversity in this region remains unresolved. Therefore, we propose to contribute to the taxonomic and evolutionary knowledge of the genus *Philodendron* through phylogenetic studies, complemented by exhaustive sampling in Central America, as well as a deep documentation of the vegetative and reproductive morphology. Fieldwork was made between 2020 and 2023 in Mexico, Costa Rica, Panama, and Colombia. We generated a total of 354 new sequences of three non-coding plastid regions (petD, rpl16, and trnK/matK) from 70 taxa (130 accessions, mostly from Central America). Together with the data from Canal *et al*. (2018, 2019) we have 243 taxa from three subgenera (*Pteromischum, Meconostigma* and *Philodendron*). The phylogenetic results support the monophyly among the three subgenera. In the case of the subgenus *Philodendron*, there are 12 clades, 10 of which consist of species from the Amazon, Andean region, and the Chocó Biogeographical Region. Additionally, we found two distinctive Central American clades, which consist mostly of Central American species, southern Mexico, and three species from the Chocó Biogeographical Region. The phylogenetic results suggest that the endemic taxa from the Isthmus of Panama are nested in four clades, however, two clades contain the majority of the species with other exclusively Central American taxa.

A REVIEW OF TAXONOMIC STUDIES IN ARACEAE

Thomas B. Croat

Considerable progress has been made in our knowledge of the Neotropical aroids justifying a review of that information. In the Central American, all species have been fully revised with 826 species and full revisions have been published or are being published for those genera not previously revised for Central America including *Monstera, Rhodospatha, Spathiphyllum, Stenospermation, Syngonium* and *Xanthosoma*. In addition, both *Anthurium* and *Philodendron*, greatly modified since the last revision, will be republished. Lucid Keys have been completed for *Adelonema, Anthurium, Dieffenbachia, Dracontium, Philodendron, Rhodospatha, Spathiphyllum, Stenospermation* and *Xanthosoma*. Full revisions have been completed for *Chlorospatha* and *Rhodospatha* while for *Xanthosoma* only Brazil and the Southern Cone remain. Floristic surveys have been published for the West Indies, Central America, Argentina, Ecuador, the Guianas, Peru and Venezuela while smaller floristic studies have been made in parts of Colombia, Ecuador and Peru. In Brazil considerable revisionary progress has been made with *Anthurium* sect. *Urospadix* and with *Philodendron*. Molecular studies on *Anthurium* and *Philodendron* have been published and are being reconsidered. Considerable number of new species have been published for South America but much work remains to be finished on most South American genera, especially *Anthurium, Dieffenbachia, Monstera, Philodendron, Spathiphyllum* and *Stenospermation*.

FERMENTING FRUIT MIMICKING FLOWERS WITH SUGARY REWARDS IN TWO CO-FLOWERING *ANTHURIUM* SPECIES (ARACEAE)

Corinna Ehn*, Florian Etl, Raimund Thenhaken,
Wolfgang Wanek, Erika Salas, Stefan Dötterl
and Jürg Schönenberger

Some plants produce floral scents that mimic non-floral resources such as, for instance, the breeding substrate for specialized insects. While carrion and dung mimicry systems are better known, fruit mimicry has been studied less frequently. In this study, two co-flowering Neotropical epiphytes, *Anthurium brownii* and *A. clavigerum* (Araceae) are investigated. Their inflorescences produce floral scents reminiscent of fermenting fruits. In addition, they also produce liquid exudates on their flowers. We chemically analyzed their floral scents, using GC/MS (gas chromatography/ mass spectrometry) and the sugar and amino acid composition of floral exudates via (U-)HPLC/MS [(ultra-)high-performance liquid chromatography/ mass spectrometry]. We recorded the timing of floral anthesis and inflorescence visitors and we conducted bioassays with fermented fruits and with synthetic substances. The studied *Anthurium* species differed in their floral scent composition but shared some main compounds like ethyl acetate and acetic acid. Synthetic compounds (e.g., acetoin, 2,3-butanediol) attracted several floral visitors. Floral exudates, containing a variety of sugars and amino acids, were consumed by individuals from more than 100 insect species. The visiting insect community of *A. brownii* consisted mainly of diurnal insects while in *A. clavigerum* nocturnal insects dominated. *A. brownii* and *A. clavigerum* exhibit two specialized fermenting fruit mimicking systems that are reproductively isolated in their diurnal patterns of flowering time, by their different scent bouquets and, thus, different pollinators. Reproduc-

tive barriers between closely related species are important factors of speciation, and adaptations to different pollinator niches might be one reason for the high species-richness in the genus *Anthurium*.

EVIDENCE FOR THE RECRUITMENT OF FLORIVOROUS PLANT BUGS AS POLLINATORS

Florian Etl*, Christian Kaiser, Oliver Reiser, Mario Schubert,
Stefan Dötterl and Jürg Schönenberger

Angiosperm flowers and their animal visitors have co-evolved for at least 140 Ma, and early flowers were likely used mainly as mating and feeding sites by several groups of insects, including beetles, flies, true bugs, and thrips. Earlier studies suggested that shifts from such neutral or antagonistic relationships toward mutualistic pollination interactions between flowers and insects occurred repeatedly during angiosperm evolution. However, the evolutionary mechanisms and adaptations, which accompanied shifts toward effective pollination, are barely understood, and evidence for such scenarios has been lacking. Here, we show that *Syngonium hastiferum* (Araceae), a Neotropical representative of an otherwise beetle-pollinated clade, is pollinated by plant bugs (Miridae; Heteroptera), which are florivores of *Syngonium schottianum* and other Araceae species. We found that *S. hastiferum* differs in several floral traits from its beetle-pollinated relatives. Scent emission and thermogenesis occur in the morning instead of the evening hours, and its pollen surface is spiny instead of smooth. Furthermore, the floral scent of *S. hastiferum* includes a previously unknown natural product, (Z)-3-isopropylpent-3-en-1-ol, which we show to have a function in specifically attracting the plant bug pollinators. This is the first known case of a specialized plant bug pollination system and provides clear evidence for the hypothesis that the adoption of antagonistic florivores as pollinators can drive flower diversification.

ADVANCES IN THE KNOWLEDGE OF COLOMBIAN ARACEAE

Alejandro Zuluaga

Recently, Colombia was found to be the most significant knowledge gap in plant diversity, and the family Araceae is not the exception. Colombia is believed to house the highest aroid diversity in the world, but there is no updated taxonomic list. The Checklist of lichens and plants of Colombia published in 2015 registered 23 genera and 793 native species for the country, but more than 170 new species have been described in the last ten years. We recorded 25 genera (two new for the country) and 990 species for Colombia. Besides taxonomic work, little is known about other aspects of Colombian aroid diversity, such as epiphytism, diversity patterns, and pollination ecology. We registered 278 true epiphyte species, with the Andes and the Pacific regions being the most diverse. Using 100 m^2 plots, we studied the diversity patterns in the Biogeographic Choco in detail and found levels of exclusivity between 22 and 48 % altitudinally (every 500 m in gradient) and latitudinally (100 km distance). Finally, we are advancing in studying the pollination ecology of 30 species in 5 genera, describing the floral fragrances, thermogenesis patterns, and floral visitors and pollinators. We produced the first fragrance profile and recorded the pollinators of the genus *Chlorospatha*, the first report of *Cyclocephala* beetles as pollinators of *Stenospermation* and a new pollination system involving *Philodendron* and Diptera.

Symposium

MARANTACEAE: DIVERSITY, ECOLOGY AND EVOLUTION

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INFLORESCENCE DEVELOPMENT IN MARANTACEAE: THE ENIGMA OF THE FLOWER PAIRS

Regine Claßen-Bockhoff* and Sarah Deobald

Marantaceae are characterized by unique, modularly organized inflorescences with different degrees of complexity. Instead of flowers, the smallest unit is a pair of mirror image flowers terminating a short shoot with a sterile prophyll and an interphyll from which branching is monochasially continued. Inflorescence architecture is known for a long time, but its interpretation still controversial. It ranges from a pleiothyrse to a compound spike system, i.e., from an inflorescence with cymose vs. racemose branching. The unsatisfactory situation is due to three reasons: terminological incongruences, appearance of sterile prophylls, and contrary opinions about the flower-pair. The latter has been interpreted as a dichasium (`open cyme´), two-flowered monochasium and two-flowered spikelet, respectively. Based on ontogenetic studies, we add a further alternative. We propose to interpret the flower pair as a flower equivalent (floral unit) originating from a flower-like meristem through the process of fractionation (splitting). We also question the interpretation of the monochasium as a racemose branching. Though shoot connection originates from the second bract and not from the prophyll, the overall developmental pattern resembles a cyme. Comparing the typological vs. ontogenetic approach, we conclude that the inflorescences of Marantaceae cannot be easily assigned to one of the common inflorescence types. We rather prefer a descriptive approach: The basic structure in Marantaceae is a compact or loose `spike´ with components each of them including one or several, monochasially connected biflorous elements. The spikes are solitary or parts of a moderately to richly branched inflorescence. Whether the inflorescence is called a pleiospica, thyrse or `thyrsopleiospicate system´ depends on the reference framework. Interpreting the flower-pairs as floral units originating from flower-like instead of inflorescence meristems introduces the ontogenetic concept of inflorescence diversification to monocots. This concept draws attention to analogous inflorescence structures and new evolutionary conclusions.

ENVIRONMENTAL HETEROGENEITY AND SEED DISPERSAL SYNDROMES: IMPLICATIONS TO MARANTACEAE SPECIES DISTRIBUTION

Flávia Delgado Santana* and Flávia Costa

Herbs represent at least 25% of plant species richness in tropical forests. However, we still poorly know most of the herb´s biology and dispersal, and thus how these affect species distribution in tropical environments. Local spatial patterns of plant distribution can be either determined by dispersal or establishment limitation. We evaluated the spatial pattern and demography structure of four Marantaceae species that differed by diaspore size and leaf mass area (LMA). We used Moran's I to assess species aggregation patterns within habitats, while the spatial aggregation at the landscape scale was accessed using Local Indication of Spatial Association (LISA). Finally, we asked how population abundance changes across life stages, habitat heterogeneity and their interaction. The two large-seeded species were widely spread in the landscape while small seeds had a large but local (within habitats) aggregation of abundances. Environmental filtering, possibly leading to recruitment limitation, was strong for all species but water supply was the most important predictor of abundances of most species and life-stages. Light conditions did not affect the population structure as previously suggested, and while seed size predicted spatial pattern at landscape scale, local aggregation patterns inversely differed according to the LMA values of the species. The abundance distribution patterns are partially associated with the dispersal and to the interaction of growth strategies and the landscape patterns of water and light distribution in the heterogeneous landscape. The low LMA of small seeds herbs should explain their association to a fast-growth strategy but not necessary as shade tolerant species. This vegetative strategy ends up breaking the expected link between re-

productive strategies and light environments for understory herbs in a heterogeneous landscape with strong contrasts in water supply.

Symposium

MONOCOT DIVERSITY, RESEARCH AND CONSERVATION IN COSTA RICA

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STATE OF KNOWLEDGE OF MONOCOT DIVERSITY IN COSTA RICA

Mario A. Blanco

Costa Rica is a country with an area of only 51,000 km^2. It has been botanically explored since the mid XIX century, with higher intensity since the early XX century. Several floristic projects at the national level have sequentially augmented the national plant inventory. The last such large-scale effort, the Manual de Plantas de Costa Rica, published its monocot family treatments in 2003, amounting to 2985 monocot plant species (native, naturalized or cultivated on a large scale), documented in the country with at least one voucher herbarium specimen. A survey was carried out to account for the monocot species that have since been added to the Costa Rican flora under the same criteria, filtering (as much as possible) new synonymies and previous misidentifications. The current national total of monocot plant species is 3468 (16% increment), in 40 families and 9 orders (out of 77 monocot families and 11 orders worldwide, according to the Angiosperm Phylogeny Website classification, similar to the APG IV system). The largest angiosperm family in the country, Orchidaceae (1684 spp.), also shows the largest increment (23%), in large part because of the existence of a national research center (Lankester Botanical Garden) focused on this group. Poaceae is the second largest monocot family in the country (510 spp., with ca. 23% of them being naturalized or widely cultivated exotics, by far the largest proportion for any monocot family), but only with a 5% increment. Araceae (306 spp.) is the third largest monocot family in the country and has the second highest increment (20%). Cyperaceae (221 spp.) and Bromeliaceae (205 spp.) are the fourth and fifth largest monocot families, with a 5% increment each. Four families (Asteliaceae, Liliaceae, Musaceae and Pandanaceae) do not have native representatives.

SAFEGUARDING MONOCOT DIVERSITY IN COSTA RICA: IS COSTA RICA'S NATIONAL SYSTEM OF CONSERVATION AREAS EFFECTIVE?

Diego Bogarín

Costa Rica is known for its robust system of conservation areas, which protects nearly 30% of the national territory across 169 protected areas, including national parks, biological reserves, and protective zones. The country also has a long-standing tradition of botanical research, and its flora is among the best documented in the Neotropics. However, there is still a knowledge gap regarding the biodiversity protected in these sites.

Monocots are a significant component of Costa Rica's biodiversity, with over 3,500 species representing 4% of the global diversity of this group in just 0.01% of the Earth's surface. In Costa Rica, monocots are represented by 38 families, including approximately 700 endemic species. This study focuses on three critical aspects of monocot conservation in Costa Rica: 1) What is protected in situ regarding monocot species diversity? 2) Which endemic monocot species are not protected or recorded in protected areas? 3) What is the status of IUCN Red List assessments of monocots in Costa Rica?

Data were gathered from various sources, including taxonomically curated and verified databases such as, GBIF, iNaturalist, Tropicos, POWO, and the major herbaria of Costa Rica, as well as international herbaria known for holding important plant collections from the country. The georeferenced records were filtered and verified, then mapped in QGIS onto Costa Rica's Protected Areas (PAs) map to determine which species are represented in PAs and which have not yet been recorded.

A total of 47,676 occurrence records were obtained, of which 45,654 (95%) were within PAs and 2,022 (5%) were outside these areas. Regarding monocot species diversity, 82% of the recorded species are found in PAs. However, 31% of the endemic species have not yet been documented in any PA.

Regarding IUCN Red List assessments, only 8% (287 species) of monocots have been evaluated. Of these, 1% of the evaluated endemic species include six endangered and six critically endangered species, with one listed as critically endangered. Notably, 1% of the endemic species not recorded in any PA have already been evaluated.

