

# ABSTRACTS

6TH INTERNATIONAL CONFERENCE  
ON COMPARATIVE BIOLOGY OF MONOCOTYLEDONS



# MONOCOTS VI

N A T A L - B R A Z I L

**“Monocots for all:  
building the whole  
from its parts”**

**Natal, Brazil, October 7th-12th, 2018**

2nd World Congress of Bromeliaceae Evolution – Bromevo 2

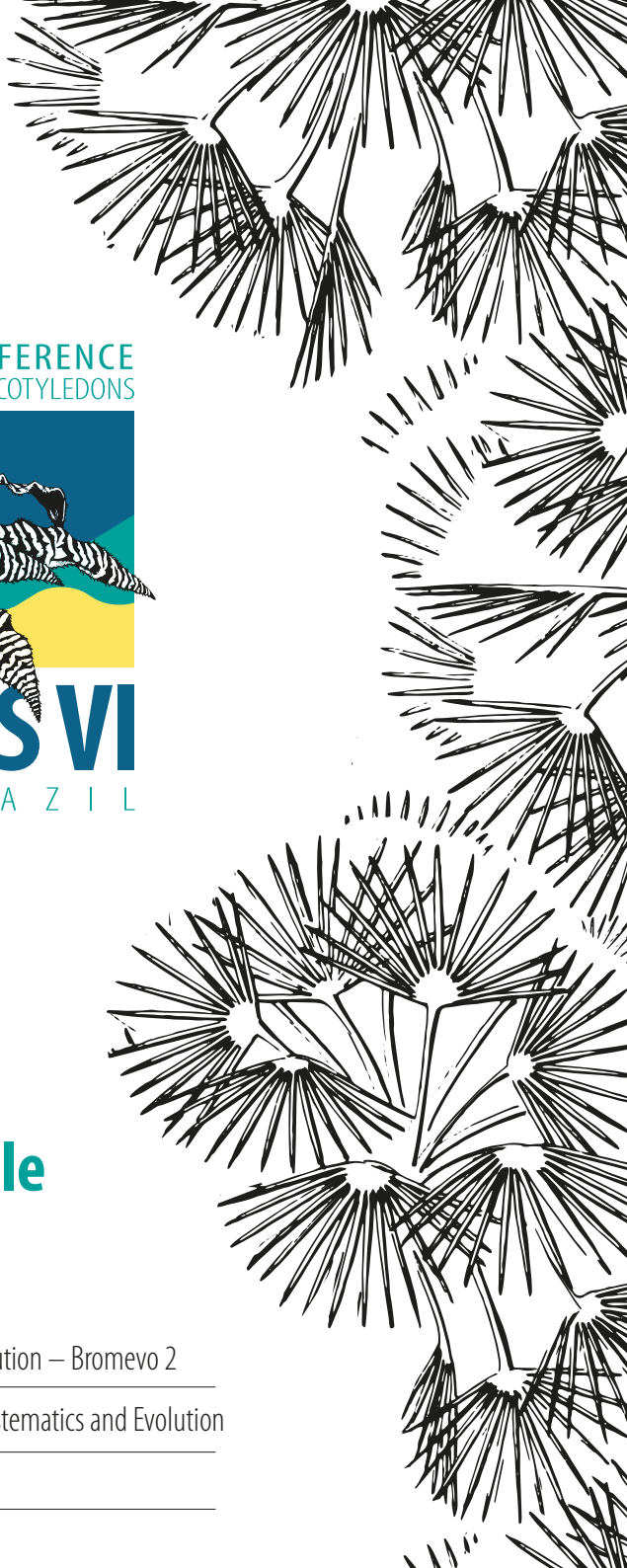
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7th International Symposium on Grass Systematics and Evolution

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III Symposium on Neotropical Araceae

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# **ABSTRACTS OF THE MONOCOTS VI**

Leonardo M. Versieux & Lynn G. Clark (Editors)

**6<sup>th</sup> International Conference on the Comparative Biology of  
Monocotyledons**

**7<sup>th</sup> International Symposium on Grass Systematics and  
Evolution**

**2<sup>nd</sup> World Congress of Bromeliaceae Evolution – BromEvo 2**

**III Symposium on Neotropical Araceae**

**Natal, Brazil  
07 - 12 October 2018**

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Front cover: *Cryptanthus zonatus* (Vis.) Vis. and the Carnaúba palm *Copernicia prunifera* (Mill.) H.E. Moore

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# ABSTRACTS

**Keynote lectures** **p. x**

**Symposium Talks** **p. xx**

**Posters\*** **p. xx**

Morphology, development and cellular biology **p. xx**

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Economic botany, plant breeding and agriculture **p. xx**

---

Ecology and conservation **p. xx**

---

Monocots in society and tools to spread knowledge about monocots **p. xx**

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Genetics, genomics and bioinformatics **p. xx**

---

Physiology and biochemistry **p. xx**

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Systematics, evolution, biogeography **p. xx**

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**\*Organized in alphabetical order, 1<sup>st</sup> author, given name**

## Symposia: in alphabetical order - organizers

1. **Advances in molecular phylogeny, systematics and evolution of Dioscoreales** - Juan Viruel
2. **Advances in the anatomy of the large Poales clade** - Aline Oriani, Thales D. Leandro and Vera L. Scatena
3. **Applied botany: Use of comparative data in horticulture, reproductive biology and systematics of Bromeliaceae** - Leonardo M. Versieux
4. **Biogeography, ecology and macroevolution of grasses** - Maria Vorontsova, Lynn Clark and Elizabeth Kellogg
5. **Ecophysiology of Bromeliaceae** - Helenice Mercier
6. **Evolution and diversification in Cyperaceae** - Wayt Thomas
7. **Genomic innovation through genome duplication: Examples from across Monocots** - Michael McKain and Alex Harkess
8. **Grass systematics and evolution—Plastome phylogenetics and the BOP Clade** - Lynn Clark, Elizabeth Kellogg, R. Patrícia de Oliveira and Pedro Viana
9. **Grass systematics, evolution and development—The PACMAD clade** - Elizabeth Kellogg, Lynn Clark, R. Patrícia de Oliveira and Pedro Viana
10. **Growing knowledge on monocot vegetative anatomy** - Thales D. Leandro, Aline Oriani and Vera L. Scatena
11. **How can anatomy contribute to understanding monocot evolutionary patterns?** - Aline Oriani, Thales D. Leandro and Vera L. Scatena
12. **III Symposium on Neotropical Araceae - systematics and evolution** - Livia Godinho Temponi and Ivanilza Moreira de Andrade
13. **III Symposium on Neotropical Araceae – floristics, morphology and evolution** - Livia Godinho Temponi and Ivanilza Moreira de Andrade
14. **Linking macro- and microevolution in Bromeliaceae** - Clarisse Palma da Silva
15. **Monocot mats on Gondwanan inselbergs: binding taxonomy, ecology and molecular aspects under a biogeographic view** - Luiza de Paula and Stefan Porembski
16. **Monocot phylogenomics I** - Thomas Givnish
17. **Monocot phylogenomics II - new insights on genome evolution, diversification and biogeography** - Oscar Alejandro Pérez Escobar, Thomas Givnish, Wolf L. Eiserhardt, William J. Baker
18. **Monocots in society and tools to spread knowledge about monocots** - Fernanda Antunes Carvalho and Leonardo M. Versieux
19. **Neotropical Eriocaulaceae: answering evolutionary questions and supporting sustainability** - Livia Echternacht
20. **Orchid ecology and conservation** - Edlley Pessoa
21. **Palms – The ecologically most diverse tropical plant family?** - Wolf L. Eiserhardt
22. **Recent advances in Bromelioideae systematics, taxonomy, and evolution** - Georg Zizka, Elton Leme and Juraj Paule
23. **Recent advances in the systematics of Bromeliaceae** - Leonardo M. Versieux
24. **Setaria as a model system for monocot development and biotechnology** - Adriana Pinheiro Martinelli and Marcio Alves-Ferreira
25. **Systematics, evolution, and biogeography of Orchidaceae** - Edlley Pessoa
26. **The next generation of research on the evolution of Crassulacean acid metabolism: integrating physiology, ecology, and genomics** - Karolina Heyduk
27. **Understanding Amaryllidaceae evolution using different approaches** - Antonio Campos-Rocha, Alan William Meerow and Julie Henriette Antoinette Dutilh
28. **Zingiberales I - Evolution** - Thiago Andre and Fernando Figueiredo
29. **Zingiberales II - Diversity** - Thiago Andre and Fernando Figueiredo

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## The road to evolutionary success: insights from *Mauritia flexuosa*

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*Mauritia flexuosa* has one of the widest distributions of all palms, covering millions of hectares across northern South America, where it forms extensive, high-density stands. How does a species reach this wide distribution and high abundance in the face of strong competition in hyperdiverse tropical forests as well as persist through extreme landscape and climate changes throughout the Cenozoic (last ca. 65 mya)? Evolutionary success is related to historical contingency, genetic variation, and demography. Here I present a robust approach to understanding diversification in the tribe Lepidocaryeae, showing how clade competition contributed to persistence through geological time. Within the *Mauritia* lineage, I show extinction patterns using the pollen fossil record, leading to low species diversity in the genus. I also review how climatic change during the Quaternary influenced the extant demography and distribution of *M. flexuosa*. *Mauritia flexuosa* presents significant genetic differentiation among different river basins and between the Amazonian and Cerrado biomes. Touching on environmental correlations across the genome, I conclude by contrasting adaptive selection with genomic plasticity. Our work provides new insights into the historical factors that affected geographical distribution and structured genetic diversity, contributing to long-term evolutionary success.

**Key words:** biome, demography, genetic diversity, outlier loci, phylogeny.

## A sceptic's view on scientific "facts" and "concepts"

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Absorbed by our daily science routine and the struggle for survival ("publish or perish") we rarely pause to contemplate the way we do science, established "facts" are rarely questioned, and we easily overlook common pitfalls and problems. I want to guide the audience through a stimulating and possibly provocative journey that addresses the topics data quality, continuous vs. discontinuous variation, ambiguous data interpretation, premature generalisations, and a number of other conceptual issues. I will use numerous examples, mostly from studies with monocots, to illustrate my points. I finish my talk with a few, rather optimistic remarks on science in the times of global change and alternative facts.

**Key words:** global change, hemiepiphytes, heteroblasty, ontogenetic drift, velamen radicum.

## 3D Biology: What we can learn from the "flat" grasses

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Organisms are 3-dimensional and interact with other organisms and the environment as such, but they are frequently studied in only two dimensions. Our goal is to integrate computer technology with biology to study morphology in three dimensions to gain unique insights into plant function and evolution. We discuss two examples from the grasses, in which 3D models of chlorenchyma cells and spikelets were created using paraffin sections and computer-aided design (CAD) software. We surveyed chlorenchyma cell shape for 215 species of grasses. Five major morphotypes were identified; cell shape was significantly correlated with both photosynthetic pathway and light regime. Several descriptors (e.g., cell length:width) had significant phylogenetic signal. Prior assumptions about the extent and configuration of intercellular leaf spaces require reevaluation. We explored the junction of flower form and function by using virtual spikelet models for computational fluid dynamic simulations to understand the aerodynamics of anemophily and investigate to what extent it has shaped spikelet morphology. Preliminary results indicated that a forest species creates faster wind speeds around the stigmas relative to a congeneric prairie species, and that stigma position is critical. The ability to model cells or structures with CAD software has opened new pathways for the study of morphology.

**Key words:** cell shape modeling, computational fluid dynamics, grass leaf anatomy, pollination, 3D modeling.

**Funded by:** National Science Foundation

### **Monocots in the Brazilian Flora 2020: facilitate access to plant diversity**

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The Global Strategy for Plant Conservation (GSPC) was established by the Conference of Parties in 2002 to decrease the loss of plant diversity, reduce poverty and contribute to sustainable development. To achieve this overarching goal, the GSPC has established a series of targets, one of which is to ensure that plant diversity is well understood, so that it can be effectively conserved and used in a sustainable manner. Brazil hosts more than 32,000 species of Angiosperms representing one of the most biodiverse countries on Earth, and playing a key role in the GSPC. To meet the GSPC goals of Target 1 and facilitate access to plant diversity, Brazil committed to preparing the List of Species of the Brazilian Flora (2008–2015) and the Brazilian Flora 2020 (2016–present). Overall, the system contains information on 47 families, 740 genera (89 of them are endemic to Brazil), and 8,991 native and naturalized monocot species (5,359 endemic), many of them under some level of threat. Almost 40% have been described including identification keys, field images and botanical illustrations. The Atlantic Forest has the highest diversity of monocot species in the country and holds 4,801 species, 60% of them are endemic to the Domain.

**Key words:** systematics, Brazilian Flora, GSPC, Atlantic Forest, conservation.

**Funded by:** CNPq, MMA, MCT

## **Monocots in the Anthropocene: species interactions in a rapidly changing world**

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The complexity of life on earth is a product of the diversity of species multiplied by the diversity of their interactions. An understanding of species diversity, which requires correct species identification and patterns of evolution, is a prerequisite to accurately interpreting and understanding their interactions. Investigations of plant-herbivore and plant-pollinator interactions have provided classic examples of the interdependent relationships that exist among species within and across ecosystems. Within the Monocots the charismatic tropical Zingiberales, including bananas, birds-of-paradise, heliconias, ginger, and prayer plants, offer illustrative and often surprising insights into the ecology of the animals with which they have coevolved. Specifically, the ecological interactions between coleopteran herbivores and hummingbird pollinators with their Zingiberalean hosts reveal intricate patterns of evolutionary history and how these species may respond to today's rapidly changing planet. The geographic mosaic of these relationships across tropical islands, fragmented landscapes, and elevational gradients suggests that human-caused habitat alterations, biological invasions, and climate change may significantly modify and disrupt through time and space the historical patterns of ecological interactions. As scientists we must recognize and address the past and present causes of these disruptions. The future of the Earth's biological complexity in the Age of Humans, in the Anthropocene, depends on our solutions.

**Key words:** complexity, interactions, herbivory, pollination, Zingiberales.



### **Phylogenomics of *Dioscorea*: the influence of polyploidy on its evolution**

*Juan Viruel*<sup>1</sup>, *Marybel Soto*<sup>2</sup>, *Lisa Pokorny*<sup>1</sup>, *Oriane Hidalgo*<sup>3</sup>, *María Conejero*<sup>1</sup>, *Felix Forest*<sup>1</sup>, *Ilija J. Leitch*<sup>1</sup>, *Barbara Gravendeel*<sup>4</sup>, *Sean Graham*<sup>2</sup>, *Michael Kantar*<sup>5</sup> & *Paul Wilkin*<sup>1</sup>

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With ca. 650 species mainly distributed in the tropics, *Dioscorea* stands out among Dioscoreales for its high species diversity and for including crops that feed tens of millions worldwide. Up to seven species are widely cultivated worldwide in an extensive variety of agronomic environments and social contexts. Its economic importance is complemented by other species which accumulate secondary steroidal compounds of pharmacological interest. Evolutionary patterns within *Dioscorea* have been explored using one nuclear region and five plastid regions for only 20% of the species. *Dioscorea* constitutes a focus of interest in evolutionary studies due to the enormous distribution range of its lineages and whole genome duplications (WGD). WGD is a common phenomenon in *Dioscorea*, with polyploidy reaching up to 14-ploid superimposed on a dysploid series with  $x = 9-10$ . However, the importance of WGD as a driving force in its evolution has still not been explored. We have generated sequence data for 303 low-copy nuclear genes and full plastomes using the HybSeq method (a combination of target enrichment and genome skimming), which allowed us to resolve phylogenetic relationships among 300 species of *Dioscorea*, to identify Crop Wild Relatives, and to further understand WGD in a phylogenomic context.

**Key words:** Dioscoreales, HybSeq, low-copy nuclear genes, whole genome duplication, yams.

**Funded by:** Marie Skłodowska-Curie Individual Fellowship (704464 - YAMNOMICS - MSCA-IF-EF-ST) and a Kew-funded pilot study (YAMAGASY).

### ***Dioscorea* as a window into human cultural evolution: from the cognitive revolution to the sexual revolution and beyond**

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*Dioscorea* has shaped human biology and culture across continents and millennia. Consumption of yam tubers has been invoked as a factor in the evolution of human brain size going back almost two million years. Much later, agriculture revolutionized human societies and in the process at least ten *Dioscorea* species were domesticated across four continents. Yam domestication remains an ongoing activity in both Africa and America and I present a modern example from the Colombian Amazon. I also explore the role of yams in the trans-Atlantic slave trade. Yams are still celebrated in traditional festivals and fertility rituals across diverse cultures, but their greatest influence on fertility came in the mid-20<sup>th</sup> century when Mexican wild yams constituted the chief raw material for the production of birth control pills, the widespread availability of which was a major factor leading to the sexual revolution of the 1960s. At this time, yams also created a capitalist boom,

followed by a national labor revolt, in Mexico. In today's technological revolution, wild yams are marketed online to alleviate symptoms of menopause, while in laboratories in Colombia and elsewhere, *Dioscorea* is emerging in the line of defense in the global fight against diabetes and obesity.

**Key words:** crop domestication, *Dioscorea*, economic botany, medicinal plants, yams.

## **Towards a reclassification of Dioscoreales using phylogenomics and morphology**

*Marybel Soto Gomez*<sup>1</sup>, *Paula J. Rudall*<sup>2</sup>, *Vivienne K.Y. Lam*<sup>1</sup>, *Qianshi Lin*<sup>1</sup> & *Sean W. Graham*<sup>1</sup>

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Despite being a relatively small order with ~865 species, Dioscoreales display a diverse range of habits, and vegetative and reproductive morphologies. Its ~24 genera encompass the economically important *Dioscorea* yams, lineages with reticulate venation, and mycoheterotrophic taxa whose loss of photosynthesis has resulted in modified or reduced morphology. Previous studies have produced a basic phylogenetic framework for the order, but multiple unresolved higher-order relationships persist, and some familial circumscriptions remain in a great deal of flux. The latest Angiosperm Phylogeny Group classification recognizes three families (Burmanniaceae, Dioscoreaceae, Nartheciaceae), with all mycoheterotrophs grouped in Burmanniaceae. However, they also noted the need for more data to assess whether Thismiaceae should be differentiated from Burmanniaceae, and Taccaceae from Dioscoreaceae. Here we present results from organellar phylogenomic analyses using a genus-level taxon-sampling focus. Our analysis resolves most higher-order relationships with strong support. Neither Dioscoreaceae nor Burmanniaceae is monophyletic, as photosynthetic *Tacca* is sister to mycoheterotrophic *Thismia* and mycoheterotrophic *Afrothismia* is sister to neither. We review options for a revised family-level classification of Dioscoreales and recommend a system that recognizes more than two to three families. We conducted ancestral-state reconstructions of morphology to evaluate possible family-level synapomorphies.