This study highlights the urgent need for more Red List assessments, and emerging tools like artificial intelligence may help expedite this process. Although monocot diversity is relatively well protected in Costa Rica, 31% of the endemic species have yet to be recorded in any PA. Finally, distribution models are suggested to predict the potential presence of unprotected endemic species within PAs, thus contributing to more effective conservation efforts and increasing knowledge of protected monocot diversity.

DO YOU KNOW WHERE THE TYPES ARE? THE MONOCOT TYPE SPECIMENS FROM COSTA RICA

Silvia Lobo Cabezas

Locating and accessing type specimens is an essential activity for any taxonomist, but can be a limiting step, as taxa have been described for more than 300 years and their types are sometimes separately archived in multiple locations, they are, in some cases, not deposited in the herbaria cited in the original description, authors do not annotate the specimens as types, etc., making difficult to detect and to register. Fortunately, worldwide efforts in collection digitization and imaging makes now possible to locate, consult and study type specimens. But, as you can see, from saying to fact there is a long way. This study presents information about monocot type specimens collected in Costa Rica, as part of the updating and registration of the type specimens present in the National Herbarium of Costa Rica (CR) that is carried out periodically. According to the protologues, at least 2026 monocot taxa have been described from (type) material collected in Costa Rica since the mid-19th century. Orchids register the largest number of taxa (65.25%) followed by aroids (9.52%) and bromeliads (7.35%). At least 2514 type specimens of 59% of the taxa are deposited in 70 different collections outside the country of origin (Costa Rica), mainly in the United States (AMES, USNH, MO) and Europe (W, B), accounting for 1054 holotypes and 811 isotypes. On the other hand, 1736 type specimens are housed in three Costa Rican collections (CR, JBL, USJ), 335 holotypes and 408 isotypes. Differences were found in the information recorded between the sources consulted: GBIF registered 3764 type specimens in 61 institutions, 1408 taxa, 1026 holotypes and 1017 isotypes; JSTOR includes 2765 specimens deposited in 66 institutions, 822 holotypes and 1280 isotypes.

MODERN FLORISTICS AND COLLABORATIVE TAXONOMY

Franco Pupulin

While in recent decades molecular biology has brought about invaluable progress regarding the natural relationships between the main orchid groups and is today allowing ever greater precision in the evaluation of relationships between closely related species, the need to correctly understand taxonomic concepts and interpret the circumscription of species in light of their natural variation has become even more urgent. This is particularly critical in the case of those taxa described at the origins of the modern nomenclatory system, which often lack original materials of unambiguous interpretation, as well as for complexes of species of wide distribution, whose effective understanding requires a comparable level of documentation through distant geographical areas, impossible for the individual botanist to complete. At least in the specific case of the Orchidaceae, with which I deal more directly - but I suppose that the topic is generally transversal to systematics -, a methodologically common approach in the documentation of the species has shown that in many cases it can replace direct observation, made impossible by the location of the populations of interest. A "mapping" of the morphological characteristics expressed in individuals of the populations of type- localities, and their comparison with similar documents created across the supposed distribution ranges of the species would be able to provide much more accurate answers to our questions relating to the identity of the species and the real diversity of the groups under study. This points to the formation of a network of botanists who share a minimum common set of concepts and techniques to carry out shared taxonomic work. "Shared taxonomy" constitutes a new frontier for the study of complex groups of plants, and the formation of the network or networks necessary to accomplish it is one of the major challenges of contemporary botany.

Symposium

MYCOHETEROTROPHIC MONOCOT BIOLOGY

PHYLOCOMPARATIVE ANALYSES OF PLASTID GENOME MOLECULAR EVOLUTION IN MONOCOT MYCOHETEROTROPHS

Craig F. Barrett*, Sean W. Graham, Cameron W. Corbett,
Izai Kikuchi, Nathaniel Klimpert, Vivienne K.Y. Lam,
Marybel Soto Gomez, Philippa Stone, Hana L. Thixton-Nolan,
Vincent S.F.T. Merckx and John V. Freudenstein

Mycoheterotrophic plants obtain some or all of their carbon from soil-fungal associates instead of via photosynthesis. This trophic shift has occurred at least 50 times in land plants, with an unusually high rate of transitions found in monocots, including Afrothismiaceae (*Afrothismia*), Burmanniaceae, Corsiaceae, Iridaceae (*Geosiris*), Orchidaceae, Petrosaviaceae (*Petrosavia*), Thismiaceae, and Triuridaceae—typically with accompanying reduction/remodeling of their plastid ("chloroplast") genomes. The families Burmanniaceae and especially Orchidaceae have experienced multiple losses of photosynthesis. Models of plastid genome evolution were developed based on observed regularities in plastid gene content across heterotrophic lineages, although plastid genome (plastome) changes are not limited to massive gene losses. Here we present new data and review current knowledge on monocot plastomes, documenting the convergent, divergent and unique ways that they can evolve. Major topics include: (i) patterns of gene and intron loss/retention in plastid-encoded genes; (ii) genome structural changes, including rearrangements, inverted repeat losses and noncoding region compactification; (iii) DNA substitutional rate acceleration, and changes in purifying selection in retained genes; and (iv) changes in the plastome genetic code. We show that these distinct phenomena do not necessarily evolve in concert.

Orchids in particular provide a microcosm of the possible types of plastid genome evolutionary change, with over half of all origins of full mycoheterotrophy found among the family's >28,000 species. Representative plastomes of nearly all fully mycoheterotrophic orchid genera have now been sequenced, although gaps remain, including a general lack of intrageneric and intraspecific sampling. We gathered publicly available data and sequenced several new orchid plastomes, including previously unsequenced taxa representing nearly all hypothesized transitions to full mycoheterotrophy in the family. We synthesize information from orchids and other monocots in the context of recent models of plastome degradation in heterotrophs, providing novel insights into patterns, tempo, modes and trajectories of plastome evolution.

INTEGRATIVE ANALYSES OF VARIATION IN FULLY MYCOHETEROTROPHIC, NORTH AMERICAN ORCHIDS

Craig F. Barrett*, Hana Thixton-Nolan and John Freudenstein

Mycoheterotrophic plants display drastic reduction in genomic and morphological features, making them attractive subjects for systematic study. These attributes have presented challenges for their taxonomic placement, complicating conservation efforts in these often rare or endangered species. Mycoheterotrophs are useful models for testing general hypotheses on the evolution of parasitism; specifically, potential tradeoffs between extreme host specificity, morphology, reproduction, physiology, and maintenance of genomic stability. Combining data from multiple sources provides a holistic representation of variation for evolutionary biology, integrative taxonomy, and defining conservation units. Here we use three leafless, fully mycoheterotrophic species—*Cephalanthera austiniae, Corallorhiza maculata,* and *Corallorhiza striata*—as models for implementing integrative analyses. In the *Corallorhiza striata* complex, integrative analysis of plastomes, nuclear SNPs, fungal host ITS sequences, and abiotic niche data clearly differentiate *C. bentleyi, C. involuta,* and the widespread *C. striata*. Analyses within the latter reveal divergence among four entities: 1) *C. striata* var. *striata*, 2) *C. striata* var. *vreelandii*, 3) Sierra Nevadan populations (California, USA), and 4) Coast Range/Cascade populations (California, Oregon, USA). Each comprises a strongly supported clade based on >27,000 SNPs generated via ISSRseq. However, there is a lack of fixed differences in "extended phenotype" data (i.e. morphology, niche, fungal associates) among these four entities, as required by the "lineage + role" species concept applied here, so they are best described at the infraspecific level. Similar integrative analyses of plastid genomes, nuclear SNPs, morphology, and fungal

associates are being conducted in *Corallorhiza maculata* and *Cephalanthera austiniae*. By analyzing multiple data streams for these species, with emphasis on potential adaptive variation, we hope to deepen understanding of their conservation status, and extend this framework more broadly in other mycoheterotorphs.

POLLINATION AND REPRODUCTIVE STRATEGIES IN MONOCOT MYCOHETEROTROPHS

John V. Freudenstein* and Craig F. Barrett

Holomycotrophic orchids are often described as being primarily autogamous and this is true for many, but not all. Here we describe the reproductive biology of *Corallorhiza* and suggest that, as a group that is relatively early in the transition to holomycotrophy as indicated by plastome size, it retains more reproductive strategies than many other holomycotrophic groups, ranging from obligate outcrossing to cleistogamy. *Corallorhiza* comprises 13 species occurring primarily in North America but with one circumboreal species and a recently described species from China. Two species in particular are significant in their reproductive strategies. *Corallorhiza striata* is one of the two largest-flowered species in the genus (along with *C. macrantha*). It is a widespread species with variation that is recognized at the varietal level and, with *C. bentleyi* and *C. involuta*, comprises the sister clade to the remainder of the genus. The flowers attract males of an ichneumonid wasp, *Pimpla pedalis*, through scent and visual cues. This is the first pseudocopulatory orchid known in North America, one of only two genera to utilize ichneumonids for this purpose, and one of few epidendroids known to use sexual deceit. The broader implications of this situation are that this species is a "double parasite", narrowly specializing on two groups of other organisms (the other is the fungus *Tomentella*). Along with deceit-pollinated species of holomycotrophs *Gastrodia* and *Danxiaorchis*, these exemplify an evolutionary scenario predicted to be highly improbable. The other species of note is *C. odontorhiza*, which has an obligately outcrossing form and much more common autogamous forms. Here, outcrossing may be limited by pollinator range, with partial peloria providing a mechanism for generating morphologies that greatly extend the range of the species.

TARGET-ENRICHMENT-BASED PHYLOGENOMIC ANALYSIS OF MYCOHETEROTROPHIC LINEAGES IN THE YAM ORDER, DIOSCOREALES

Izai Kikuchi*, Juan Viruel, Vincent S.F.T. Merckx
and Sean W. Graham

Fully mycoheterotrophic plants remain among the most enigmatic plant groups due to their rarity and frequent morphological divergence. The loss of photosynthesis in mycoheterotrophs may also lead to gene loss and/or rate elevation, for example in photosynthesis-related genes. More importantly, the often substantial rate elevation in retained genes can contribute to misleading estimation of their phylogenetic placement. This has been documented for plastid genes in the yam order Dioscoreales, which has experienced multiple independent losses of photosynthesis across the three distinct lineages that include fully mycoheterotrophic plants: Burmanniaceae (which includes both photosynthetic and fully mycoheterotrophic lineages), Afrothismiaceae and Thismiaceae. Here we used a phylogenomic approach to re-examine phylogenetic relationships in Dioscoreales, with a focus on the mycoheterotrophic lineages. We used a nuclear-focused target enrichment approach (Angiosperms353), combined with dense taxon sampling aided by substantial use of herbarium material. Our study includes a variety of fully mycoheterotrophic lineages that represent different ages for the origin of full mycoheterotrophy (and corresponding loss of photosynthesis), to investigate how timing of trophic mode shifts may have affected nuclear gene evolution. We also address the effectiveness of the Angiosperms353 panel to study mycoheterotrophic plants and document recovery and analysis of unbaited plastid genome sequences.

PHYLOCOMPARATIVE TRANSCRIPTOMICS IN *CORALLORHIZA*: CHARACTERIZING DIFFERENTIAL GENE EXPRESSION ALONG A MYCO-HETEROTROPHIC GRADIENT

Brandon T. Sinn* and Craig F. Barrett

Recent whole genome sequencing has improved our understanding of gene family evolution in a few species of mycoheterotrophic orchids. Our work aims to improve our understanding of changes in gene expression that can proceed gene family evolution and the eventual large-scale gene losses that have been characterized from fully-mycoheterotrophic orchid lineages. Here we describe the results of a phylotranscriptomic investigation of differential gene expression using four species which together comprise a trophic gradient in *Corallorhiza*, a clade of early-transitional mycoheterotrophic orchids. We sequenced total mRNAs using the Illumina HiSeq platform from above and belowground tissues in a bioreplicated design from *C. trifida, C. wisteriana, C. maculata,* and *C. striata* populations. De novo assembled transcriptomes were compacted by collapsing all assembled isoforms for a putative gene into a single gene model containing all possible exons. The assembled gene space for each species was evaluated using BUSCOs conserved in monocots and by comparison with Viridiplantae-conserved orthologues in the Plaza database. We identified 22,275 putative gene models, which represent at least portions of 92.4% and 97.4% of expected BUSCO and Viridiplantae orthologues, respectively. The recovered number of putative genes was similar to recent estimates published for other orchids, and 68% were taxonomically assigned to Orchidaceae. Our work identified statistically significant changes to gene expression between both above and belowground tissues between both pairs of earlier- and later-transitional species. GO terms that were significantly enriched in aboveground tissues of *C. trifida* and *C. wisteriana* were associated with photosynthesis-related processes, while those of *C. maculata* and *C. striata* were associated with defense and immune responses as well as oxidation and lipid metabolism. Belowground, *C. maculata* and *C. striata* were characterized by stress responses not enriched in the earlier-transitional species. Our results showcase dramatic changes in the expression of stress, defense, and metabolism-related genes that occurred along the path to full-mycoheterotrophy.

THE PLASTID GENOME OF *OXYGYNE SHINZATOI* AND THE EVOLUTION OF THISMIACEAE

Petra Svetlikova*, Filip Husnik and Kenji Suetsugu

Thismiaceae is a monocot family consisting entirely of full mycoheterotrophs. These plants fully rely for their nutrition on a narrow spectrum of mycorrhizal fungi. The family includes four or five genera but the plastid genomes and plastid-based phylogeny of only two of them, *Thismia* and *Haplothismia*, have been revealed to date. The phylogenetic studies of the nuclear 18S rRNA gene suggest *Oxygyne* a sister group to the rest of the family. Therefore, determining the phylogenetic position of *Oxygyne* may be crucial for studying the evolution of Thismiaceae. The *Oxygyne* genus is not only phylogenetically important, but also has a disjunct distribution in Japan and Africa. Here, we focused on *Oxygyne shinzatoi*, an endemic species of Okinawa. We sequenced its genome with Illumina paired end reads and assembled the plastid genome. The plastid genome of *Oxygyne shinzatoi* is reduced in size and gene content, however the extent of the genome reduction is lower than in the *Thismia* species (30kbp vs. 6–18kbp; 27 vs. 6–16 genes). Our results allowed us to carry out a detailed comparison of the Thismiaceae plastomes and update the plastid-based phylogeny of the family with *Oxygyne shinzatoi*.

A POPULATION PERSPECTIVE ON PLASTOME EVOLUTION IN THE MYCOHETEROTROPHIC *CORALLORHIZA MACULATA* SPECIES COMPLEX

Hana L. Thixton*, Mathilda V. Santee, John V. Freudenstein
and Craig F. Barrett

Mycoheterotrophic plants exploit fungi for most or all nutritional needs, providing a compelling model system to address the evolutionary consequences of genome reduction due to the relaxation of purifying selection on photosynthetic function. We are using the widespread *Corallorhiza maculata* complex to quantify plastome variation across the geographic range. Previous studies based on a limited sampling of complete plastid genome sequences provided robust branch support for relationships within the complex, with a single, shared loss of photosynthesis in the fully mycoheterotrophic North American members (*C. mertensiana, C. maculata vars. maculata* and *occidentalis*). To address the extent and degree of plastome structural modification in the *C. maculata* complex, we have sequenced Illumina genomic libraries from 89 samples from populations across North America for the fully mycoheterotrophic members *C. mertensiana*, *C. maculata* var. *maculata*, and *C. maculata* var. *occidentalis*. We assembled and annotated plastid genomes and compared these to the partially mycoheterotrophic species from Mexico (*C. bulbosa, C. maculata var. mexicana,* and *C. macrantha*). We found structural variation in the large single copy, which has been rarely documented below the species level. Length variation was present in the large single copy, with the smallest being 80,041 bp in *C. maculata* var. *maculata* and the largest at 84,374bp in *C. maculata* var. *mexicana*. We identified a 16kb and 22kb inversion event between *C. maculata* vars. *maculata* and *occidentalis*, with two structural variants present in the former. Potential reconstruction suggests a 16kb inversion followed by expansion to 22kb, or

the 22kb inversion was followed by a reinversion to the 16kb. We further quantified the extent of plastid gene loss, pseudogenization, and analysis of selective regimes for protein-coding genes across the complex. Our analyses provide a population-based perspective on the process of rapid genome evolution, extending beyond what is known above the species level.

Symposium

NEW INSIGHTS INTO NEOTROPICAL BAMBOO BIODIVERSITY

MOLECULAR SYSTEMATICS OF THE NON-WOODY BAMBOOS (POACEAE: BAMBUSOIDEAE: OLYREAE) AND ITS TAXONOMIC IMPLICATIONS

Reyjane P. Oliveira*, Iasmin Laiane C. Oliveira,
Maria Luiza S. Carvalho, Fabrício M. Ferreira,
Cassiano A.D. Welker, João P. S. Vieira, Marcos C. Dórea,
Alessandra S. Schnadelbach and Lynn G. Clark

In the last three decades, the use of molecular systematics has assisted the resolution of taxonomic issues in bamboos and other Poaceae, including decisions on the circumscription of genera and species and also revealing unknown relationships. The main lineage of non-woody (i.e., herbaceous) bamboos comprises the tribe Olyreae, which includes c. 130 species almost entirely restricted to Neotropical forests. Information from isolated regions of plastid and nuclear DNA have recovered its monophyly and identified three main lineages of herbaceous bamboos, with subtribe Buergersiochloinae sister to the Olyrinae + Parianinae clade. Since the first molecular study was conducted in this group, aiming to resolve generic circumscriptions within Olyrinae focusing on *Raddia*, much new information about the tribe has been gathered, supported by macro- and micromorphological data, as well as by biogeographic patterns. Three genera (*Piresiella, Miniochloa* and *Ekmanochloa*) were transferred from Olyrinae to Buergersiochloinae, enlarging the circumscription of the last subtribe to four genera and in Parianinae we described *Parianella* and one other new genus, also increasing its generic number to four. However, most of the novelties come from Olyrinae, the largest subtribe, with two newly described genera (*Brasilochloa* and *Taquara*) as well as five others in revision for publication, based on confirmation of the paraphyly of *Olyra*. Thus, molecular studies indicate that non-woody bamboos currently encompass 30 genera, with several endemic and threatened species, mainly from Brazil. Despite all these advances, the diversity of the Olyreae continues to be underestimated and understudied due to the absence of some rare species in the phylogenies and the complexity of this group´s evolutionary processes, including reticulation and incomplete lineage sorting. Phylogenomic studies based on plastomes are ongoing, to better understand the actual diversity of genera within Olyreae, as well as in Bambusoideae as a whole, and how they are related evolutionarily.

POPULATION GENETICS OF TWO AMERICAN SPECIES OF *GUADUA* (POACEAE: BAMBUSOIDEAE: GUADUINAE)

Eduardo Ruíz-Sánchez, María de la Luz Pérez-García,
Jessica Perez-Alquicira, Yessica Rico, Ofelia Vargas-Ponce
and Lia Monti

Guadua is a genus of bamboo endemic to the Americas, comprising 34 species with a neotropical distribution. It ranges from northeastern Mexico to northern Argentina and southern Brazil. We analyzed the diversity, genetic structure, and the influence of environmental and landscape factors in two species of the genus that are located at the northern and southern extremes of their distribution. *Guadua velutina* is the northernmost species, endemic to Mexico and distributed in the states of Hidalgo, San Luis Potosí, Tamaulipas, and Veracruz, while *G. trinii* has a wide distribution in northern Argentina, southern Brazil, Paraguay, and Uruguay. Both species are riparian, characterized by high population density, even in landscapes fragmented by anthropogenic disturbances. Regarding their flowering cycles, *G. velutina* exhibits sporadic flowering cycles between populations, whereas *G. trinii* displays gregarious massive flowering events occurring every 30–33 years. In total, we examined 169 individuals from 11 populations of *G. velutina* in the Huasteca Potosina, Mexico, and 143 individuals from nine populations of *G. trinii* in the province of Misiones, Argentina. We amplified nuclear microsatellites developed for Guadua species, including eight for *G. velutina* and nine for *G. trinii*. For landscape analysis, we utilized raster layers representing bioclimatic conditions, elevation, vegetation, and rivers. Our findings reveal that both species exhibit moderate to high genetic diversity, low genetic structure, and a high degree of gene flow, with rivers serving as the primary corridors for gene flow. Importantly, it appears that landscape fragmentation has not significantly impacted gene flow between populations in either species. Given the potential of *G. velutina* and *G. trinii* in construction, it is crucial to preserve their habitats, especially rivers and bodies of water, to safeguard their genetic diversity.

AMERICAN BAMBOOS, A NEW RESOURCE FOR IDENTIFICATION OF BAMBOO BIODIVERSITY IN THE AMERICAS

Christopher Tyrrell

Bamboos (Poaceae: Bambusoideae) make up a sizable component of tropical and subtropical ecosystems. The western hemisphere is home to 457 woody and over 150 non-woody species. The number of described species of bamboo has been on an exponential increase, with over half the species and nearly a quarter of the genera being first described in the last 20 years. Bamboo identification often requires examining several different organs (such as culm leaves, branch complements, branch leaves and inflorescence parts). Assigning a name to a specimen can be complicated when the plant is only in a vegetative state. The long-interval, gregarious, monocarpic life history of most woody bamboos means there is low probability of encountering a bamboo in flower at any given time. Moreover, for most species, there are very few herbarium specimens available. In this talk, I present new and developing specimen-based online tools to aid in bamboo identification in the western hemisphere. In addition, these tools allow visitors to visualize species ecological envelopes to facilitate new population discovery, conservation assessments, and natural resources planning. Preliminary results are based on 36,400 specimens representing 636 taxa and demonstrate that most species of bamboo can be found growing from sea level to over 4,000 meters in elevation and in habitats ranging from semi-arid (receiving ca. 350 mm of annual precipitation) to wet (> 13,000 mm) environments. These new resources will hopefully reduce barriers for native bamboo identification and facilitate future research on their diversity, distribution and conservation status.

Symposium

ORCHID FLOWERS: THEIR POLLINATION, ECOLOGY AND EVOLUTION

IMPORTANCE OF THE POLLINATION BY FLIES IN ORCHIDACEAE: A CASE OF STUDY ON THE POLLINATION ECOLOGY AND REPRODUCTIVE BIOLOGY OF *MASDEVALLIA STRIATELLA* (PLEUROTHALLIDINAE)

Noelia Belfort-Oconitrillo

Studying pollination and reproductive strategies in Orchidaceae has been considered key to elucidating evolutionary processes and pollinators' role in the family's diversification. Diptera (flies) interact with the family's most diverse subtribes, potentially pollinating over 8000 orchid species. However, significant information gaps persist, particularly in the Neotropical region. Pleurothallidinae is a fly-pollinated megadiverse subtribe, with *Masdevallia* as the third most diverse genus (with more than 600 species). Despite its diversity, knowledge about the pollination biology of *Masdevallia* remains limited. Evidence of effective pollinaria removal is scarce, and there is little documentation on the diversity of secretory glands associated with pollination mechanisms. Additionally, few studies have investigated the reproductive systems within the genus. Understanding the pollination and reproductive ecology of *Masdevallia* species can contribute to a better understanding of evolutionary relationships in Pleurothallidinae and Diptera's role in the group's radiation. This work focuses on *Masdevallia striatella*, an endemic species from Costa Rica and western Panama. We documented a wide range of floral visitors, including representatives from three classes (Insecta, Arachnida, and Gastropoda) and seven orders of invertebrates. Diptera was the most abundant visitor group. However, effective pollinators were restricted to the family Lauxaniidae (Diptera), with at least three species in the genera *Poecilominettia* and *Xenochaetina*. The perianth of *M. striatella* displays a wide variety of papillary and glandular trichomes, and stomata, with contents that vary between proteins, lipids, insoluble polysaccharides, and simple sugars. It suggests several potential sites for producing and releasing volatile compounds to attract visitors and guide pollinators into the flower's interior. In addition, this study evidences the presence of nectar composed of glucose and fructose accumulating at the base of the lip, suggesting a myophily pollination syndrome. Finally, *M. striatella* is highly self-compatible but unable to self-fertilize autonomously. The species exhibits mechanisms that prevent self-pollination, ensuring cross-pollination as a key feature of its reproductive strategy.

AREAS OF ENDEMISM OF THE ORCHIDS OF MEGAMEXICO: HOTSPOTS OF BIOTIC INTERACTIONS WITH POLLINATORS

Brandon E. Gutiérrez-Rodríguez* and Victoria Sosa

The ecological and evolutionary processes involved in areas of endemism are complex and reflect interactive aspects of climatic, geological factors, biogeographic and evolutionary history. Based on the hypothesis that areas of endemism acted as refugia, we investigated whether areas of endemism of the orchids of Megamexico are hotspots of biotic interactions by comparing the orchid–pollinator interactions with those of adjacent areas. Patterns of functional signatures and phylogenetic signal were estimated, using pollination syndromes as a proxy for functional attributes. Phylogenetic signal was estimated by coding pollinator groups for every orchid recorded. Metrics of the interaction networks and the phylogenetic signal were compared with those obtained from adjacent areas. Our results show that areas of endemism exhibit higher significant differences in the phylogenetic signal compared to adjacent areas. It can be explained by the many distantly related orchid lineages sharing attributes related to pollination. Network size and robustness differed statistically between the areas of endemism and the adjacent areas. The same configuration of modules in interaction networks was found in the areas of endemism; however, remarkably, the composition of species in large genera differed in these areas. Areas of endemism harbor more orchid lineages that closely interact with many groups of insects. The southerly areas of endemism in Chiapas and Central America are prominent, with the most significant phylogenetic signal and networks metrics. The results indicate that areas of endemism for the orchids of Megamexico represent hotspots of biotic interactions. Strategies for conservation must take this biotic interaction into account.

POLLINATION AND SEED-DISPERSAL STRATEGIES IN NEOTROPICAL *VANILLA*

Adam P. Karremans* and Charlotte Watteyn

Vanilla is one of the most highly valued spices worldwide. Little is known about the pollination and seed-dispersal mechanisms of species belonging to the pantropical orchid genus *Vanilla* (Orchidaceae). More than one hundred species are found in Asia, Africa and America. But vanillin, the organic compound responsible for the much appreciated vanilla aroma, is only found in the fruits of certain Neotropical species. These fragrant *Vanilla* species show a series of intricate interactions with other organisms required for pollination and seed-dispersal. Based on sporadic records, orchid bees (Euglossini) have been observed visiting flowers and fruits of Neotropical *Vanilla* species. Our research aimed at better understanding the pollinator attraction mechanisms involved by identifying floral visitors, documenting their behavior, measuring morphological traits, analyzing nectar presence and composition, and profiling floral fragrances. We found that *Vanilla pompona* is pollinated by *Eulaema* bees, and displays a dual pollinator attraction mechanism that combines floral fragrance rewards with food deception to induce pollen removal. *Vanilla hartii* rewards its floral visitors with sucrose-rich nectar, and is pollinated by *Euglossa* bees which fit within the labellar tube. We were also able to answer a longstanding question regarding the role played by animals in seed dispersal among fragrant *Vanilla* species and the importance of their aroma. Animal-mediated seed dispersal is a lesser-known phenomenon in the orchid family. In *Vanilla*, aromatic compounds play a pivotal role in the multimodal seed dispersal. Ectozoochory occurs in dry, dehiscent fruits, whose seeds are dispersed by (i) male euglossine bees collecting the fruit's vanillin aromatic compounds and (ii) female stingless bees collecting the fruit's mesocarp. Endozoochory occurs in (iii) highly nutritious, indehiscent fruits consumed by terrestrial mammals or (iv) fleshy, dehiscent fruits whose mesocarp is consumed by arboreal mammals.

MACROEVOLUTION OF FLORAL SCENT ACROSS RADIATIONS OF MALE EUGLOSSINE BEE-POLLINATED PLANTS

Jasen Liu*, Paulo Milet-Pinheiro, Günter Gerlach, Carlos Nunes,
Isabel Alves-dos Santos and Santiago Ramírez

Floral volatiles play key roles as signaling agents that mediate interactions between plants and animals. Despite their importance, few studies have investigated broad patterns of volatile variation across groups of plants that share pollinators, particularly in a phylogenetic context. The "perfume flowers", Neotropical plant species exhibiting exclusive pollination by male euglossine bees in search of chemical rewards, present an intriguing system to investigate these patterns due to the unique function of their chemical phenotypes as both signaling agents and rewards. We leverage recently-developed phylogenies and knowledge of biosynthesis along with decades of chemical ecology research to characterize axes of variation in the chemistry of perfume flowers, as well as understand their evolution at finer taxonomic scales. We detect pervasive chemical convergence, with many species across families exhibiting similar volatile phenotypes. Scent profiles of most species are dominated by compounds of either the phenylpropanoid or terpenoid biosynthesis pathways, while terpenoid compounds drive more subtle axes of variation. We find recapitulation of these patterns within two independent radiations of perfume flower orchids, in which we further detect evidence for rapid evolution of divergent floral chemistries, consistent with the putative importance of scent in the process of adaptation and speciation.