**Key words:** *Afrothismia*, APG update, combined molecular and morphological data, non-photosynthetic monocots, plastid and mitochondrial phylogenomics.

**Funded by:** This work was supported by a UBC (University of British Columbia) Four-Year Fellowship, an NSERC (Natural Sciences and Engineering Research Council of Canada) postgraduate fellowship, and an NSERC Michael Smith Foreign Study Supplement to M.S.G

## **Yam phylogenetic relationships, conservation and use: what do we know, and what do we need to know?**

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We will review knowledge of phylogenetic relationships in *Dioscorea* (yam) diversity generated through the Sanger sequencing era and identify current knowledge gaps, especially in Asian and Neotropical taxa. This knowledge will be linked to data on conservation status information across currently available trees, with published and in development conservation assessments being dominated by taxa from Madagascar, southern Africa and the Enantiophyllum clade. The contrasting patterns among species covered, with elevated threat levels against the flowering plant mean of 20% threatened in some clades contrasting with much lower threat levels in others. The

dearth of assessments in some clades will be noted. The presentation will conclude with a review of current knowledge of the relationships of crop species and their wild relatives and potential sources of natural products, with a view to developing better resources to guide yam agriculture and high value compound exploitation via genomic science (including genome assisted breeding) and biotechnology.

**Key words:** conservation, *Dioscorea*, exploitation, phylogenetics, yam.

## Evolution and biogeography of Neotropical *Dioscorea* and key morphological traits

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Based on wide sampling phylogenies, biogeography and key morphological characters were inferred in the evolution of Neotropical *Dioscorea* species. Four Neotropical clades can be identified, demonstrating at least four events of arrival to the American continent. The two richest clades originated between Eocene and Oligocene in the southern Andes and in a large disconnected area (Central America, northern Andes, and southeast America). Several expansions occurred from these regions to the rest of America, including to Central America before the closure of the Panama isthmus. The 'Dry Diagonal' region was occupied by both lineages after the Miocene, with New World-II being more closely related to forest environments. Exchanges between forest habitats and 'Dry Diagonal' occurred at different times and a “gray-zone” between these areas can still be observed. In addition, is observed a connection between Madagascar and the less diverse Neotropical lineage (*D. mollis*), probably a long-distance dispersal event. As for the morphology of this group, it is possible to highlight the annual tubers present only in the New World-III clade, the shape of the seed wing separating the two largest clades and characters such as the number of stamens, twining direction, and type of inflorescences, by their internal importance in the biggest clades.

**Key words:** Dioscoreaceae, Dioscoreales, *inham*, phylogeny, yam.

**Funded by:** PhD scholarship from Capes - Coordenação de Aperfeiçoamento de Pessoal de Nível Superior.

## Welcome to the dark side: Organellar genome evolution in heterotrophic Dioscoreales

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The yam order includes mycoheterotrophic lineages that rely on fungal partners (instead of photosynthesis) for some or all of their carbon budget. With at least ten independent losses of photosynthesis, Dioscoreales provides the richest examples of this major evolutionary transition outside Orchidaceae. Most of these photosynthesis losses happened in Burmanniaceae s.s., with additional losses associated with Thismiaceae. These independent events provide opportunities for examining models for the evolution of heterotrophic plant plastid genomes (plastomes), and for characterizing divergent patterns of evolution following individual losses. Mitochondrial genes seem largely unaffected, although substantial rate elevation occurred in Thismiaceae. The plastomes

of both families also have elevated rates of substitution. We characterized changes in patterns of selection in retained plastid genes. Plastid group II introns of some genes are retained despite loss of *matK*, whose intron maturase is thought to splice this intron class. We also characterized patterns of plastid genome evolution in fully mycoheterotrophic Dioscoreales, including examples of extreme genome compaction and several non-canonical plastome structures. The latter include parallel losses of the plastid inverted repeat (IR), major IR boundary shifts, and the evolution of large direct repeats. Burmanniaceae plastomes display divergent degrees of gene loss before and after photosynthesis loss.

**Key words:** genome structural evolution, mitochondrial genes, molecular evolution, plastid genome, relaxed selection.

**Funded by:** NSERC (Natural Sciences and Engineering Research Council of Canada)

## Advances in the anatomy of the large Poales clade

### The evolution of spikelets and flowers in Cyperoideae (Cyperaceae): A floral developmental approach

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Cyperaceae is a large grass-like family in Poales with two subfamilies, namely the small Mapanioideae and the species-rich Cyperoideae. Based on scanning electron microscopic observations, we studied the development of the flowers and inflorescences of representatives in a wide range of genera of Cyperoideae. In Cyperoideae, the compound inflorescences are basically panicles of spikelets. The spikelets are composed of wind-pollinated, trimerous, monocotyledonous flowers. Reduction of the number of flowers in the spikelet, loss of the spikelet prophyll, metatopic displacements of the bracts subtending a flower, and reduction of the floral parts of the flowers are main evolutionary trends. Within the flowers, spatial constraints may result in the reduction of the number of stamens to three instead of six, delaying or totally reducing the development of the abaxial floral parts and the loss of the perianth. Moreover, developmental fusion and reduction processes result in a large diversity of gynoecial forms. In the most derived subtaxa of Cyperoideae, the compacted inflorescence tends to take over the floral function, forming dense heads consisting of single-flowered spikelets. The question remains whether these evolutionary trends were driven by mechanical forces or by pollination strategies, or a combination of both.

**Key words:** Cyperoideae, evolution, floral reduction, ontogeny, spikelet.

### Floral development and vasculature in Rapateaceae and Mayacaceae

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Floral development and vasculature were studied in Rapateaceae and Mayacaceae to better understand interfamilial relationships in Poales. Both families were recently included in the cyperid clade, although with low support. In both families the floral vascular system is organized into six central complexes from which the traces of all floral parts diverge. In Rapateaceae sepals receive

more than three vascular traces and petals receive three traces, whereas in Mayacaceae both sepals and petals receive three traces. In sepals and petals of most Rapateaceae and in sepals of Mayacaceae the median and lateral bundles do not originate from the same vascular complex. In petals of Mayacaceae the median and lateral bundles originate from the same vascular complex. In Rapateaceae ventral carpellary bundles originate from the six central vascular complexes, whereas in Mayacaceae these bundles originate from only three central vascular complexes. Reduction of the inner stamen whorl, gynoeceum development from an annular primordium, and initiation of petals after the stamens link Mayacaceae to the cyperids. In Rapateaceae the gynoeceum originates from three carpel primordia and its formation is a combination of postgenital and congenital fusion processes, supporting the early-diverging position of this family in Poales.

**Key words:** anatomy, cyperids, flower, ontogeny, Poales.

**Funded by:** CAPES, CNPq, FAPESP

## **The phylogenetic and ecological correlates of variation in root anatomy of the African Restionaceae**

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Root architecture varies massively in the African Restionaceae, one of the ecologically dominant and taxonomically diverse elements of the Cape flora, with deep-growing water-roots, shallower fibrous roots, apogeous feeding roots and cluster roots. Root variation may hold the key to the co-existence of the rich Cape restio diversity. The root anatomy is equally diverse: there is substantial variation in the cortex (which either collapses in older roots or persists as an aerenchyma), endodermis (in relative size, shape of the endodermal cells, and degree of wall thickening), pericycle (from 1– 10 cells, varying from unthickened to massively thickened), and the metaxylem vessels (5 to more than 100, organized in a ring or scattered). Almost all root anatomical characters are phylogenetically constrained, similar to the culm anatomical characters, which are considered to be phylogenetically informative. It is possible to recognize groups of genera with broadly similar root anatomies, and the many exceptions indicate some evolutionary lability. The variation in root anatomy may contribute interesting insight into the evolution of this unusual group of plants, most likely via complex interactions between roots and culms.

**Key words:** anatomy, ecology, phylogeny, Restionaceae, roots.

## **Evolution of embryological traits in xyrids**

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Embryo development was comparatively studied in species of Xyridaceae (*Abolboda*, *Orectanthe* and *Xyris*) and Mayacaceae (*Mayaca*) in order to better understand the evolution of some embryological characters in the xyrids (Poales). The results show that in Xyridaceae the embryo development is of the Asterad-type, *Muscari* variation, whereas in Mayacaceae it is of the Onagrad-type, *Juncus* variation. In both families the embryo exhibits a vestigial suspensor. A cellular cap between the embryo and the seed coat was observed at the seed micropylar region in Xyridaceae and Eriocaulaceae, which may be pointed out as synapomorphy of the xyrid clade including only

Xyridaceae and Eriocaulaceae. In Mayacaceae an aleurone layer is originated from the peripheral cells of endosperm, a trait also present in other cyperid families. Embryo development of the Asterad-type, differentiated embryo and presence of a persistent suspensor are the ancestral conditions in Poales. The undifferentiated embryo and the vestigial suspensor probably had independent origins in Xyridaceae and Mayacaceae.

**Key words:** aleurone layer, cellular cap, embryo, embryogenesis, suspensor.

**Funded by:** Funded by: CNPq (#160640/2014-8), FAPESP (#2011/11536-3), CAPES-PNPD.

## **Evolutionary precursors, convergent evolution, and the origins of C<sub>4</sub> leaf anatomy in grasses**

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C<sub>4</sub> photosynthesis boosts productivity in tropical conditions through the coordinated action of multiple anatomical and biochemical components that work together to concentrate CO<sub>2</sub> within the leaf. Despite its complexity, the C<sub>4</sub> trait is a prime example of convergent evolution, having evolved more than 60 times in flowering plants, and at least 22 times just in grasses. How did such a complex trait evolve so many times? Understanding the origins of the C<sub>4</sub> complex trait requires atomizing it, and analysing each of its constituents independently. This approach shows that no single trait related to cell sizes or numbers consistently differentiates C<sub>4</sub> from non-C<sub>4</sub> leaves of grasses. Using a phylogenetic framework, the changes that preceded C<sub>4</sub> evolution can be inferred, which points to anatomical preconditions in some clades. The transition to C<sub>4</sub> anatomy then involved few key modifications, and the anatomy was later optimized during the rich history of C<sub>4</sub> grasses. Overall, evolutionary precursors, key development transitions, and convergent as well as divergent evolution were involved in the evolution of leaf anatomies allowing an efficient C<sub>4</sub> pathway in grasses.

**Key words:** C<sub>4</sub> photosynthesis, evolution, grasses, leaf anatomy, phylogenetics.

## **Vegetative and reproductive outstanding characters in Bromeliaceae**

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Anatomical and embryological features of Bromeliaceae have been analyzed in a phylogenetic perspective to understand evolutionary trends. In the genus *Nidularium* a schizogenous-type of aerenchyma occurs in the ovarian mesophyll. *Edmundoa lindenii* has trichomes on the ovary, as a probable autapomorphy. *Canistrum aurantiacum* has unique features in the ovules such as four cell layers in the outer integument, non-elongated cells in the nucellar epidermis, and three or four cell layers in the parietal tissue. Specialized structures in the ovary wall occur in Brocchioideae, Puyoideae and Bromelioideae. Regarding the integuments, the outer one showed an increased number of cell layers in late diverging taxa. The inner integument is more conservative, and only *Hechtia* and some Tillandsioideae and Bromelioideae have an increased number of cell layers. The plesiomorphic condition of the ovule chalazal appendages is elongate and acuminate. The multiple appendages in *Catopsis* and the lateral appendage in *Puya* are exclusive. The appendages are reduced in Bromelioideae and in certain Tillandsioideae they are vestigial or do not develop. Our

results show that some anatomical features of the ovary and ovule are stable within genera or subfamilies and besides of provide evolutionary trends, these features are useful for systematic approaches.

**Key words:** chalazal appendages, embryological characters, flower anatomy, ovary, ovule.

**Funded by:** CNPq/CAPES.

### **Fusoid cells in the grass family Poaceae and their homologies within the Graminid clade**

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In mature grass leaf blades as seen in cross section, oblong cell-like structures have been interpreted most recently as intercellular gas spaces delimited by successive collapsed fusoid cells. These cells have been reported in at least seven of 12 subfamilies of Poaceae and they are considered as a synapomorphy for the family. A developmental study including LM, SEM, TEM, and CSEM techniques was therefore carried out, comprising 20 species in three families (Flagellariaceae, Joinvilleaceae, and Poaceae), representing the earlier diverging and derived branches within the Graminid clade and Poaceae. Developmental results show that, in mature leaf blades as seen in cross section, one apparent fusoid cell is typically a cavity resulting from the collapse of the initial fusoid cell and its internal divisions, which are interpreted as derivative cells with formation of cell plates only. Fusoid cells in all studied Poaceae species originate from the ground meristem as do the colourless cells in *Joinvillea ascendens* (Joinvilleaceae), suggesting that these cells are homologous. Within Poaceae, the meristematic origin of fusoid cells is the same in the early-diverging lineages, the BOP clade and the Panicoideae, and thus they are homologous within the family.

**Key words:** Graminid clade, grasses, leaf blade anatomy, mesophyll cells, ontogeny.

**Funded by:** CAPES, CNPq, NSF.

Applied botany: use of comparative data in horticulture, reproductive biology and systematics of Bromeliaceae

### **Conservation strategies and new research approaches for the *Ananas* genus**

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The genus *Ananas* (Bromelioideae, Bromeliaceae) is native to South America, and Brazil presents the highest variability in its territory. The conservation of this important germplasm demands efficient strategies to keep these genes with their functionalities. Recent studies have proposed an integrated conservation system considering the microbiome associated with these plants in areas of

natural occurrence and brought a differentiated approach to germplasm conservation. On the other hand, the genus has been little studied and explored for uses other than food. In recent years, several studies have been carried out and resulted in applications in the sectors of floriculture, ornamental, pharmaceutical and new materials industries. Ornamental pineapples have been developed for use as cutting plants, potted plants or for landscaping; molecules with antioxidant activity or inhibition capacity of cancer cells may generate new drugs. And finally, some species of the *Ananas* genus have fibers with noble properties for use as reinforcement in different products, improving their quality. This work will address the different conservation strategies and their improvements, as well as the present state of art of new research approaches on the *Ananas* genus.

**Key words:** *Ananas comosus*, Bromeliaceae, fiber, ornamental, microbiome associated.

**Funded by:** CNPq; FAPESB, CAPES PROCAD, PNPd, CAPES/Embrapa

### **Pollination ecology of the bromeliads from the Itatiaia National Park, Brazil: binding nectaries to ecology**

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Among the floral attributes present in plants with biotic pollination, nectar has an important role in attracting pollinators, since it is a source of energetic resources for several animals. Nectar production dynamics may show inter- and intraspecific variations, characteristics that may be associated with environmental and ecological factors, as well as with the ultrastructural diversity of the floral nectary. In this context, this work aims to evaluate nectar production dynamics from a morphofunctional perspective, using as a case study bromeliad species pollinated by different groups of animals. Floral visitors were observed for about 70 hours in two flowering periods (2016/2017). Nectar production pattern and daily availability were determined through three treatments: 1) standing crop; 2) pattern of nectar secretion (accumulated nectar); and 3) nectar removal effects. Three variables were measured: volume, concentration, and sugar mass (% sucrose, mass/total mass). Anatomical studies of the floral nectary were done by light microscopy and scanning and transmission electron microscopy. The results indicated that the species invest in this production in different ways in order to attract their pollinators (fast or slow producer), and also that the anatomical characteristics of the nectaries "controlled" the quality of the nectar produced throughout the floral anthesis.

**Key words:** Bromeliaceae, morphoanatomy, nectar production dynamics, pollinators, ultrastructure.

### **Bromeliad hybridization: potential and limits for the horticultural trade investigated using microscopy**

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Bromeliads are tropical plants with a wide variety of colors and shapes that make them widely and extremely appreciated. Plant hybridization plays a strategic role in the development of new cultivars of interest for the horticultural trade, and is a central phenomenon in plant speciation processes.



This study aimed to perform different interspecific and intergeneric hybridizations in Bromeliaceae to investigate possible reproductive barriers through microscopic techniques. A total of 931 interspecific and intergeneric hybridization crosses were conducted in 80 different combinations involving 16 species belonging to five genera *Aechmea*, *Ananas*, *Pseudananas*, *Alcantarea*, and *Vriesea*. Fluorescence microscopy was used to evaluate pollen tube growth in the pistil. Crosses were successful in 43% of the attempts, corresponding to 32% of the species of the *Ananas* genus. Among the intergeneric crosses, only *V. michaelii* × *Al. nahoumii* and *V. simplex* × *Al. nahoumii* succeeded. Possible causes of incongruity in these crosses are related to different impediments to the pollen tube growth. The results provide important contributions to studies of hybridization in bromeliads, aiming to produce new hybrids for the ornamental plants market, as well as contributions to the characterization and conservation of this important family of plants.

**Key words:** Bromeliaceae, fluorescence microscopy, interspecific and intergeneric hybridizations, ornamental plant, reproductive barriers.

**Funded by:** FAPESP, CNPq, CAPES, PROCAD, PNPD

### **Can vegetative organ anatomy help to disentangle species complexes? The cases of *Neoregelia bahiana* and *Vriesea oligantha***

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Delimitation of Bromeliaceae into genera or species is often problematic, as observed for the *Neoregelia bahiana* and *Vriesea oligantha* complexes, distributed throughout the rocky fields of the Espinhaço mountain range, Brazil. Considering that the anatomical characterization of different organs is potentially important for taxonomic and ecological interpretation of these species complexes, roots, stems, leaves and inflorescence axes (peduncles) from individuals of different populations were analyzed with light and scanning electron microscopy. As the main results, the leaf morphology and anatomy, in combination with the plant architecture, proved to be useful to distinguish populations. For *Neoregelia bahiana*, the lamina shape, distribution of the peltate scales, and number of cell layers forming the water-storage hypodermis distinguish three of 12 populations studied. For *Vriesea oligantha*, the leaf morphology, tank diameter, and the presence of fiber strands in the mesophyll grouped the individuals from the 15 populations studied into four clusters. All these data suggest the existence of more than one taxon in both species complexes.