THE POLLINATION MECHANISMS AND THEIR EVOLUTION IN *TELIPOGON* ORCHIDS: FROM DECEPTION TO HONESTY

Carlos Martel

Telipogon is a highly diverse neotropical orchid genus. This genus accounts for more than 250 species, most of which have evolved recently. The recent explosive diversification of *Telipogon* seems to be related to their pollination strategies. To this date, four pollination mechanisms have been recorded in *Telipogon*: (i) tachinid pollination by sexual deception involving pseudocopulation, (ii) tachinid pollination by sexual deception without involving pseudocopulation, (iii) wasp pollination involving nectar reward, and (iv) spontaneous self-pollination. The first two mechanisms involved male tachinids, which are sexually attracted to the centre of the flowers by a combination of chemical, visual and tactile stimuli. *Telipogon* species that evolved these mechanisms are pollinated when males display premating or mating behaviour on the flowers. The third mechanism involves Braconidae male wasps that pollinate the flowers when feeding from the nectar released by the labellum. The fourth mechanism seems to be a backup strategy also developed by the wasp-pollinated *Telipogon* species, in which the stipe is bent to allow the pollinium to contact the stigma. Based on the morphology and chemistry of the flowers, I have predicted their pollination mechanisms and used them to reconstruct their evolutionary history in *Telipogon*. Sexual deception involving pseudocopulation seems to be the ancestral condition and the more widespread strategy among *Telipogon* species. Only a few species of *Telipogon* seem to have evolved sexual deception without involving pseudocopulation and might have evolved more than once. Wasp pollination in combination with spontaneous self-pollination seems to have evolved twice. Nevertheless, to fully understand the evolution of pollination mechanisms and how they affected the diversification of *Telipogon*, field pollination studies are needed.

FLORAL SCENT REGULATES FINE-SCALE NICHE PARTITIONING OF BEE POLLINATORS AND REPRODUCTIVE ISOLATION AMONG SYMPATRIC *GONGORA* ORCHIDS

Santiago Ramírez

Pollinators have profoundly influenced the evolution and diversification of flowering plants. Shifts in pollinator specificity, for example, can facilitate floral adaptation and the formation of reproductive barriers among plant populations. However, how plants adapt to different pollinators remains understudied. Because floral displays often target multiple sensory modalities—vision, olfaction, taste—identifying or isolating the key traits that mediate pollinator specificity remains challenging. Here we take advantage of a potent chemical signaling system to uncover the mechanisms of pollinator attraction and pollinator specificity in a specialized plant-pollinator mutualism. Flowers of *Gongora* orchids emit scent that exclusively attracts male euglossine bees in exchange for pollination service. In turn, male bees collect and store volatile compounds in hind-leg pockets to subsequently release during courtship display. Thus, floral scent is both an attractant and a reward in this mutualism. We test whether variation in scent chemistry controls reproductive isolation through pollinator attraction and pollinator specificity in *Gongora* orchids. We reconstructed bee-orchid pollination networks by combining direct pollinator observations, analyses of floral scent, genotyping of pollen masses recovered from bees, population genetic analyses and phylogenetic analyses in two geographically isolated communities of co-flowering orchids. We identified seven previously unknown cryptic orchid lineages that emit distinct floral scents (chemotypes), each tightly associated with non-overlapping bee pollinators. Our results demonstrate that scent finely controls pollinator attraction, pollinator specificity and pollinator network architecture. These findings suggest that rapid evolution in volatile chemistry played a key role in the early stages of speciation in this specialized mutualism.

DISENTANGLING THE ORCHID-EUGLOSSINI INTERACTION NETWORKS IN THE TROPICAL FOREST IN COSTA RICA

Nicolás Rengifo Alfonso*, Adam P. Karremans, Paul Hanson
and Mauricio Fernández Otárola

Orchids are one of the most diverse groups of plants. They have sophisticated reproductive mechanisms that have allowed their diversification as well as of their pollinators. The study of orchid reproductive interactions based on pollinarium attached to their bee pollinators provides a clear understanding of the specificity and strength in this mutualism. It also allows the indirect study of the plant reproductive phenology and the seasonal variation in the interactions themselves. In addition, many bee species have a clear seasonal variation in their abundance in certain ecosystems, which puts pressure on the orchids that depend on these pollinators for reproduction. Studying the phenology of these mutualistic interactions can generate information on reproduction and evolutionary mechanisms that have led to the temporal specialization of pollinators by orchids. The objective of this work was to characterize the reproductive phenology of orchids and the Euglossini pollinator bee community. The work was carried out at La Selva Biological Station, Costa Rica. During oneyear, monthly samplings of three days were carried out, using different chemical attractants to orchid bees (Euglossini), and only those observed with pollinators or accessory structures on their bodies were captured. The orchid pollinators were identified at the Lankester Botanical Garden and the bees at the School of Biology, University of Costa Rica. Gongora and Euglossa were the most abundant orchid and bee genera. During the dry season, bee abundance was higher than during the rainy season and flowering species varied significantly from one season to the other, as did bee species. This work presents the first bipartite network between orchids and Euglossini bees, considering the rainfall seasonality and the phenological interactions between both biological groups.

UNVEILING THE ‘BUZZ’ IN AUSTRALIAN SUN ORCHIDS

Daniela Scaccabarozzi* and Nina Sletvold

Certain reward-less plants have evolved floral mimicry to resemble other plants that provide rewards to pollinators. This phenomenon is observed in 32 plant families, including approximately one-third of Orchidaceae. Sun orchids, a group of Australian orchids, are believed to mimic flowers of model plants that require bee wing vibrations for pollen release (buzz pollination). We focused on mimicry, particularly on stamen mimicry in *Thelymitra macrophylla* and *T. crinita*, in two southwestern Australian orchids. To test the function of false anthers, we manipulated the false anthers, including removal and colour obscuring, and we compared orchid fruit set on manipulated and control flowers, in sites with and without model plants for *T. macrophylla* and with low/high model plant abundance for *T. crinita*. We assessed floral trait similarity using spectral reflectance measurements and bee visual perception-based on false-colour photography. Bee behaviour observation during attempts to manipulate false anthers/anthers on orchids and model plants was compared. Preliminary results indicate that intact false anthers enhance orchid success, with higher fruit set in the presence of model plants and higher model plant frequencies. Bees from Halictidae, *Leioproctus* (Colletidae), and *Exoneura* (Apidae) attempted false anther manipulation, buzzing on model plants. Orchid and model plant colours were perceived similarly by bees. *Thelymitra macrophylla* and *T. crinita* are likely pollinated through mimicry of buzz pollinated plants. This study suggests stamen mimicry may be widespread in the *Thelymitra* genus, offering opportunities to explore novel ecological and evolutionary adaptations in deceptive and buzz pollinated plants.

MODELING REPRODUCTIVE SUCCESS OF LONG-SPURRED ANGRAECOID ORCHIDS

Melissa Whitman

In 1862, Darwin observed the exceptionally long nectar spur of the Malagasy orchid *Angraecum sesquipedale* and surmised that it must have co-evolved with a pollinator with a proboscis of comparable length, discovered decades later to be the hawkmoth *Xanthopan morganii praedicta*. Angrecoid orchids have since been used as a model system to study orchid-hawkmoth coevolution. First, I expand on the concept of morphometrics as a predictor of plant-pollinator relationships by investigating potential trade-offs between specialization versus network stability. Information on orchid spurs and hawkmoth proboscis length was obtained from literature or collector records. The coefficient of overlap was calculated for all known interactions, as well as potential pairings, and used to create a larger predictive pollination network of ~400 species to assess possible pollination guilds, network structure, and extinction risk. Using ranked distribution of traits as well as degree of network modularity, I found that generalist associations were common, shaped by diffuse co-evolution, with only a small number of pairings representing highly specialized relationships. Second, I modeled the reproductive success of the long-spurred orchid *A. sororium* (n = 94), using classification and regression trees and data collected in-situ in the highlands of Madagascar. Fruit-set (%) was used as the response variable, and various metrics, including number of flowers, flower height, distance to the nearest neighbor, relative reproductive effort, and whether the site had signs of disturbance, were used as predictors. The highest fruitset (39%) occurred within unburned sites, amongst individuals that were close together (i.e. successful outcrossing), with diminishing returns for individuals with more than 5 flowers. The lowest fruitset success

(4%) was within burned areas, with greater distance between plants (i.e. stochastic visitation) and potential stressors contributing to higher seedpod abortion. This study helps to identify potential conservation priorities, with applications towards future modeling of complex co-evolutionary systems

Symposium

Perspectives on historical and recent hybridization in the evolutionary history of Monocots

BIOGEOGRAPHY HELPS UNDERSTAND DEEP-TIME PHYLOGENETIC RETICULATION

Marcos V. Dantas-Queiroz*, Eliška Záveská
and Pavel Trávníček

As genomic data increasingly pervades phylogenetic studies, reticulation events among terminal lineages or deep time reticulation (DTR) has become more prevalent. Genetic introgression and speciation through hybridization are well-documented phenomena across various domains of life. The coexistence of these processes with DTR in a phylogeny suggests that horizontal transfer of genomic material across ancestral lineages might play a more significant role in biodiversity generation than previously considered. To explore the role of ancient hybridization in the diversification of extant lineages, we employed targeted enrichment-based Illumina sequencing and a suite of genome-wide methods to reconstruct the evolutionary relationships within *Curcuma* (Zingiberaceae) an economically important tropical genus as a model. With approximately 128 species spanning South and South-East Asia, China, Australia, and the Southern Pacific, *Curcuma* offers a diverse and widespread empirical system for exploring the intricate relationship between DTR and biogeography, since hybridization and polyploidization events have been identified as pivotal factors influencing *Curcuma*'s evolutionary trajectory. Our study further investigates the spatial and temporal dynamics necessary for ancient reticulations to occur. By estimating ancestral area probabilities for reticulating nodes, we seek to unravel the geographical dimensions of the hybridization process. Ultimately, this multidisciplinary exploration of the *Curcuma* genus promises to enhance our understanding of the evolutionary significance of ancient hybridization events and their contribution to the rich tapestry of biodiversity.

INTROGRESSIVE HYBRIDIZATION PLAYS AN ESSENTIAL ROLE IN PERIPHERAL, FOUNDING, AND EXPANDING POPULATIONS OF WAX PALMS (GENUS *CEROXYLON*)

María José Sanín* and Sara Carvalho Madrigal

Wax palms, genus *Ceroxylon* (Arecaceae: Ceroxyloideae), form a group of 13 species endemic to the Tropical Andean region. They live in isolated populations, in extreme, high-elevation environments for palms. Despite their patchy distributions, species can overlap in some localities, coexisting as morphologically unique, syntopic entities. Three of these species were shown to share a main pollinator by flowering at different times of the year, suggesting they are reproductively isolated. However, the demographic history of these species also revealed historical interspecific gene flow. Through hybrid capture, we sequenced ca. 4,000 nuclear regions in populations of six species of wax palms, five of which form a monophyletic group. Our work shows that peripheral populations are backcrosses of other species. Also, introgressed regions do not seem to be under positive selection and their retention in a population is likely the result of inefficient reproductive barriers and drift. The magnitude of introgressive hybridization is likely determined by demographic factors, such as population size and interspecific pollen availability. This points to the species likely functioning as an 'evolutionary syngameon' where expanding, peripheral, small, and isolated populations maintain diversity by crossing with individuals of other available wax palms. In mountain contexts, species can benefit from the enhancement of population sizes and genetic diversity by recreating an interspecific common genetic pool. In general, introgressive hybridization is not geographically widespread and can play an essential role in the evolutionary history of founding and expanding populations.

PERVASIVE HYBRIDIZATION AND ADAPTIVE RADIATION

Gil Yardeni, Clara Groot Crego, Michael Barfuss, Walter Till,
Christian Lexer, Thibault Leroy and Ovidiu Paun*

Hybridization is often cited as an evolutionary driver of speciation and adaptation, contributing to biodiversity increase. Evolutionary radiations, generating a large number of species within a particular clade, often happen over short times and are associated with colonization of an array of ecological niches. *Tillandsia* subgenus *Tillandsia* (Bromeliaceae) provides an attractive system to study the drivers and limits of species diversification. This species-rich Neotropical monocot clade includes predominantly epiphytic species displaying vast phenotypic diversity. Recent in-depth phylogenomic work revealed that the subgenus originated within the last 7 MY, later expanding through one major event from South into Central America. However, disagreements between phylogenies and lack of resolution at shallow nodes suggested that hybridization occurred throughout the radiation, together with frequent incomplete lineage sorting and/or considerable gene family evolution. We produced high quality reference genomes and used whole-genome resequencing data to explore the evolutionary history of the subgenus employing both tree-based and network approaches. Our results indicate that lineage co-occurrence does not predict relatedness and confirm significant deviations from a tree-like structure, coupled with pervasive gene tree discordance. Focusing on hybridization, ABBA-BABA and related statistics were used to infer the rates and relative timing of introgression, while topology weighting uncovered high heterogeneity of the phylogenetic signal along the genome. High rates of hybridization within and among clades suggest that, in contrast to previous hypotheses, the expansion of subgenus *Tillandsia* into Central America proceeded in several dispersal events, punctuated by episodes of diversification and gene flow. Network analysis revealed reticulation as a prominent propeller during radiation and establishment in different ecological niches. We finally discuss the possible contribution of hybridization to adaptive trait shifts during this radiation. Our results contribute a plant example of prevalent hybridization during rapid species diversification, supporting the hypothesis that interspecific gene flow facilitates explosive diversification.

ANCIENT HYBRIDIZATION IN ZINGIBERACEAE – ACCELERATOR OR BRAKE IN LINEAGE DIVERSIFICATIONS?

E. Záveská*, J. Leong-Škorničková, O. Šída, Z. Chumová,
M.V. Dantas-Queiroz, K. Hlavatá, A.D. Poulsen and T. Fér

Hybridization plays a pivotal role in the evolution of plants, and its investigation is crucial for unraveling the diversification processes of numerous taxonomic groups. Recently, more attention is focused on the role of ancient hybridization that has repeatedly been shown to trigger evolutionary radiation, although in some cases it can prevent further diversification. The causes, frequency and consequences of ancient hybridization remain to be explored. Here we present an account of several ancient hybridization events in the Zingiberaceae, a tropical family of c. 2000 species, many of which are of high economical importance such as ginger, turmeric and cardamom. We analyzed 1094 targeted low-copy genes and plastomes obtained by next-generation sequencing of 250 species from the Zingiberaceae family, representing the vast majority of known genera and their geographical distributions. Using phylogenetic-network analysis we revealed multiple instances of ancient hybridization, often preceding the diversification of entire genera or larger lineages within the Zingiberaceae family. Such a prevalence of the ancient hybridization explains the long term issue with unresolved relationships among and within some genera and highlights the importance to take historical hybridization events into account when reconstructing phylogenies, resolving taxonomic issues or reconstructing biogeographical history. We further discuss distinct circumstances of ancient hybridization events that might be influenced, for instance, with (in)congruence in chromosome counts of the parental lineages.

Symposium

PHYLOGENOMICS OF NEOTROPICAL ORCHIDACEAE

PLASTID PHYLOGENOMICS RESOLVES RECALCITRANT RELATIONSHIPS IN SPIRANTHINAE (ORCHIDACEAE, CRANICHIDEAE)

Lidia I. Cabrera*, Sandra I. Vera-Paz, Rubi N. Meza-Lazaro,
James D. Ackerman, Federico Rizo-Patrón, Eric Hagsater,
Carolina Granados Mendoza, Susana Magallon
and Gerardo A. Salazar

The reduction of time and cost to obtain genomic data resulting from the rapid development of high-throughput parallel sequencing allows the generation of information at the genomic scale for non-model organisms, including complete plastid genomes (plastomes), facilitating the comparative study of their structure, composition, and gene order, as well as their use in phylogenetic reconstruction. In this study, we de novo assembled and annotated full plastomes of 28 species of 19 genera of Spiranthinae and five species of other subtribes of "core Cranichidaeae" as outgroups. Nine additional plastomes, including both ingroup and outgroup taxa, were downloaded from GenBank. The assembled plastomes exhibit two structural haplotypes (A and B), and a typical quadripartite organization: large single copy (LSC), small single copy (SSC), and two inverted repeat regions (IRA, IRB). Alignment of B haplotypes showed conserved synteny. Plastome length variation (139,811–156,188 bp) was observed, with 36–37% GC content. The greatest variation in length occurs in the SSC (9,166–18,342 bp), mainly due to the loss of genes from the ndh family. Potential pseudogenization of some members of the ndh, rpl, rpo, and rps gene families and some photosynthetic genes was observed. The loss of ndh family genes is especially pronounced in the clade including epiphytic Eurystyles and Lankesterella. A maximum-likelihood analysis of the aligned plastomes strongly supported the monophyly of Spiranthinae and its four major clades, in agreement with previous analyses based on a few Sanger-sequenced nuclear and plastid markers. Moreover, our analysis resolves, with maximum bootstrap support, the relationships among the four major clades of Spiranthinae as [[*Eurystyles* clade – *Spiranthes* clade] – [*Stenorrhynchos* clade – *Pelexia* clade]], in contrast with the undecisive results of prior phylogenetic studies.

PHYLOGENY OF PLEUROTHALLIDINAE BASED ON TARGET ENRICHMENT NGS SEQUENCING AND EVOLUTION OF GENOME-WIDE TRAITS

Jan Ponert*, Pavel Trávníček and Zuzana Chumová

Pleurothallidinae is the most diverse orchid subtribe, comprised of about 5500 recognized species in 48 genera, constituting about 20% of total orchid diversity. Interestingly, this subtribe is only about 20 million years old making it perhaps the largest recent radiation in angiosperms. Despite such a recent origin, Pleurothallidinae possess extreme morphological variability and their taxonomy is highly challenging. We aim to study possible relationships of their diversification with evolution of genomes. We focused on genome size, nucleobase composition (GC content), chromosomal variation (including polyploidy) and the type of endoreplication. We used a target enrichment NGS sequencing approach (HybSeq) to refine the phylogenetic relationships in Pleurothallidinae. Our results mostly support the recent taxonomic classification based on previous results of morphology- and ITS-based phylogenies, but we found some discrepancies, especially in the genera *Diodonopsis*, *Karma* and *Specklinia* which we found to be polyphyletic. The results also support separation of *Rubellia* from *Platystele*. While some lineages are highly supported, others have very low concordance between gene trees, likely indicating rapid radiation events in these lineages. The genera of the Pleurothallidinae were divided into six main groups based on the congruent topologies of multiple gene trees. Genome size diversity was mostly independent of profound chromosome count variation but tightly linked with the overall content of repetitive DNA elements. Our results demonstrate that proliferation of repetitive DNA elements in species with partial endoreplication drive the genome size diversity in Pleurothallidinae. Our multi-loci NGS sequencing approach applied for the first time at the level of the entire Pleurothallidinae brings a new level of precision and accuracy to phylogenetic reconstructions, providing an opportunity to resolve taxonomically intricate complexes.

MITOGENOME EVOLUTION IN ORCHIDS, THE IMPORTANCE OF THE HORIZONTAL GENE TRANSFER

Janice Valencia-D.* and Kurt Neubig

Organellar genomes are remnants of more complex endosymbiotically inherited bacterial genomes, reduced until they reached the simplest and most efficient content. While plastome gene content is nearly constant throughout flowering plants, mitogenomes are less well understood and prone to drastic changes in genome size and content. One mechanism that has importantly affected the mitogenome is the horizontal gene transfer (HGT). The consequential transformation of the host genome reveals a unique opportunity to study the evolution of the genomic content. Here, we present how the new acquired material evolves in the mitogenome and its phylogenetic informativity. This talk will contribute to the understanding of the evolution of orchids by exploring the significance of the HGT in the mitogenomes.

Symposium

SYMBIOTIC SECRETS: EXPLORING MYCORRHIZAL FUNGI FROM NEOTROPICAL EPIPHYTIC ORCHIDS

* * *

MYCORRHIZAL ASSOCIATIONS IN THE THREATENED ORCHID *CATTLEYA PURPURATA* (EPIDENDROIDEAE, ORCHIDACEAE): MORPHOLOGY, ULTRASTRUCTURE, SYMBIOTIC GERMINATION, AND OTHER CONSIDERATIONS

Eliana de Medeiros Oliveira

Tiny orchid seeds carry only a poorly differentiated embryo, which depends on specific mycorrhizal fungi for germination and the development of protocorms. The nutritional supply of these fungi is also essential in the adult stage. Seeking to understand details of the symbiotic interaction between mycorrhizal fungi and *Cattleya purpurata*, an orchid of ornamental importance and threatened with extinction in natural environments, symbiotic germination tests, morphological and ultrastructural studies of the initial stages of development, and also of the roots of adult plants, were carried out using different microscopy techniques. The fungus of the genus Tullasnella, isolated from roots and protocorms, was effective in promoting the germination and development of protocorms. Hyphae entered through the suspensor cells, and formed pelotons only in the medial and apical portions of the embryo. In embryonic cells, mitochondria and protoplasts become more numerous, and protoplasts differentiate into amyloplasts and then into chloroplasts. In adult plants, the roots showed a mycorrhization rate of 18%, and a morphological pattern equal to that of protocorms, where the ultrastructure of the pelotons showed the absence of the plant cell wall, demonstrating the contact between the plant cell's plasma membrane and the fungal wall. Mitochondria and vesicles are often associated with the perifungal membrane. The digestion of the hyphae followed a pattern, where hyphae with electron-transparent cytoplasm were observed in the intact pelotons, and then these cells be-

came more electron-dense and accumulated packets of membranes, myelin-like structures, in the hyphal compartment. So this process culminates in an amorphous mass and electrondense, which is the final moment of digestion of the hyphae, what we call degraded peloton. This morphological pattern resembles that observed in the fungal cell death process. These events may be associated with the fungal renewal process, with plant cells digesting senescent hyphae.

ISOLATION AND MOLECULAR IDENTIFICATION OF ENDOPHYTIC FUNGI FROM EPIPHYTIC ORCHIDS BASED ON AXENIC CULTURES

Carlos Moya Villalobos

Endophytic fungi provide orchids with nutrients, water, and growth regulators, accompanying the plants throughout their development. Many of these fungi form endomycorrhizae, which assist in crucial functions such as germination, growth, and reproduction. Despite relying on endophytic fungi for these essential functions, knowledge of the identity and diversity of orchid endophytes is limited. This project aims to isolate and molecularly characterize by barcoding of the internal transcribed spacer region (ITS) 100 cultures of endophytic fungi from tropical epiphytic orchids in five orchid species, of which 50 have already been characterized. Among these, 12 fungi families have been found, including Ceratobasidiaceae, Xylariaceae, Nectriaceae, and Psathyrellaceae, among others. The last three have been reported in the literature as putatively mycorrhizal. Based on the cultures and information collected, the first collection of Neotropical orchid endophytic fungi cultures has been created. This collection will open up a wide range of possibilities for orchid research, cultivation, and conservation. In a last step, the formation of orchid pelotons will be evaluated with two orchid species from where these fungi were isolated, to document and provide evidence of their symbiotic role.

Symposium

UNTANGLING THE VINE: UNDERSTANDING THE EVOLUTION OF THE ORCHID GENUS *VANILLA*, FROM MORPHOLOGY TO GENOMES

VANILLOMICS: TOWARDS A PHYLOGENOMIC MONOGRAPH OF NEOTROPICAL *VANILLA*

Alexander Damián-Parizaca

The biodiversity of the Neotropics provides an excellent opportunity for investigating various evolutionary phenomena. Early divergent lineages, such as the genus *Vanilla*, show promise as a system for testing these processes. Despite their unique characteristics, including a wide floral diversity and specialization in complex ecological interactions, many aspects of the evolution, taxonomy, and ecology of the genus remain understudied. This study aims to generate an extensive and detailed phylogeny of Neotropical vanillas through an unprecedented international collaborative effort that includes broad taxonomic sampling and the use of high-throughput sequencing techniques, specifically the Angiosperm353 probe set. We will use the generated species tree as a framework to examine the evolutionary relationships of the group, the diversification rate of major clades, their biogeography, and the evolution of certain characters. Simultaneously, we will propose a modern monograph of the group by integrating the molecular data we generate with field studies and an extensive review of herbarium collections. Among the preliminary results, noteworthy are: a tissue bank comprising more than 200 samples, covering >80% of the total Neotropical species, with 70% resulting from the authors' fieldwork efforts; and a matrix of over 2500 specimens (including herbarium samples and flowers in alcohol) from 33 examined herbaria. This project illustrates how regional collaborative work and the exchange of capabilities at different levels can contribute to the study of our Neotropical flora, the most diverse on the planet.

RESOLVING CHALLENGING TAXONOMIC ISSUES IN *VANILLA*

Michel Grisoni* and Pascale Besse

The concept of species is pivotal in biology, but a polysemic notion considered from various and evolving perspectives over time. Though, accurate taxonomic identification of species is crucial for population studies, particularly in evolutionary biology or ecology, and holds implications for commercial activities as well. Despite the growing influence of DNA based methods, integrative approaches, combining different taxonomic perspectives (such as morphology, ecology, geography, chemotaxonomy, behavioral traits, phylogenetics) is nowadays preferred for species differentiation. We exemplify this through the taxonomic deciphering of two *Vanilla* groups of species, highlighting the intricate nature of species delineation in contemporary biological research.

The complexity of the leafless *Vanilla* species in the South-West Indian Ocean (SWIO) region poses taxonomic challenges due to limited morphological differentiation and chloroplast sequence variation. Comprising seven morphospecies, including Malagasy endemics and others in Comoros and Seychelles, previous genetic studies revealed additional clusters in Madagascar. Integrating microsatellite, morphological, and ITS sequencing data, in a comprehensive taxonomic approach, we identified nine species in SWIO, with two new Malagasy species. The leafless *Vanilla* group likely originated in Madagascar from an African leafy ancestor, displaying three subsequent colonization events to other SWIO territories.

The aromatic *Vanilla* species form a complex taxonomic group characterized by a vegetative reproduction mode combined with intra- and inter-specific hybridizations, and polyploidy events. Genotyping-By-Sequencing was employed to assess genetic diversity in *V. planifolia* and *V. × tahitensis*, along with seven related species and nineteen hybrids. Examining 133 vanilla accessions using 2004 SNPs revealed strong genetic structuring among the nine species. The high-density genomic data confirmed the genetic differentiation of *V. sotoarenasii* from *V. planifolia*, previously inferred from significant morphological and biochemical differences, but weak nuclear and plastid sequences divergence. Additionally, Bayesian clustering confirmed kinship in hybrids, and suggested *V. sotoarenasii* and *V. odorata* as the potential parents of *V.* × *tahitensis*.

THE IDENTITY CRISIS OF THE ‘MEXICAN’ *VANILLA*

Adam P. Karremans

Upon publishing the genus name *Vanilla* Mill. in 1754, Philip Miller did not designate a type species. He did mention that it was the fruit of these plants which the “Spaniards in America” called *Vanilla* and which was “much used by them to scent their Chocolate”. In 1768, When Miller updated his definition of *Vanilla*, proposing the name *Vanilla mexicana* Mill. for the species cited above. Naturally, *Vanilla* was typified with the name *V. mexicana*, a species which became associated with vanilla of commerce. However, it later became apparent that early authors had confused different aromatic and non-aromatic *Vanilla* species under the name *V. mexicana* and unfortunately the name actually refers to a species that neither grows in Mexico, nor is aromatic and ofcommercial interest. The aromatic Mexican *Vanilla* thereafter became known as *V. planifolia*. In Mexico, *Vanilla planifolia* seems rather rare in nature, and the name has been heavily associated with the aromatic vanillas in cultivation. Wild populations in Central America, which have somewhat smaller flowers and fruits, have even been suggested to be a different species. But it is these, and not the commercially grown varieties, which in fact are more similar to the original drawings of the type of this species. We propose recognizing these wild forms of *V. planifolia* as typical for the species and have made a formal proposal for substituting the type of genus *Vanilla*, from the non-Mexican and non-aromatic *V. mexicana* to the well-known and aromatic *V. planifolia*.

THE PUZZLING GENOME OF *VANILLA*: CURRENT KNOWLEDGE AND APPLICATIONS

Quentin Piet

Vanilla planifolia is the orchid species cultivated to produce the world's most popular natural flavor. Despite its economic importance, this species has long lacked the genomic resources that are decisive in modern diversity studies and breeding programs. Though, several attempts were made recently to reconstruct its 4.09 Gb diploid genome composed of 16 chromosomes pairs.

While the first two attempts to reconstruct this genome brought a better understanding of some of its characteristics such as genes (59,000 protein-coding genes) and repeats content (72% of the sequence), none of these succeeded in reconstructing the entire sequence. The most complete assembly was only 82% of the expected size and only one-third of its sequence was anchored on a total of 14 pairs of pseudo-chromosomes instead of 16. In addition to frequent aneuploidy and a high rate of repeats, the main cause of the difficulties encountered seems to be the phenomenon of partial endoreplication, specific to the orchid family in plants. This phenomenon, which appears to be evolutionarily conserved, involves several replication rounds of a fraction of the genome - that varies according to the species studied and the tissue within a given species - without cell division.