**Key words:** Bromeliaceae, leaf, Poales, root, stem.

**Funded by:** CAPES - Coordenação de Aperfeiçoamento de Pessoal de Nível Superior and CNPq - Conselho Nacional de Desenvolvimento Científico e Tecnológico (#455510/2014-8).

### **Floral development in *Aechmea*: understanding floral change in a large polyphyletic genus**

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*Aechmea* is a large bromeliad genus with controversial systematics and distinct pollinators. Here we characterize aspects of flower development and anatomy of *Aechmea correia-araujoi* and *A. gamosepala* contributing to a better understanding of its variable morphology and radiation. We analyzed flower buds from 1 up to 14 mm in size and followed developmental landmarks of whorls using SEM and LM. Both species showed similar sequence of whorls development, but the final morphology of the flowers and petal appendages present rather variable characters, not as simple as previously defined. While in *A. gamosepala* the appendages are formed late in flower development, in *A. correia-araujoi* it is an early process. Both species present a complex morphological process for appendage formation, including positional changes, possibly favoring insects rather than vertebrates. In both taxa the nectar is released through three basal orifices apically on the ovary and we documented the link between the nectary region, through discrete channels, upward to the conduplicate lobes of the wet stigma, suggesting that small amounts of nectar are released on the stigmatic surface. Such interpretations may affect studies of pollinator shifts in the core Eu-Bromelioideae, explain part of their polymorphic flowers and be significant in their adaptive radiation.

**Key words:** Bromelioideae, conduplicate-spiral stigma, floral anatomy, petal appendage, septal nectary.

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### **The tank-inflorescence of *Nidularium*: 3D architecture and the role of water using Finite Element Analysis based on $\mu$ CT**

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The *Nidularium* inflorescence prompts some harshness regarding its correct interpretation, especially its architecture and the role of water on its tank. This study focuses on the development of the *Nidularium* inflorescence, combining different approaches to understand such issues. Individuals were split into two groups: with (control) and without (experimental) water provision in inflorescence. Another specimen was prepared and scanned with X-ray microtomography ( $\mu$ CT) generating a high-resolution, 3D model that was used in a heat-transfer Finite Element Analysis (FEA). Thermal effects of the presence and absence of water were simulated and compared, using an accurate morphology in a realistic environmental condition. The system is polytelic; the main axis ends in the main inflorescence and bears lateral paraclades with coflorescences. No prophylls were observed in the system. In the 3D reconstruction, the tank volume was approximately 2.4 times larger than the encompassing plant material. Flower size in the control group was significantly larger, and many injuries were observed in the experimental group. FEA data showed that the presence of water in the tank led to lower temperature variations in the plant when compared with the waterless scenario. Water acted as a temperature stabilizer for the inflorescence, and its absence initiated physiological stress responses.

**Key words:** Bromelioideae, flower development, Nidularioids, temperature stress, water deficit.

**Funded by:** This work was supported by the National Council for Scientific and Technological Development (CNPq) and the Coordination of Improvement of Higher Education Personnel (CAPES).

### **Reversibility of breeding systems in bromeliads: history indicates paths to diversification**

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Bromeliaceae (3578 spp.) is one of the five largest monocot families, among the largest of all the angiosperms, mostly exclusively of the Neotropics. It arose in Guayana ca. 100 million year ago, but most of its current species diverged recently from each other, 15 to 10 mya. Ecological features such as epiphytism, water tank, absorptive trichomes and CAM photosynthesis have been investigated to explain diversification paths. It is widely accepted that outbreeding is evolutionarily advantageous by favoring genetic variability and that inbreeding lineages lose genetic diversity, increasing the extinction rate. Here we reviewed the breeding systems literature available for all bromeliads and investigated its patterns of evolution. The literature supported that self-incompatibility could be lost, but only rarely regained during angiosperm diversification. Most of bromeliad species are hummingbird pollinated and traditionally considered to be outcrossing. However, our literature survey showed that bromeliad species are predominantly self-compatible, which is discordant with trends that outcrossing is the prevalent breeding system, especially for a large angiosperm family. We reconstructed the evolution of the breeding systems for Bromeliaceae on Bayesian phylogenetic analyses, which showed that the selfing breeding system is reversible with multiple origins. Selfing promoted reproductive assurance and may have allowed bromeliads to invade new adaptive zones and could also be important in the beginning of the diversification process.

**Key words:** diversification, outcrossing, reproductive assurance, reproductive isolation, selfing.

**Funded by:** CNPq and CAPES.

### Biogeography, ecology and macroevolution of grasses

#### **Historical contingency and the origin of tropical-temperate niche transitions in the grass family (Poaceae)**

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Biotic and abiotic factors interact to restrict the distribution of taxonomic groups between specific boundaries that comprise their ecological niche. Whereas a large part of a taxon's ability to penetrate these boundaries will be dictated by local selection pressures and novel ecological opportunities, niche evolution will also be constrained internally through the inheritance of adaptive, neutral, or even maladaptive ancestral trait complexes and their underlying mechanisms. Flowering plants evolved when the Earth was largely tropical. However, although the majority of species have remained in the tropics, there are multiple examples of evolutionary shifts into both

northern and southern temperate regions. Using the grass family (Poaceae) as a model, we aim to pinpoint key traits that have facilitated tropical-temperate boundary crossings, and assess the degree to which such transitions have been facilitated by changes in lineage-specific versus deeply conserved developmental modules. Our data so far support a combination of these forces at play in the evolution of flowering time and cold acclimation, and highlight the importance of phylogenetic history in shaping the evolutionary potential of taxa to cross major ecological boundaries.

**Key words:** grasses, low temperature adaptations, Pooideae, temperate regions, vernalization.

**Funded by:** National Science Foundation, Norway Research Council, United States Department of Agriculture - HATCH

## Grasses and grasslands of Madagascar

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Anthropogenic destruction has long been blamed for 65% of Madagascar's land area being covered by grassland and savanna. Over the last six years, via new collections of Malagasy grasses and taxonomic work, we have found that an estimated 217 of Madagascar's 541 grass species are endemic, a level of endemism consistent with other subtropical islands. Madagascar contains 70 endemic grass lineages that colonised Madagascar primarily from Africa, with a mean age of 3.5 million years; 50% of grass dispersals were C<sub>4</sub> lineages pre-adapted to open canopy habitats. The High Plateau is home to a diverse grass flora, where a grass checklist of the Itremo Protected Area found 20% of grass species restricted to the High Plateau. Grass community composition suggests *Tapia* woodlands, historically perceived as degraded forest, are a savanna and that the grass and tree functional traits of this ecosystem diverge from gallery forests. Phylogenetic diversity within grassy ecosystems decreases with strong physical disturbance, such as grazing, indicating ecosystem dynamics typical of natural assemblages. Madagascar is home to an ancient and diverse grass flora, with local species assemblages functioning similarly to savanna ecosystems of Africa, indicating that pre-human Madagascar was home to tropical grasslands and savannas.

**Key words:** Africa, evolution, Madagascar, Poaceae, savanna.

**Funded by:** GBIF BID; British Ecological Society; UK SynTax award scheme supported by BBSRC and NERC; National Geographic Society; Bentham-Moxon Trust; Kew Madagascar Conservation Centre; Madagascar National Parks; Direction Générale des Forêts; Parc Botanique et Zoologique de Tsimbazaza

## The Viking syndrome—why grasses are so successful

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Poaceae is highly successful, occurring in (almost) all ecosystems and ecologically dominating many, and high species richness. The success of grasses may be due to their capacity to colonize, persist, and transform environments (the “Viking syndrome”). This results from combining effective long-distance dispersal, efficacious establishment biology, ecological flexibility, resilience

to disturbance and the capacity to modify environments by changing the fire and mammalian herbivory regimes. We identify a diverse set of functional traits linked to dispersal, establishment and competitive abilities. These include unique features such as the spikelet, the awned lemma, the precocious embryo and large starch reserves. Other potentially important traits are wind pollination, widespread polyploidy, gametic self-incompatibility, C<sub>4</sub> photosynthesis, frost tolerance, and a sympodial growth-form. Grasses have traits that facilitate frequent fire and tolerate grazing. We trace the accumulation of these traits since the late Cretaceous grass origin, and link these to the several phases in the grass success story: Cretaceous dinosaur fodder, to occasional late Palaeogene tropical grassland patches, to extensive Miocene C<sub>3</sub> grasslands, to dramatic Pliocene expansion of tropical C<sub>4</sub> savannas and grasslands, and finally Pleistocene C<sub>3</sub> steppe grasslands.