Despite its incompleteness, this assembly has provided valuable support for genetic mapping endeavors and the identification of Quantitative Trait Loci associated with resistance to *Fusarium oxysporum*. Thus allowing the discovery of resistance related candidate genes.

Most recent efforts, supported by an informed choice of tissues to work with and a combination of technological and methodological advances, are finally leading to a complete and more contiguous assembly of this complex genome. This assembly and its refined annotation – and, more generally, the recent increase of *Vanilla* related omics data - should offer new opportunities in several fields such as *Vanilla* diversity studies and varietal improvement, among others.

GENETIC DIVERSITY AND CLONAL STRUCTURE IN WILD POPULATIONS OF *VANILLA ODORATA* AND *VANILLA POMPONA* IN COSTA RICA

Maria Alejandra Serna-Sánchez*, Eric J Fuchs
and Adam P. Karremans

Around a dozen species of wild *Vanilla* have been identified in Costa Rica, but genetic information about these populations remains limited. This information is crucial, especially considering that three commercially important species are present in the country: *Vanilla planifolia, V. odorata,* and *V. pompona*. Identifying genetic diversity in these species is of great importance to discover genetic variants for breeding or restoration efforts. Both *Vanilla odorata* and *Vanilla pompona* are categorized as endangered species by the IUCN, and the lack of studies on genetic diversity and population structure hampers their management and conservation. Limited genetic diversity in *Vanilla* populations has been suggested due to their potential formation by few individuals with clonal reproduction. However, the interactions among the plant, its pollinators, and dispersers influence the genetic diversity of their populations. To assess the genetic diversity and structure of wild *V. pompona* populations, 117 individuals from eight populations along Costa Rica's Pacific region were collected. Microsatellites (SSRs) previously proposed for different *Vanilla* species were used; of the 28 SSRs tested, 20 were transferable, and 11 were polymorphic. Locating *V. odorata* individuals in the field has proven challenging, with only 54 individuals collected in the Osa Peninsula. Eighteen SSR were transferable, and only 6 showed polymorphism. High genetic diversity was observed in wild *V. pompona* populations (HT = 0.588) with moderate genetic structure (FST = 0.194). As previously suggested, greater genetic diversity was observed among populations rather than within populations, primarily due to their extensive clonal spread. The notable genetic diversity may be attributed to their presence in well-preserved forests, supporting large effective population sizes over extended periods and mitigating the impact of genetic drift. This study is crucial for guiding ex situ and in situ preservation strategies by providing a comprehensive understanding of the genetic diversity in wild *Vanilla* populations.

IN SEARCH OF CLIMATE RESILIENT *VANILLA* SPECIES: COMBINING SPECIES DISTRIBUTION MODELLING AND POPULATION GENETICS TO GUIDE THEIR CONSERVATION

Charlotte Watteyn*, Adam P. Karremans, Eric Fuchs Castillo,
Diego Asvestas, Ruth Madrigal Brenes, Steven Janssens
and Bart Muys

Both staple food and cash crops provide livelihoods to millions of smallholder farmers in the tropics, with cash crops offering great opportunities for rural development. *Vanilla* is a high value tropical cash crop and a globally very important spice used in a broad spectrum of food products. Future vanilla bean supply is, however, at risk due to the combined effect of a genetically eroded crop species with an over-intensified farming system. The negative effects associated with low genetic diversity are expected to exacerbate under climate change. As a consequence, there is an urgent need to enhance vanilla production resilience through diversification at both crop and system level. Despite the promising role of the wild relatives of the crop species *Vanilla planifolia*, an in-depth evaluation to integrate these so-called crop wild relatives into breeding programs with improved plant performance under climate change, is currently lacking. Our study seeks to guide the development of a climate-smart vanilla production strategy that involves novel *Vanilla* genetics. We apply an innovative, interdisciplinary approach that combines state-of-the-art techniques within the field of species distribution modelling and population genetics to (i) formulate a conservation scheme for priority conservation areas (i.e. areas holding populations with great climate adaptive capacity), and (ii) outline a *Vanilla* provenancing strategy by labelling populations or genetic variants that may serve as donors for crop improvement and breeding.

Lectures

A PRELIMINARY OVERVIEW ON WETLAND INHABITING ORCHIDS IN THE NEOTROPICS

Rafael Acuña-Castillo*, Mario A. Blanco, Miguel Artavia,
José Esteban Jiménez and Diego Bogarín

Orchidaceae, a highly diverse family of angiosperms, exhibit remarkable ecological and morphological adaptations, with the majority of species being epiphytic or terrestrial. Nonetheless, their occurrence in aquatic and wetland habitats in the tropics is relatively uncommon, with only a few species adapted to these environments. Consequently, our current understanding of orchids inhabiting wetland ecosystems is limited. In the present contribution we aim to provide a preliminary overview, mostly based on a revision of the literature, about the taxonomic diversity of neotropical orchids occupying wetland habitats.

So far we found 37 genera in 11 tribes and 4 subfamilies. Orchidioideae with 38 species associated with wetlands was found to be the most diverse subfamily in these habitats. We recognize that our results are still preliminary and that a more in depth analysis of the literature, herbarium material and field observations will most likely increase the number of orchid species associated with wetlands.

RECONCILING ANTHROPOLOGY AND GENETICS IN AGRICULTURAL RESEARCH FOR THE WEST AFRICAN MILLET FONIO

George Burton*, Maria Bat Vorontsova and Tiziana Ulian

Reconciling ethical and sustainable practice with scientific progress is an ever-present challenge when conducting research to improve food security. Using the millet Fonio (*Digitaria exilis*, Poaceae) as a case study, we inspect how human-crop relationships can be more effectively integrated with genetic studies to bridge this gap in future-proofing agricultural systems for rural communities. Our starting point is a regional investigation of landrace diversity in the Fouta Djallon region of Guinea, a known hotspot for both infra-specific diversity and production. Working with farmers and local experts we produce an ethnobotanical survey of descriptors for over 20 varieties, assessing cultivation practices, plant traits, food preferences, and changes over the past 50 years. We then establish growth tolerance thresholds for heat and drought stress representative of current and future conditions across cultivation regions in West Africa, using rigorous in-vitro germination trials. RNA-seq analysis allows us to detect the gene regions most heavily upregulated during exposure to these stressors, and predict which landraces might be useful to farmers under future climatic scenarios. Alongside, we explore the phylogenetic structure of the wider *Digitaria* genus using historical botanical collections, tracing geneflow and hybridisation associated with selected-for traits in wild and domesticated species (i.e., developed abscission zones for low shattering, or inflorescence shape for higher yields). We propose this multi-dimensional project workflow as a pipeline to approach Fonio and other neglected and underutilised crops which have great future potential, while keeping human priorities local needs, and ethnobotany at the forefront of our research.

LOOKING BACK TO THE ORIGIN OF AMARYLLIDACEAE: A PHYLOGENETIC AND BIOGEOGRAPHIC ANALYSIS

Zoë Dennehy-Carr*, Alastair Culham, John David,
Chris Yesson, Joy Singarayer and Kálmán Könyves

Amaryllidaceae sensu APG III (2009) has a cosmopolitan distribution and contains several horticulturally important genera including *Amaryllis, Nerine, Agapanthus,* and *Allium*. The family comprises three subfamilies: Agapanthoideae, Allioideae, and Amaryllidoideae, with approximately 90 genera and over 1700 species. Previous research indicates Amaryllidaceae diverged in Africa between 62.5 – 45 MYA based on angiosperm-wide studies. Greater precision on when and where this divergence occurred is lacking due to limited sampling of Amaryllidaceae and lack of fossil history.

Using 78 plastome protein-coding genes from a total of 91 samples covering 56 genera we estimated the age and geographic origin of Amaryllidaceae, all subfamilies, and recognised groups. Due to the scarcity of fossils within Amaryllidaceae, we used molecular sequences from the dated Monocotyledon phylogeny by Givnish *et al.* (2018) to place four fossils and five secondary calibration points across a wider Asparagales phylogeny. The broader taxon sampling of the family, the greater number of genes, and the placement of fossils within Asparagales has made it possible to estimate lineage divergence with greater confidence.

Our results show that Amaryllidaceae diverged 48.6 MYA in southern Africa during the early Eocene, a period of warmer and wetter conditions than present. Our biogeographical analyses indicate that taxa migrated from Africa via the Arabian Peninsula dispersing to Temperate Asia and beyond during the Miocene, an epoch of progressive cooling and aridity. We report the biogeographical pathways that resulted in the cosmopolitan distribution of Amaryllidoideae, linking our knowledge of paleoclimates and paleogeography to dispersal and diversification.

RELATIONSHIP BETWEEN TOTAL PHENOLIC COMPOUNDS, MICROBIOME, MYCORRHIZATION, AND SOIL NUTRITIONAL CONTENT IN DIFFERENT SPECIES OF THE GENUS *GUADUA* (POACEAE) IN COLOMBIA

Lucía Ana Díaz Ariza*, Hair Santiago Puentes Lozano,
Juan José Sánchez, Sergio Díaz, David Villamarín
and Ximena Londoño

Guadua spp. produces several biologically active phenolic compounds that can modify the interactions, composition, and function of the plant microbiome. Some flavonoids were reported to be shared among such as *G. aculeata, G. angustifolia, G. incana, G. uncinata,* and *G. amplexifolia*. They act as signaling and chemostimulatory molecules that mediate communication and association between plant and soil microorganisms. This is particularly true and relevant for rhizospheric symbiosis and recruitment of plant growth-promoting microorganisms. Therefore, understanding the composition of secondary metabolites in the plant, in addition to the its genotype, phenological stage, and soil associated with the roots, is necessary to understand the structure of the rhizobiome assemblage. In studies of natural *Guadua* plants, common bacteria were found in different *Guadua* species and in different regions of Colombia. In the case of arbuscular mycorrhizal fungi, although there are common fungi in the soil of different locations, not all of them are present in the roots of the individual *Guadua* collected. The mycorrhization of the plants exceeds 60% in all cases, and among the most common fungal groups in the soils of guadua trees in the Andean region of Colombia, are *Acaulospora* sp. and *Glomus* sp. Agronomic management of a crop, including the application of fertilizers and biological inoculants, affects the phenolic and flavonoid content of plants that produce these metabolites. *Guadua an-*

gustifolia (Guadua) responds favorably to the application of microbial inoculants. The evaluation of different chemically synthesized fertilizers on guadua has shown an increase in the chlorophyll, nitrogen, and phosphorus content of the leaves. In our studies, the combined use of DAP and *Azospirillum brasilense, Pseudomonas fluorescens,* and *Stenotrophomonas* sp. inoculants was recommended for the production of *G. angustifolia* plant material with a high content of promising biologically active flavonoids or phenolics.

POLLINATION ECOLOGY OF *DRACULA ERYTHROCHAETE* (ORCHIDACEAE): A CASE OF FOOD DECEPTION IN A FUNGAL MIMICRY SYSTEM

Karen Gil-Amaya*, Melania Fernández, David Grimaldi,
Mario A. Blanco, Miguel Benavides-Acevedo, Lizbeth Oses
and Adam P. Karremans

Pleurothallidinae is the most diverse Neotropical subtribe in Orchidaceae and is almost exclusively pollinated by insects of the order Diptera. *Dracula*, a genus of 138 species in the Pleurothallidinae, is known to attract Zygothrica (Drosophilidae) flies, common macrofungi visitors, by imitating fungal volatile compounds and lamellae. Interestingly, *Dracula* orchids do not appear to offer any rewards to their floral visitors. While brood-site imitation of macrofungi has been suggested as their pollination system, the exact behavior of flies during their extended visits to the orchid flowers is yet to be confirmed. In this study, we documented the pollination mechanism of *Dracula erythrochaete*. We characterized the floral structures involved in the mechanism using anatomical and morphological evidence. Additionally, through the application of in situ observations and camera recordings, we described the insect behavior. We show that flowers of *D. erythrochaete* share the same group of visitors as nearby macrofungi, including different *Zygothrica* species, seven of which were determined as effective pollinators. Male and female flies were attracted to the flowers and displayed a feeding behavior. Accordingly, proteins were detected in high concentrations on the papillae at the base of the movable lip and in papillary trichomes of the sepals, near the column. Brood-site imitation is debated, as no oviposition events were observed nor

were eggs found on the flowers, food deception is proposed as an alternative strategy for pollination.

NEW INSIGHTS INTO THE TAXONOMY OF HYACINTHINAE PARL. (ASPARAGACEAE, SCILLOIDEAE)

Hannah Hall*, Alastair Culham, John David
and Kálmán Könyves

The Eurasian subtribe Hyacinthinae Parl. consists of more than 200 species of mostly spring-flowering bulbs, several of which have importance in horticulture, notably hyacinths, grape hyacinths and squills. The number of genera in Hyacinthinae has varied over time. Speta in 1998 provided a comprehensive morphological investigation of the Hyacinthinae, increasing the number of recognised genera from nine in Flora Europaea to 21 based largely on bulb characteristics. The first molecular phylogenetic investigation into the subtribe, by Pfosser and Speta in 1999, using two plastid regions, showed that *Scilla* in the traditional sense, to be highly polyphyletic and recognised three main clades: *Scilla* s.str., *Fessia*/*Hyacinthus*, and *Hyacinthoides* containing genera recognised by Speta. Since this treatment of Hyacinthinae, there has been a general resistance towards the acceptance of these genera due to the lack of readily visible morphological characters and few molecular based phylogenetic investigations into the subtribe. Kew's Plants of the World Online recognises only 13 out of the 21 genera as proposed by Speta. Using whole plastome data, and sampling across the taxonomic and geographic breadth of Hyacinthinae, we present the most comprehensive phylogenomic investigation into the subtribe including 175 samples representing over 100 species across all 21 genera. Though our phylogeny of Hyacinthinae is broadly congruent with those previously published, the increased data depth and sampling breadth has revealed a polyphyletic *Hyacinthus*, with *H. litwinovii* and *H. transcaspicus* recovered within *Fessia*. Furthermore, our results reveal a polyphyletic *Othocallis*, splitting into three monophyletic groups here named *Othocallis* 1, 2 and 3. Our results suggest that further redefinition of generic boundaries is needed within Hyacinthinae.