**Key words:** dispersal, establishment biology, fire and grazing regimes, functional traits, Poaceae

## **Macrofossils, cuticles, and phytoliths: an update on the paleoecology and biogeography of the grasses**

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The timing of the origin and diversification of the grass family has become the subject of intense debate particularly with regards to the taxonomic placement of recently described grass phytoliths and cuticle from the Maastrichtian of India. Here, we report on new quantitative analyses of phytoliths from multiple sources and a newly discovered early-mid Campanian fossil grass cuticle from James Ross Island, Antarctica. Time calibrated phylogenetic analyses and ancestral area estimations using macrofossils, fossil cuticles and phytoliths support an Early Cretaceous Gondwanan origin of the Poaceae. Ancestral habitat estimations using extant taxa indicate that grasses first evolved in forest-associated habitats and may have occupied key positions in forest margins, allowing lineages to more readily evolve into either deep shade or open habitats. In the Late Cretaceous or Paleocene, the PACMAD and Pooideae moved from forest associated ecosystems to open habitats, more than 30 Ma before the spread of grass-dominated vegetation in the Oligocene-Miocene. Our temporal estimations also suggest that C<sub>4</sub> photosynthesis evolved first in the Chloridoideae in the Eocene or Oligocene, approximately 15-20 Ma earlier than other C<sub>4</sub> PACMAD lineages and long before the rise to dominance of C<sub>4</sub> grasslands within the last 10 Ma.

**Key words:** ancestral state estimation, fossils, paleobotany, phylogenetics, Poaceae.

## **C<sub>4</sub> grass species assemblages in savannas: a biogeographical approach**

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Modern savannas are species-rich biomes, which include many modern mammal lineages and are dominated by C<sub>4</sub> grass (Poaceae) species. These systems are thought to have evolved by the late Miocene-early Pliocene and have spread worldwide 8 mya. However, the assembly of grass species

seems to indicate massive grass intercontinental dispersal but its role in savanna species assemblies is unknown. To resolve the question of how the modern subfamily composition of the savannas in each disjunct region was assembled, we ask (i) on which continent did C<sub>4</sub> clades evolve and (ii) whether dispersal from the area of origin accelerated with the ecological dominance of savannas. We used the largest time-corrected species-level phylogenies of PACMAD grasses to optimize ancestral biogeographical areas using the Dispersal-Extinction-Cladogenesis model and computed relative dispersal rates for each clade through time. We show that dispersal rates for Andropogoneae increased by 8 mya, which coincided with an increase in the isotopic signal. However, the major C<sub>4</sub> clades started dispersing before the onset of savanna expansion in the middle Miocene. The presence of C<sub>4</sub> clades by the early Miocene in Africa, Tropical Asia and Australasia suggests that local C<sub>4</sub> patches were followed by intercontinental dispersal in the late Miocene.

**Key words:** biogeography, C<sub>4</sub> grasses, intercontinental dispersals, Miocene, spread of savannas.

## Ecophysiology of Bromeliaceae

### Part 1: Ecophysiology of Bromeliaceae

#### **Ecophysiological strategies in Bromeliaceae: how distinct traits favored their radiation into a wide range of environments**

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Bromeliaceae is a monophyletic family that occupies many resource-poor environments; however, bromeliads possess traits that allow their efficient water and nutrient use and uptake, including the presence of foliar trichomes, phytotelmata (tanks with water), the diversification of the carbon metabolism and contrasting growth forms. Besides distinct subfamilies, bromeliads are divided into five ecophysiological types according to their sources of nutrients (soil, tank) and their way to access them (trichomes, roots). In this context, new researches are showing distinct physiological, anatomical and molecular traits among Bromeliaceae subfamilies and ecophysiological types. For example, bromeliads have a trade-off between the use of nitrogen for soluble protein production and immediate growth or to store it in amino acids for transamination reactions during nutritional stress. In another example, *Guzmania monostachia* performed a weak CAM but kept the integrity of the PSII, suggesting a metabolic strategy to maintain plant fitness even in water deficit conditions. Additionally, the expression of genes associated with the nitrogen metabolism and the resistance to oxidative stress appears to be different in bromeliad leaf portions, suggesting high foliar adaptation to nutritional and water variations. These results highlight the ecophysiological plasticity of bromeliads to deal with a wide range of environments.

**Key words:** ecophysiological types, foliar adaptation, nitrogen use efficiency, resource-poor environment, water use efficiency.

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#### **Cold tolerance in tropical bromeliads**

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Despite the predicted global warming scenario for the next decades, models of climate simulation reveal that cold events will persist in the 21st century. Plants from temperate climate tend to be more tolerant to cold and present greater survival when exposed to temperatures between 0 and 5 °C than tropical species, which may be stressed at temperatures below 10 °C. In general, tropical plants are considered vulnerable to cold. However, the great diversity of tropical species growing in regions subject to sudden temperature changes suggests the presence of efficient mechanisms of temperature tolerance. Since 2004, our group has been studying the physiological behavior of Bromeliaceae species growing in tropical areas with a broad temperature range. Bromeliads encompass 58 genera and 3,200 species and can be found from sea level to the Andes, inhabiting humid regions such as the Atlantic Rainforest or dry areas, with temperatures ranging from 0 to 40 °C. Therefore, considering their thermal plasticity we aimed to study the cold tolerance of Brazilian bromeliads, investigating their physiological, biochemical and anatomical characteristics. Our findings indicate that nitric oxide levels, sugar content and antioxidant enzymes, change in the first hours of cold exposure and subsequent de-acclimation, allowing the bromeliads to rapidly adjust its metabolism to thermal changes. Taking into account the increase in the expected thermal amplitude due to climatic changes, with the onset of episodes of sudden cold, investigations about the effects of temperature on tropical species become an important indicator of the impact that global changes on the tropical biodiversity.

**Key words:** antioxidants, Bromeliaceae, CAM, carbohydrates, NO, sudden cold

**Funded by:** FAPESP – Fundação de Amparo a Pesquisa do Estado de São Paulo

### **Effects of drought and water supply on the performance of tank bromeliads**

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The epiphytic habit represents a stressful environment for bromeliads. Drought stress has been studied mainly on atmospheric-epiphytes because of their inability to trap external water in a tank. However, future climate scenarios forecast up to 50% decline in rainfall, which could also impact tank-epiphytes. The objectives were to evaluate water relations and water stress between two tank-epiphytic species from C<sub>3</sub> and CAM metabolisms. Plant performance was compared, based on a drought gradient and water supply experiments, using a set of functional traits related to plant growth, resource capture and resource-use strategies. We demonstrated that the performance of both species declines with increasing drought stress but with some contrasting physiological responses between species at intermediary stress. Additionally, we showed that these two species had contrasting functioning in leaf vs. roots for water and nutrient uptake. While the roots of the C<sub>3</sub> tank-epiphyte played only a marginal role in the uptake of water, in the CAM species the roots did contribute to plant water and nutrient balance. The occurrence of CAM and C<sub>3</sub> metabolisms in tank-epiphytes do have implications in the response to drought gradient and for water and nutrient acquisition.

**Key words:** drought, functional traits, photosynthetic metabolism, tank-bromeliads, water and nutrient uptake.

## Exploring the physiological and molecular aspects of some functional pathways along the leaves of *Guzmania monostachia* (Bromeliaceae)

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Bromeliaceae includes species spanning a wide diversity of habitats throughout Neotropical ecosystems. Nearly half of all bromeliads are epiphytes, and their representatives often display highly specialized adaptations to survive in extreme environments. Many adaptations exist in epiphytic bromeliads and they represent key strategies to efficiently intercept, absorb, and store water and nutrients. Among them, one of the most studied is CAM photosynthesis. However, the molecular basis behind the morpho-functional biology of those important plants remains to be discovered. We have chosen the epiphytic-tank bromeliad *Guzmania monostachia*, the only well-recognized CAM facultative species amongst Bromeliaceae, to perform a deep sequencing, transcriptome assembly, followed by differential expression analysis across several tissues along the proximal-distal leaf axis (base, middle, apical leaf portions) in response to varied conditions of water and/or nutrient availability. The transcriptional profiles obtained in our RNA-seq analysis highlight the existence of a clear morpho-functional heterogeneity along leaf blades of epiphytic-tank bromeliads with a specialization gradient typically varying from the base to the leaf apex, chlorophyll-rich regions being more related to metabolic-assimilatory processes and the leaf bases to nutrient responses.

**Key words:** CAM photosynthesis, epiphyte, nutrient responses, RNA-seq, water availability.

**Funded by:** FAPESP/CNPq

### *Part 2: Adaptations and conservation of Bromeliaceae in Brazilian hotspots*

#### **Evolution and conservation of bromeliads from hotspots: a case study from the cerrado-pantanal-chaco species**

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Data generated by population genetics research can be used to define conservation units and priorities for the management of genetic resources, indicating populations of importance for the conservation of species. The present study described patterns of population structure, genetic diversity and gene flow of *D. ferruginea*, which occurs in Cerrado areas in Mato Grosso do Sul, Central Brazil. Six microsatellite markers were used for both nuclear ( $H_O = 0.218$  and  $H_E = 0.787$ ) and plastidial ( $H_E = 0.917$  to  $H_E = 1.000$  and 39 haplotypes) genomes. There were significant



deviations from the Hardy-Weinberg Equilibrium, with an inbreeding coefficient of 0.760. A large genetic variation was observed within the populations for both nuclear (88.39%) and plastid (91.87%) microsatellites, with moderate genetic structure ( $F_{ST} = 0.116$ ). Moderate levels of genetic differentiation between populations were found considering  $F_{ST}$  comparisons. The number of migrants per generation ranged from 0.299 to 1.308, which maintains the cohesion of populations although with low gene flow. There is no record that *D. ferruginea* is included in Conservation Units or on any list of endangered species. Therefore, species conservation strategies are necessary to ensure that *D. ferruginea* will be protected as well as the Cerrado ecosystem.