CONTRASTING REPRODUCTIVE TRAITS AFFECT THE GENETIC DIVERSITY AND FINE-SCALE GENETIC STRUCTURE OF TWO SYMPATRIC EPIPHYTIC BROMELIAD SPECIES

Sofía Huerta-Fahara*, Eric J. Fuchs-Castillo,
Afredo Cascante-Marín, E. Jacob Cristóbal-Pérez,
Ruth Madrigal-Brenes and Mauricio Quesada-Avendaño

The effect of reproductive traits on genetic diversity and its distribution in space has been poorly studied in epiphytic angiosperm species, in which different patterns can be expected compared to terrestrial plants because of their distribution within phorophytes trees. In this study we determine how reproductive traits of two epiphytic plant species of the family Bromeliaceae: *Aechmea mariae-reginae* and *Werauhia ampla*, affect genetic diversity and fine-scale genetic structure in a Costa Rican montane forest. *Aechmea mariae-reginae*, a dioecious plant, with obligate outcrossing, pollinated by hummingbirds and dispersed by birds and *W. ampla*, a hermaphrodite, self-compatible plant, pollinated by bats and dispersed by wind. 8 polymorphic microsatellites determined that genetic diversity was higher in the dioecious species with obligate outcrossing and pollinated by hummingbirds *A. mariae-reginae*, (Ne= 2.497, He= 0.577, Fis= 0.11) compared to the bat-pollinated self-compatible hermaphrodite species *W. ampla* (Ne= 1.730, He= 0.401, Fis= 0.175*, *p<0.05). Spatial autocorrelation analyses showed that fine genetic structure was higher in the dioecious, hummingbird-pollinated, bird-dispersed species *A. mariae-reginae* (GST=0.099 G'ST= 0.225, Sp=0.0242) compared to the self-compatible, bat-pollinated, wind-dispersed hermaphrodite species *W. ampla* (GST=0.0748, G'ST= 0.1254, Sp=0.0084). *Aechmea mariae-reginae* has high genetic diversity values because it is a dioecious species and because of the ability of hummingbird-mediated pollen dispersal, the low genetic diversity in *W. ampla* is due to the high potential for self-fertilization possibly because the low abundance of pollinators. Limited seed dispersal of *A. mariae-reginae* by social birds generates high genetic structure in the population, whereas wind dispersal of *W. ampla* seeds does not generate genetic structure.

THE EVOLUTIONARY HISTORY OF TEPALOID MONOCOTS: AN EMPHASIS ON LILIACEAE (LILIALES)

Joo-Hwan Kim

The families of the monocot order Liliales exhibit highly contrasting characteristic of photosynthetic and mycoheterotrophic life histories. Although previous phylogenetic and morphological studies of Liliales have been conducted, they have not examined molecular evolution associated with this contrasting phenomenon. Here, we conduct the first comparative plastome study of all ten families of Liliales using 29 newly sequenced plastid genomes analyzed together with previously published data. We also present a phylogenetic analysis for Liliales of 78 plastid genes combined with 22 genes from all three genomes (nuclear 18S rDNA and phyC; 17 plastid genes; and mitochondrial matR, atpA, and cob). The newly sequenced plastid genomes and combined three-genome data revealed Smilacaceae as sister to Liliaceae instead of Philesiaceae and Ripogonaceae. Additionally, we propose a revised familial classification system of Liliales that consists of nine families, considering Ripogonaceae a synonym of Philesiaceae. The ancestral state reconstruction indicated synapomorphies for each family of Liliales, except Liliaceae, Melanthiaceae and Colchicaceae. A taxonomic key for all nine families of Liliales is also provided.

We also conducted molecular phylogenetic analyses, time estimations and biogeographical analyses to confirm generic relationships, discuss their circumscription for classification and clarify the evolutionary history of Liliaceae. A phylogenetic analysis is presented as a combined data set from four plastid genes for 142 taxa representing all the genera of Liliaceae. Medeoloideae were newly defined as an independent subfamily from Lilioideae. Tricyrtis of Calochortoideae is embedded in Streptopoideae, despite its unique characteristics. The crown age of Liliaceae was calculated at c. 85 Mya, and Liliaceae are considered to have originated in temperate Asia in the late Cretaceous and to have expanded their distribution via dispersal with the occurrence of repetitive vicariance events during their evolution.

UNFOLDING ORCHID DIVERSITY THROUGH PAPER FOLDING

Cekouat Elim León Peralta

Exotic and elegant, orchids have inspired different kinds of artistic expression for millennia. Origami, the ancient Japanese art of paper folding, has not been an exception. However, very few artists have explored this subject so far, specially following orthodox origami principles, i.e. using uncut squares of paper only by folding. In this talk, I will explain the mathematical principles and fine-art methods I have applied over a decade to develop a series of realistic origami orchid designs representing species from the five subfamilies in the Orchidaceae. Through examples of selected workshops and exhibitions, I will highlight how origami can be a tool for teaching concepts of orchid morphology, ecology and diversity.

THE PHYLOGENY AND EVOLUTION OF THE PALEOTROPICAL WOODY BAMBOOS (POACEAE: BAMBUSOIDEAE)

Jing-Xia Liu*, Cen Guo, Peng-Fei Ma, Meng-Yuan Zhou,
Ya-Huang Luo, Guang-Fu Zhu, Zu-Chang Xu,
Richard I Milne, Maria S. Vorontsova and De-Zhu Li

The evolutionary lability of morphological characters has been hypothesized to have been driven by multiple factors, but few studies have synthesized morphological data with phylogenetic frameworks to elucidate broader patterns of character evolution. The paleotropical woody bamboos (PWB) are distinct hexaploid with complex evolutionary history and remarkablely diverse morphology, making it an ideal clade to investigate the factors underlying morphological evolution in bamboos.

We generated a robust and time-calibrated phylogeny of the PWB using SNPs retrieved from MiddRAD-seq data, reconstructed ancestral characters with rates of transition, and explored associations between environmental variables and key morphological characters.

The PWB started to diverse across the Oligocene/Miocene boundary and formed four major clades including Melocanninae, Racemobambosinae, Hickeliinae and Bambusinae corresponding to recognized subtribes. The ancestor of PWB was reconstructed as having erect habit, indeterminate inflorescence and nucoid caryopsis. However, the evolution of all three traits was correlated with, and hence likely influenced by, aspects of climate, topography, and soil in general, leading to homoplasy across the PWB.

Our results synthesized here provide an example of how morphological characters under environmental selection can show homoplasy and hence must be used with caution at generic and higher taxonomic levels, with novel perspectives on morphological evolution of the woody bamboos.

CAREX SECT. *FECUNDAE*: A BOREOTEMPERATE RADIATION GROUP IN THE NEOTROPICS

Raúl Lois*, Anton A. Reznicek, Socorro González Elizondo, Eduardo Ruiz Sánchez, Marcial Escudero, Ana Morales, Carmen Acedo, and Pedro Jiménez Mejías

Carex, a member of the Cyperaceae family, stands as a megadiverse genus encompassing over 2000 recognized species, primarily thriving within the boreal and temperate zones of the northern hemisphere. Despite this, certain lineages of *Carex* have successfully ventured into the tropical zones of the southern hemisphere on multiple occasions. Notably, *Carex* sect. *Fecundae* emerges as one such lineage, displaying a distribution entirely within the Neotropics, predominantly within high-altitude forests and grasslands spanning from Central Mexico to Northern Argentina.

Comprising more than 30 species, *Carex* sect. *Fecundae* stands as one of the broadest lineages within *Carex*. It boasts perhaps the most intricate inflorescence patterns among its counterparts. However, akin to numerous Neotropical plant groups, the understanding of sect. Fecundae remains significantly limited. Only three treatments of this section exist to date, none of which are comprehensive.

Our research addresses this gap through a comprehensive taxonomic revision of *Carex* sect. *Fecundae*, reviewing more than 1000 specimens and statistically testing more than 100 characters. We established morphometric boundaries for species within the section by selecting the most distinguishing characters, such as inflorescence conformation, utricle, glumes, and achene dimensions. Furthermore, we undertook the first complete molecular phylogeny of the section, employing HybSeq to unravel the evolutionary relationships within *Carex* sect. *Fecundae*.

Our combined analysis of morphological and molecular data led to the discovery of 18 previously overlooked species within the section. Additionally, we dated the recent origin of this section to approximately 5.77 Mya, affirming the rapid adaptive radiation witnessed within *Carex* sect. *Fecundae*. Through biogeographical analysis, we inferred the most recent common ancestor of the section between the Neotropical and South American Transition Zones. This origin was followed by two independent rapid radiations—one in Mexico and the other in South America—further elucidating its evolutionary history.

COMPREHENSIVE MANAGEMENT IN EL PARAÍSO DEL BAMBÚ Y LA GUADUA: EDUCATION, SCIENCE AND INNOVATION

Ximena Londoño

Bamboo has a long history in the world and is closely related to people's lives. In El Paraíso del Bambú y la Guadua, given the deep conviction of its mentor, that bamboos are plants that can transform the lives of millions of people and that they have advantages over dicotyledons, such as their rapid growth, their self-reproduction and their high economic value, she decided to consolidate a life project, more than 30 years ago, at Finca El Bambusal, located in the Department of Quindío, the heart of the Colombian coffee region. El Paraíso del Bambú y la Guadua opens to the public in 2014 with the aim of offering experiential tourism, through educational, sensory and territorial assessment processes; is a business model with a triple impact strategy: social, environmental and economic, where innovation, entrepreneurship, environmental and financial sustainability are combined. El Paraíso del Bambú y la Guadua has one of the most extensive collection of bamboos in Latin American countries and develops research projects in partnership with national and foreign universities. The management of bamboo and the agro-industrial development in El Paraíso del Bambú y la Guadua tends to maintain a sustainable model, where productivity, environmental conservation and innovation are its main objectives. As a result, this small park did not only popularize bamboo knowledge and culture, but also provided large amount of mother stocks for reforestation and ornamental projects. Up to date, El Paraíso del Bambú y la Guadua educated 8700 visitors since May 2014 to August 2023. The proposed talk will highlight the relation between this giant monocot and El Paraiso del Bambú y la Guadua.

A DIFFERENCE IN STRATEGY: HOW HABITAT AFFECTS FUNCTIONAL TRAITS RELATED TO COLONIZATION AND PYROGENICITY IN BOLIVIAN *GUADUA*

Elizabeth K. McMurchie*, Joseph W. Veldman,
Maira T. Martinez-Ugarteche and Lynn G. Clark

The Neotropical woody bamboo genus *Guadua* contains many of the most ecologically important bamboos of the Neotropics. Although primarily limited to the lowlands, *Guadua* have colonized a broad variety of habitats, including tropical rainforests and dry forests, seasonally-inundated river margins, and, among a few species, fire-dependent savannas. In each of these habitats, *Guadua* can have dramatic effects on surrounding vegetation. Large stands of *Guadua* can dominate entire forests and river margins, and some can affect successional patterns by colonizing gaps and killing surrounding trees. *Guadua* that predominantly live in savannas are often the dominant grass in these habitats, and can survive and quickly resprout following fires that limit growth of trees. While savanna species of *Guadua* appear to maintain savanna habitats and take advantage of disturbance, they seem unable to invade undisturbed forest habitats and convert forests to savannas. The limited ability of savanna *Guadua* to spread into forests may be related to trade-offs in the functional traits of this species: the traits that indirectly affect fitness through their effects on survival, reproduction, and growth. We compared the functional traits related to ability of bamboos to survive and persist after fire and those related to colonization between *Guadua* from Bolivia, including *G. paniculata* of savannas, *G. chacoensis* of forests, and tree-killing gap-colonizer *G. weberbaueri*. Preliminary results indicate that there are significant differences in some of the fire- and colonization-related functional traits of these species, including culm height, culm diameter at base, internode wall thickness, aboveground biomass, and number of rhizomes produced. However, no significant differences were found in rhizome length, a functional trait related to colonization potential. Only the savanna species *G. paniculata* was observed to produce large resprouts of the original culm when the culm was burned or cut near ground level.

EXPLORING THE DIVERSITY AND EVOLUTION OF CLIMBERS IN THE NEOTROPICS: ONGOING STUDIES IN THE GENUS *SMILAX* (SMILACACEAE)

Thomas H. Murphy* and Lucas C. Majure

Smilax, a monocot genus of primarily climbers, exhibits a near-cosmopolitan distribution and is comprised of 200–350 species. Neotropical taxa belong to a well-supported New World Clade, but the species diversity and evolution in this clade remains poorly understood relative to the remainder of the genus. Ongoing research is demonstrating that species diversity is vastly underestimated under current taxonomic frameworks. Not only has the discovery of new species added to basic taxonomic knowledge of the genus, but they also reveal intriguing biogeographic patterns and unusual morphological characters that have gone previously undetected and undescribed. Many morphotypes, which likely represent new species, cannot be described or sampled in molecular phylogenies until additional fieldwork is conducted. Lastly, integrating data from phylogenomics, morphometrics, and distributions is discussed as a promising avenue to study the interaction between leaf shape evolution and niche transition in the Neotropics.

A SUBGENERIC RECLASSIFICATION OF *MASDEVALLIA* (ORCHIDACEAE: PLEUROTHALLIDINAE) BASED ON GENETIC DATA

Lizbeth Oses* and Adam P. Karremans

The horticulturally important genus *Masdevallia* is one of the most species-rich groups in the Pleurothallidinae (Orchidaceae) subtribe. Historically, species assigned to the genus have been classified under various, sometimes conflicting, generic and subgeneric systems. None of which have been supported by molecular data, prompting instability in the nomenclature of this challenging group of species. In this study, we evaluate the phylogenetic position of 124 species of *Masdevallia* based on ITS sequences and matK sequences analyzed using Bayesian inference and maximum likelihood. Previously proposed generic and subgeneric segregates are evaluated, and mostly found to be inconsistent and non-monophyletic. Both the Bayesian inference and Maximum Likelihood analyzes reflect little support for most *Masdevallia* segregates. Phylogenetic relationships are found to be highly correlated with geographic distribution, while floral morphology is often found to result from convergence rather than relatedness. Genus *Masdevallia* is recircumscribed in a broad sense, with highly supported clades prompting the recognition of 4 subgenera and 15 sections established based on their phylogenetic relationships, carefully redified using morphological and ecological features.