**Key words:** conservation, gene flow, microsatellites, Pitcairnioideae, population genetics.

**Funded by:** CNPq, CAPES, FUNDECT

## **Drivers of bromeliad trait variation across a latitudinal gradient in the Brazilian Atlantic Forest hotspot**

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The hyperdiverse Atlantic Forest stands out as a biodiversity hotspot in which bromeliads underwent a significant rapid diversification. We investigate size and shape variation of leaves and floral bracts of three closely related *Vriesea* taxa across a latitudinal gradient in the biome to test the contribution of genetics, climate, and topography as driving forces. Morphology was assessed using geometric morphometrics. Genetic structure was assessed using two cpDNA regions. Environmental variables most important to explain variation were selected through PCA. We tested the influence of the predictors using linear mixed-effect models. We found that shifts in shape correlate with climate and chloroplast divergence, whereas size reflects local environment conditions. We also detected a phylogeographic break in species distribution between the northern and southern portions of the forest, from where species likely diverged around 4 Mya during the Late Quaternary.

**Key words:** biogeography, climatic shifts, floral bract shape, geometric morphometrics, leaf shape.

**Funded by:** The study was supported by the Coordenação de Aperfeiçoamento Pessoal (Capes), the Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ) and the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) through fellowships and financial help for field and lab works.

### Wind to insect pollination in *Rhynchospora* (Cyperaceae)

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Cyperaceae are one of the few families in which the change from wind to insect pollination happens regularly. We analyzed pollination systems in *Rhynchospora* species, testing the role of involucre bracts in the attraction of insects, and the contribution of different pollen vectors to fruit set in ambophilous species over time and in different locations. Floral traits of 79 species of *Rhynchosporae* were analyzed and mapped onto a phylogeny of the group. The pollination systems of each species were classified into anemophilous (5% of species), ambophilous (70%), or entomophilous (25%). Scent emission in flowers of the genus was recorded for the first time. Where species with green and with white involucre bracts co-occur, insects are attracted first to species with white bracts. The participation of wind and insects in ambophilous species can vary temporally, depending on rainfall and wind conditions. Traits related to entomophily appeared independently in each of the four clades of the tribe. We suggest that ambophily is a stable pollination system and important for the reproductive success of many species of *Rhynchospora*. This breaks the paradigm that this genus is anemophilous and suggests that we reconsider pollination systems in other genera of Cyperaceae.

**Key words:** adaptation, ambophily, anemophily, Cyperaceae, entomophily.

**Funded by:** CNPq, Facepe and CAPES

### Evolution and structure of holocentric chromosomes in Cyperids: The case of *Rhynchospora* (Cyperaceae)

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Species from the Cyperid clade, as well as other plant and animal groups, have chromosomes with centromeric activity along their length, known as holocentrics, instead of a localized centromere observed in monocentrics. Since holocentric chromosomes may break and fuse, and still segregate normally during cell division, karyotype evolution in groups with holocentric chromosomes is considered more dynamic than in groups with monocentric chromosomes. We have investigated chromosome structure and evolution in *Rhynchospora* Vahl (Cyperaceae), a genus that comprises around 380 species with chromosome numbers varying from  $2n = 4$  to  $2n = 84$ . We have reconstructed ancestral chromosome number in the genus and observed its maintenance in three out of four main clades, with polyploidy and/or fusion/fission events present in specific lineages. We have also shown that holocentromeres from *Rhynchospora* species are enriched with a satellite DNA and a retrotransposon family, which had not been detected in other holocentromeres. Other satellite DNA families are associated or not to typical heterochromatin. A particular centromere

organization was observed during its inverted meiosis. Ongoing work about evolution of repetitive DNA and centromere structure in *Rhynchospora* and other genera of Cyperaceae will be discussed. **Key words:** centromere, holokinetic, karyotype evolution, repetitive DNA, satellite DNA.

**Funded by:** CAPES and CNPq, Brazil

### **Changing generic concepts in Cyperaceae: focus on *Costularia* s.l.**

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Recent advances in elucidating relationships in Cyperaceae have uncovered new lineages requiring changes in generic concepts. An overview is provided and the case of *Costularia* s.l. (Schoeneae) is discussed. We investigated the monophyly of *Costularia* (25 species), which is distributed from southeastern Africa, in Madagascar, the Mascarenes and Seychelles, to Malesia and New Caledonia. Relationships and divergence times were inferred using a four-marker phylogeny including new sequence data. Phylogenetic reconstruction was executed using Bayesian inference and maximum likelihood. Divergence times were estimated using a relaxed molecular clock model, calibrated with fossil data. Based on our results, *Costularia* s.l. is composed of four distinct evolutionary lineages. Two lineages, one including the type species, are part of the *Oreobolus* clade: (1) a much reduced genus *Costularia* restricted to southeastern Africa, Madagascar, the Mascarenes and Seychelles, and (2) *Chamaedendron*, a newly erected endemic genus from New Caledonia based on *Costularia* subgenus *Chamaedendron*. The other two lineages are part of the *Tricostularia* clade, (1) *Xyroschoenus*, a new single-species genus from the Seychelles, and (2) *Costularia* subgenus *Lophoschoenus*. The latter are congeneric with reticulate-sheathed *Tetraria*. Together with recent changes in the delimitation of *Schoenus*, this resulted in a recircumscription of *Tetraria*.

**Key words:** *Costularia*, Cyperaceae, molecular phylogeny, monophyly, new genus.

### **Holocentric chromosome evolution and its impact on diversification, with special emphasis on sedges (Cyperaceae)**

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There are two main kinds of chromosomes in eukaryotes depending on how the kinetochore activity is distributed: monocentric chromosomes, with a single centromere located in the primary constriction, and holocentric chromosomes, characterized by the lack of a clear primary constriction and a kinetochoric activity widely spread along the chromosomes. The evolutionary effect that holocentric chromosomes might have in diversification has been largely overlooked despite some of the most diverse lineages of animals and plants (e.g., lepidopterans and sedges) possessing

holocentricity. Here we look into the macroevolutionary impact of holocentricity at different scales, from a global view in eukaryotes to the Cyperaceae family and its most diverse genus (*Carex*). Results showed that holocentricity is likely to have appeared independently at least 18 times in eukaryote evolution and that there are no significant differences between diversification rates for holocentric lineages when compared with their monocentric sister lineages. In Cyperaceae, different models of chromosome number evolution for particular lineages are best supported than a single model for the complete phylogeny alone suggesting a link between transitions in the mode of chromosome evolution and shifts in diversification rates. Moreover, chromosome number yielded a high phylogenetic signal and was clearly related to diversification rates in *Carex*

**Key words:** *Carex*, chromosome number, extinction, phylogeny, speciation.

## **A playground for *Carex*: resolving its backbone and fine-scale relationships using HybSeq**

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*Carex* (Cyperaceae) is the most biodiverse Monocot genus and one of the three largest angiosperm genera. With nearly 2000 species, the study of evolution in *Carex* has been hampered by its large size and because most traditional sections have been found to be polyphyletic. Recent well-sampled phylogenies (>50% of the species) increased phylogenetic resolution within main clades and clarified the relationships among many groups of species. However, uncertainty is still present along the backbone of the tree as well as in some recent large radiations. The lack of a well-supported hypothesis in *Carex* has hindered tackling analyses of character evolution and biogeographic questions. We used HybSeq (target enrichment combined with genome skimming) to obtain hundreds of targeted loci in a fast and cost-efficient way using fresh, silica-dried and herbarium materials. We have developed new probes based on low-copy nuclear genes that work on the genus *Carex* and across Cyperaceae. At a macroevolutionary level, the relationships of the major clades within *Carex* and among the major sections were highly supported. At a microevolutionary level, and exclusively relying in herbarium material, we were able to reconstruct the phylogenetic history of the Neotropical *Carex* sect. *Fecundae*.

**Key words:** genome skimming, low-copy nuclear genes, phylogenies, resolution, target enrichment.

## **Phylogeny and Diversity in *Rhynchospora* section *Tenues***

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Kükenthal included *Rhynchospora* sections *Laevinuces* Kük., *Luzuliformes* Kük., *Spermodontes* Kük., and *Tenues* Kük. in the *Psilocarya* group of subgenus *Rhynchospora*. Existing molecular studies have included only a few of the 41 species in these four sections and indicate that they are sister to a group that includes sections *Psilocarya* and *Dichromena*. To elucidate relationships among these four sections, we sequenced chloroplast *trnL-F* and nuclear ETS and ITS markers.

Results showed that *Laevinuces*, *Spermodontes* and *Tenues* are not monophyletic but, grouped together, they form a well-supported clade, *Rhynchospora* sect. *Tenues* Kük. Sect. *Luzuliformes* is monophyletic but not a sister clade of *Tenues*.