VARIATION OF PLASTOMES AND THEIR POTENTIAL ROLE IN ADAPTIVE EVOLUTION OF POALES

Peng-Fei Ma*, Hong Wu and De-Zhu Li

The monocot order Poales includes 16 families and more than 23,000 species, accounting for about 7% of the angiosperm and 33% of the monocot diversity, with significant value in ecology and economy. In our previous study, we reconstructed a robust phylogeny of Poales based mainly on plastomes. Substantial phylogenetic conflicts were also revealed within the plastid genomes of the order. Here, we investigated the variation patterns of its plastomes by analyses of 93 plastomes from 16 families and 5 clades of Poales. The plastomic variation of Poales was diverse, including genome size, gene content, GC content, inverted repeat (IR) type and IR shift. For example, the Poales possessed the largest plastome of monocots, having a size of 225,293 bp and about twice of the smallest one (126,519 bp) in the same order. We also found an unexpected variety of IR types, especially the three IR copies in two sampled species, which, to the best of our knowledge, is the first such report in angiosperms. The repetitive sequences in plastomes of Poales were closely related their inversions and rearrangements. We also found the loss of ndh genes and variation of GC content may be an adaptation to swampy or wetland habitats. And four plastid genes were identified as positively selected, which may be related to the diverse habitats and the C3-C4 photosynthetic transition in Poales. Our findings provide new insights into the plastomic evolution of Poales, as well as for the angiosperms as a whole.

BIOGEOGRAPHY AND CLASSIFICATION OF THE GRASSES (POACEAE)

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L.G. Clark, C.D. Tyrrell, C.A.D. Welker, E.A. Kellogg
and J.K. Teisher

Using chloroplast DNA sequences (*trnK-matK*, *trnL-trnF*, and *ndhF* loci) from 700 of the 789 current use genera in the Poaceae (89%), we reconstructed a time-calibrated phylogeny and inferred ancestral areas. The phylogeny is well resolved at the subtribal level (109) and the grasses began to diversify in the Early to Late Cretaceous (crown age of 98.54Ma) on West Gondwana before the complete split between Africa and South America. Vicariance from the splitting of Gondwana may be responsible for the initial divergence in the family. The crown node of Poaceae was inferred to have occurred in a combined area consisting of the Neotropics and Afrotropics (42%). The Afrotropics was the most probable ancestral area along much of the backbone of the family, including at the crown of the Spikelet Clade (43%), Bistigmatic Clade (77%), and Core Grass Clade (69%), leading to the crown nodes of the BOP (49%) and PACMAD (87%). The common ancestor of Pooideae and Bambusoideae was estimated to have occurred in the Indomalayan region (21%), Afrotropics + Palaearctic (15%), Afrotropics (12%) or Indomalayan + Palaearctic (12%). Within PACMAD each of the subfamilies had an inferred Afrotropical origin, including Panicoideae (stem 87%), Aristidoideae (stem 90%), Micrairoideae, and Arundinoideae (stem 38%, or Afrotropics + Indomalaya 14%), and Danthonioideae and Chloridoideae (stem 85%). The current classification of the family will be discussed with an extensive review of the extant diversity and distribution of species, molecular and morphological evidence supporting the current classification scheme, and the evidence informing our understanding of the biogeographical history of the family.

PHYLOGEOGRAPHY OF THE *POLIANTHES GEMINIFLORA* (ASPARAGACEAE) COMPLEX

Pedro Daniel Rivas-Salazar and Aarón Rodríguez*

The phylogeography studies the geographic distribution of the genetic variation within a species or among closely related species. It is a useful method for the species delimitation when they have diversified because of recent geological and orogenic events, for example, the origin of the Transmexican Volcanic Belt (TVB) and the climatic oscillations of Pleistocene. As a result, species complex arise. A species complex groups related taxa but difficult to take apart. Sequence evidence suggests the diversification of genus *Polianthes* (Asparagaceae) during these geological events. The *P. geminiflora* complex includes *P. cernua, P. geminiflora, P. graminifolia, P. multicolor, P. quilae* and *P. zapopanensis* and are distributed along the TVB. In turn, P. *geminiflora* includes *P. geminiflora* var. *geminiflora*, *P. geminiflora* var. *clivicola* and *P. geminiflora* var. *pueblensis*. The analyses of the genetic and phylogeographic structure of *P. geminiflora* complex could help to recognize its components. In addition, based on morphological differences of the vegetative structures, the varieties of *P. geminiflora* could be recognized as different species. To evaluate these hypotheses, we used the sequences of intergenic spacers rpl32-trnL, ndhF- rpl32 and 3'rps16-5'trnK of five to 12 samples in 21 populations of *P. cernua, P. geminiflora, P. graminifolia, P. howardii P. multicolor, P. quilae* and *P. zapopanensis*. The genetic diversity and structure were calculated with Hd, π and FST, while the phylogeographic structure with NST and GST. We also built a haplotype network to estimate the genealogical relations. The analysis rescued 20 haplotypes. The *P. geminiflora* complex had genetic and phylogeographic structure. The results suggest that varieties of *P. geminiflora* are incipient species.

PLEUROTHALLIDINAE INFLORESCENCES: UNDERSTANDING ITS TYPOLOGY

Gustavo Rojas-Alvarado

Understanding morphology is significantly important in taxonomy and fundamental when comparing homologies between different organisms. However, the study of the inflorescences from a typological point of view, has been overlooked in one the most diverse groups within Orchidaceae, including subtribe Pleurothallidinae. The typology of the inflorescences of this group remains largely unexplored, leading to incorrect interpretations of its structures and misapplication of terms. Through the meticulous examination of living material, covering over 82% of the subtribe's genera, this study sheds light on the morphological intricacies of Pleurothallidinae inflorescences. Detailed illustrations, photographs, and diagrams were used to the typological analysis and discussion of the inflorescences, revealing a generalized pattern characterized by an abbreviated peduncle and branch system, imperceptible to the naked eye, a rarity among other orchid groups. The findings reveal that each branch has the potential to yield coflorescences with varying lengths, bearing single or multiple flowers and diverse succession patterns that collectively define the overall appearance of the plant. Within Pleurothallidinae, two primary inflorescence types were found to occur: those with single-flowered coflorescences, prevalent in early divergent clades, and multi-flowered coflorescences, dominating higher clades. A practical classification is presented, categorizing the different coflorescence types based on length and the number of bearing flowers produced, providing a comprehensive framework for understanding this structure.

THE GENUS *RHIPIDOCLADUM*, OBSERVATIONS ON THE ESTABLISHMENT AND PREVALENCE IN FRAGMENTED LANDSCAPES OF DRY AND MONTANE FORESTS IN THE DEPARTMENTS OF MANAGUA, RIVAS AND JINOTEGA, NICARAGUA

Iris Saldívar* and Marvin Tórrez

Changes in land use and forest fragmentation are the main causes of biodiversity loss (IPBES, 2019) (FAO and UNEP, 2020) and diminish forest resilience, for scenarios like invasions of exotic species (Chooksy) (Rojas *et al.*, 2022) or massive proliferations of native species (Loic, 2009). *Rhipidocladum* is a genus of Bamboo, that presents a monoclonal reproduction strategy in its rhizomes (Canavan *et al.*, 2017). The present work compiles information on the species *Rhipidocladum pacuarense* R.W.Pohl and *Rhipidocladum racemiflorum* (Steud) McClure, observed in biodiversity monitoring plots (Juan Roberto Zarruck-Jinotega Biological Station, 2015-2020), contrasted with observations in two secondary forests (Brito-San Juan del Sur and Hacienda Inglaterra- Managua, 2022). In these studies, Riphidocladum was observed to establish itself after environmental disturbance as a result of forest fires and drought conditions. We noticed that *Rhipidocladum* species displace other species, as a result of the saturation of the soil with their underground structures, which drastically decreased the richness of species of other plants in the studied plots while aboveground structures increased the risk of forest fires. The genus *Rhipidocladum*, had enormous potential to colonize areas and prevail in fragmented landscapes of dry and cloud forests in the departments of Managua, Rivas, and Jinotega, Nicaragua during the period 2015-2022. The rapid saturation of secondary forest areas with *Rhipidocladum* in Nicaragua was documented as a potential element that could increase the vulnerability in the landscape structure and affect the establishment of other species of flora and fauna.

UNDERSTANDING SEAGRASS ECOLOGICAL AND REPRODUCTIVE DYNAMICS ARE KEY TO SUPPORT MEADOW RESTORATION EFFORTS IN COSTA RICA

Jimena Samper-Villarreal

Seagrass meadows are a key coastal ecosystem that provides benefits such as being a nursery habitat, aiding in coastal protection and climate change mitigation. Costa Rica has seagrasses on both its Caribbean and Pacific coast. In the Caribbean of Costa Rica, seagrasses are abundant and have been monitored since the mid-1990s, revealing a marked decline in seagrass cover and canopy complexity. These meadows have changed from the dominant and large climatic species to colonizing and opportunistic species, with areas of seagrass loss. Recent studies on this coast have revealed that this decline is not due to diminished water quality but from excessive grazing. This decline also leads to less carbon stored in the associated sediment of degraded meadows. As such, in the Caribbean of Costa Rica, active ecological restoration of seagrasses is urgently needed. In contrast, we know very little about seagrasses in the Eastern Tropical Pacific (ETP), with the assumption that seagrasses are rare in this understudied region. Recently however, seagrasses have been reported at many new locations on the Pacific coast of Costa Rica. Meanwhile, seagrass loss has also been reported at two locations on this coast, including a meadow which was found for a brief period in the mid-1990s but subsequently disappeared following a storm event. Given a lack of natural recovery to date at this site, and significant recent efforts towards understanding the ecological dynamics of seagrasses in the ETP, we have now started the first and only seagrass restoration effort in this region. In early 2023, we started an extensive field campaign at this site to assess seagrass species presence/absence, analyze water quality conditions, seed presence/density, and potential seed germination aided by herbivore exclusion cages and mesocosm studies. Based on our findings we will undertake field experiments for facilitated seagrass restoration at this site.

GENOMICS AND SYSTEMATICS OF THE GENUS *TRIANTHA* (TOFIELDIACEAE, ALISMATALES)

Philippa Stone*, Romulo Segovia, Qianshi Lin, Marco Todesco,
Loren Rieseberg and Sean W. Graham

Triantha occidentalis is a semi-aquatic herb found along the west coast of North America from Alaska to California. This species was recently discovered to be carnivorous, cathing insects using a sticky trap located below its inflorescence. The species is of particular interest as one of only five species of carnivorous monocots, and also represents an independent origin of carnivory in angiosperms. We confirmed the karyotype of *T. occidentalis* to be 2n = 15 using chromosome squashes, and estimate its genome size to be 1C = 2.3 Gb using flow cytometry. We sequenced its nuclear genome using PacBio HiFi technology. Our current combined-haplotype assembly for the nuclear genome of *T. occidentalis* is over 2.1 Gb, with a contig N50 of over 42 Mb and a complete BUSCO score of 98.2%. We are currently sequencing a chromosomal confirmation capture (Hi-C) library to improve the assembly. We are also annotating the genome using the BRAKER pipeline, supported by RNA-seq data from leaves, sticky trap, and inflorescence. We will use this to explore gene expression differences that may underpin the plant's ecology, evolution and function. We are also investigating the phylogeography of the genus *Triantha* (comprising 3–4 species), using a combination of plastid and nuclear data. The genus has an nearly exclusively North American distribution. Preliminary data indicate that major phylogeographic groupings of *T. occidentalis* are geographically distinct, but do not completely agree with the current treatment of the genus, especially at the subspecies level. For example, a well defined genetic subgroup found in western North America includes the sole non-North-American species, *T. japonica*. In addition to providing new insighst into its genomics, a major goal is is to combine morphological work with the phylogenetic/genetic data to update species and subspecies circumscriptions, facilitating an updated treatment for the genus as a whole.

BIOMECHANICS AND STRUCTURAL ADAPTATIONS IN THE STEMS OF THE SELF-SUPPORTING *MARANTOCHLOA LEUCANTHA*

Anaclara Visconti, Linnea Hesse and Noah Knorr*

Marantaceae are herbaceous perennials and include various growth forms such as rosulate herbs, scramblers, self-supporting groups and branch-angle climbers. These growth forms are based on a monocot typical atactostelar vascular system comprising of isolated vascular bundles, i.e. fibrous conductive tissue with mechanically stiffening sclerenchyma fiber caps, which are embedded in a viscoelastic parenchyma matrix. In Marantaceae, the vascular bundles are arranged in ring-like formations across the internode cross-section and are confined by primary growth. Hence, structural-mechanical adaptations to environmental stimuli cannot be achieved by means of a secondary vascular cambium.

We study the self-supporting *Marantochloa leucantha* to understand how the vascular system mechanically adapts to its growth across structural scales (geometry, tissue, cell and cell wall). For this purpose, we investigated the development, disposition and diversity of the individual vascular bundles along the stem and its variation at different ontogenetic stages using light microscopy and micro-computed tomography. We place a special focus on sclerenchyma tissue, as it has a relevant impact on the biomechanical properties of the stem. In addition, we carry out torsion and bending experiments on stem segments to identify changes of mechanical properties along the ontogenetic gradient of the stem. Functional nano-computed tomography is used to observe and quantify swelling and shrinking of the cell wall of sclerenchyma caps at 0% and 90% relative humidity to investigate the plasticity of the cell wall.

THE VELAMEN RADICUM – FACT AND FICTION

Gerhard Zotz

The velamen radicum, a spongy and usually multiple-layered root epidermis composed of dead cells at maturity, is a textbook example of an "adaptation" of epiphytic orchids and aroids. A thorough literature review revealed that such textbook statements are highly misleading. First, a velamen can be found throughout the monocotyledons, ranging from members of early-branching Araceae to derived Cyperaceae. Second, the association of a velamen with epiphytic growth is doubtful. Species with velamentous roots are frequent in terrestrial species and these species are often without close epiphytic relatives. Third, although epiphytic aroids are spotlighted as velamentous, just a handful of species has ever been studied. Trying to distinguish fact from fiction, we investigated the presence of a velamen in 82 *Anthurium* species that covered all currently recognized 19 sections of the genus. A velamen was indeed found in c. 90% of all species, but this was true for both epiphytic and terrestrial species. The factual basis of velamen function is similarly thin. Proposed functions are uptake and storage of water and nutrients, reduction of transpiration, photoprotection, reduced heat load and mechanical protection. None of these functions has been test extensively, some have not been studied at all. Therefore, we tested whether a velamen can really reduce the heat load in aerial roots in exposed situations. For 16 orchid and aroid species, we monitored the temperatures of root surfaces and living root tissue when exposed to IR-radiation. The average insulation capacity was just 2 °C, and the resulting temperatures in living tissue were testing its temperature tolerance. Thus, the protective role of the velamen is rather limited. I conclude that exactly 200 years after its original description we are still far from a thorough understanding of the velamen, both in terms of its distribution within the plant kingdom and its ecological role.

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