**Key words:** Cyperaceae, molecular phylogeny, *Rhynchospora*, section *Luzuliformes*, section *Tenues*.

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### **What about Cryptangieae (Cyperaceae)? Clarifying a Neotropical gap in sedges**

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The Neotropical tribe Cryptangieae is especially diverse and has high rates of endemism in Venezuelan Guayana and Brazilian *campos rupestres*. Cryptangieae include herbs with unisexual spikelets, generally with hypogynous scales opposite to the sides of trigonous achenes. The tribe is closely related to the Schoeneae and the Sclerieae+Bisboeckelerae clades but Cryptangieae diversity has been poorly sampled in phylogenies. Therefore, our goal was to clarify the monophyly and tribal status of Cryptangieae in order to better understand the relationships among the early divergent tribes of subfamily Cyperoideae. We investigated the intra- and inter-tribal relationships through a molecular approach using two chloroplast (*rbcL* and *trnL-F*) and three nuclear ribosomal (ITS, ETS and 5S-NTS) markers and compared the results to morphological data. The most probable sister-group of Cryptangieae is currently included in Schoeneae but we need more conclusive information about this diverse polyphyletic tribe. We propose Cryptangieae as a monophyletic tribe with 57 species (10 spp. nov.) distributed in six morphologically recognizable genera with new circumscriptions.

**Key words:** *Cephalocarpus*, *Everardia*, *Exochogyne*, *Lagenocarpus*, white-sand savannas.

**Funded by:** CNPq, CAPES, FAPESP, FAEPEX - Unicamp

### **Unravelling evolutionary relationships in a complex polyploid clade - southern African *Schoenus* (Cyperaceae)**

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*Schoenus* L. is a genus in tribe Schoeneae with approximately 150 species predominately restricted to nutrient-poor sandstone habitats in the temperate continents of the southern hemisphere, with the highest species diversity in Australia and southern Africa. Our work focuses on the southern African clade of *Schoenus*, where all but one species was included in either *Tetralia* P.Beauv. or *Epischoenus* C.B.Clarke until a taxonomic realignment in 2017. Ongoing taxonomic work including the description of several new species has also increased the number of species in this clade; however, the complex evolutionary relationships among these species are not yet known. Preliminary data show that genome sizes within *Schoenus* are substantially larger than those of

other Schoeneae species and are among the highest in the Cyperaceae, suggesting that aneuploidy, polyploidy and the possible accumulation of repetitive sequences are important mechanisms in this genus. We will present results from ongoing taxonomic work within the southern African clade of *Schoenus*, which includes changes in species boundaries and the description of several new species. Furthermore, we will present the results of recent phylogenetic analyses based on paired-end genotyping-by-sequencing data, and discuss genome size and chromosomal evolution within the context of this phylogeny.

**Key words:** Cyperaceae, genome size, genotyping-by-sequencing, phylogeny, Schoeneae.

## Major clades and morphological trends in *Rhynchospora* (Cyperaceae)

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Phylogenies of *Rhynchospora* derived from recent molecular studies show species grouped into five major clades. Species in each clade exhibit some morphological features in common, but often not features used in earlier classifications. Section *Longirostres* is sister to sections *Pauciflorae* plus *Pluriflorae* – plants are usually robust with large spikelets and achenes. Section *Tenues* is sister to sections *Dichromena* and *Psilocarya* – species of all three sections lack hypogynous bristles and often have transversely rugulose achene surfaces. The genus *Pleurostachys* is sister to sections *Polycephalae* plus *Paniculatae* and *Cephalotae* – many species in these sections have branched synflorescences that are repeated several times along the culm. The remaining clade comprises the 14 sections Kükenthal grouped in what he called “Eu-Rhynchospora” – this is a recently evolved group of relatively small plants with anthelate inflorescences and hypogynous bristles. Overlaying these are adaptations in each clade in response to specific ecological conditions. In each clade, for instance, there are species with adaptations for insect pollination.

**Key words:** Cyperaceae, evolution, morphological trends, phylogeny, *Rhynchospora*.

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## Genomic Innovation through Genome Duplication: Examples from across Monocots

### Genome evolution and diversification in palms

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Processes contributing to genome size variation in plants include polyploidy, tandem duplication, expansion/contraction of repetitive regions such as transposable elements, intramolecular recombination, fractionation/diploidization, and selection for smaller genomes. The palms (Arecaceae) are diverse, ecologically successful components of tropical ecosystems, with >2,500 species. Recent studies demonstrate strong correlations between genome size variation and species diversity, suggesting that polyploidy or other mechanisms of genome size change potentially confer selective advantages due to the introduction of genomic novelty. Here we aim to test that hypothesis

in palms and their close relatives, the commelinid monocots. We address relationships between chromosome number and C-value across the family, and reconstruct ancestral chromosome numbers and genome sizes. We use available genomic and transcriptomic data to investigate evidence for polyploidy among the commelinids and within the palms (at the subfamily level) by comparing the divergence distribution of paralogous sequences, and by the use of species/gene tree methods. Lastly, we investigate the mechanisms of genome size variation by exploring repeat type and content based on ‘genome skim’ data. Disentangling the causes of genome size variation in palms will help us understand the genomic conditions facilitating adaptive radiation and ecological success in other important clades.

**Key words:** Areaceae, genome size, paralog, repeat, transposable element.

## Alternative paralog recruitment in independent origins of CAM photosynthesis in monocots

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The evolution of complex traits can occur through multiple genetic mechanisms, including whole genome duplications that allow for sub- or neo-functionalization of duplicated genes that can then confer novel traits. One example of a recurrently derived complex trait is Crassulacean acid metabolism (CAM), a modified form of photosynthesis. CAM is found in about 7% of all land plants, and has arisen in multiple genera across families in the monocots, including the Orchidaceae, Asparagaceae, and Bromeliaceae. The repeated evolution of CAM allows us to interrogate whether it arose through parallel or convergent genetic evolution in independent origins. Here, we examined the gene expression and molecular evolution of key CAM genes across independent origins of CAM in the monocot order Asparagales. We further tested for alterations to rates of molecular evolution across lineages and paralogs to determine whether the same sites are under selection in independent origins of CAM. Our comparisons between *Agave* and *Yucca* (Asparagaceae) and *Erycina* (Orchidaceae) reveal that alternative paralogs of key carbon fixation genes have been recruited into the CAM pathway, even across closely related genera, and implicate the importance of gene duplication for the evolution of this complex trait.

**Key words:** gene evolution, paralogs, photosynthesis, physiology, transcriptomics.

## Genomic variation in a hexaploid prairie grass

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The North American tallgrass prairie is dominated by four grass species: *Andropogon gerardii* (big bluestem), *Schizachyrium scoparium* (little bluestem), *Sorghastrum nutans* (Indiangrass), and *Panicum virgatum* (switchgrass). Big bluestem, a hexaploid, represents more biomass than any species found in the prairie, up to 70% of all biomass in some estimates. Big bluestem has populations outside of the prairie, ranging from the dry American Southwest across the United States to Florida and up into Canada from Saskatchewan to Quebec. The climatic range of big bluestem suggests that the species may be either inherently plastic or have a wide range of genetic

diversity found across populations. Here, we share initial findings from a genome sequencing project for big bluestem, done in conjunction with the Joint Genome Institute in the United States. As part of this project, a highly resolved genome is being sequenced from a population at the center of genetic diversity for the species. Additionally, multiple populations from across the range are being resequenced to assess the diversity of the species in different habitats. A primary goal of this work is to understand the interplay between the subgenomes of big bluestem and its adaptation to various environmental stresses.

**Key words:** adaptation, *Andropogon gerardii*, grasses, prairie, whole genome duplication.

### **Gene duplication and the diversification of form and function: the evolution of the leucine-rich repeat receptor-like protein kinase (LRR-RLK) gene family**

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LRR-RLK proteins mediate cell-cell signaling in plant development and defense. For example, LRR-RLKs in the CLAVATA1 (CLV1) clade are essential regulators of meristem homeostasis and, in turn, the development of plant form. The LRR-RLK gene family has diversified to an extreme degree, both through whole genome and smaller-scale duplications. The immense size and complexity of the LRR-RLK gene family has made resolving gene trees challenging. To address this challenge, we developed an iterative search and tree-building method for capturing all the LRR-RLKs and related proteins encoded in a genome, and for resolving the evolutionary history of the gene family. Through the course of this research, we uncovered an uncharacterized gene clade related to CLV1 that has diversified in the grasses. We are assessing the function of these genes using CRISPR/Cas9 genome editing in the model grasses *Zea mays* and *Brachypodium distachyon*. We also discovered numerous instances of domain loss, domain gain through duplication and subsequent gene fusion events, and domain swapping between LRR-RLK clades. Our fine-grained history of these important genes sets the stage for understanding the diversification of signaling in the evolution of plant development and defense.

**Key words:** evo-devo, gene duplication, meristems, phylogenetics, Poaceae.

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### Grass Systematics and Evolution - Plastome phylogenetics and the BOP Clade

#### **Evolutionary systematics in dune species of *Poa* (Poaceae: Pooideae)**

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Reproductive systems in American *Poa* evolved during the Late Miocene in the Northern and Southern hemispheres, independently. Dioecy appeared associated with geological episodes such as the Andean mountain uplift, and climatic oscillations associated to glacial cycles. The monophyletic section *Dioicopoa* originated during the Pliocene to Early Pleistocene in South America by a rapid



evolutionary radiation. Coastal dunes are dynamic ecosystems due to aeolian formation and rapid modification where speciation and endemism occur. Dune environmental heterogeneity and vegetation patterns were studied using classification and ordination analyses. In this work, we studied the relationship between the presence of seed-transmitted *Epichloë* fungal endophytes and environmental characteristics on diversification of three close-related *Poa* species in coastal dunes. Through morphological multivariate analyses in a widely distributed species (*Poa lanuginosa*) and two narrowly endemic species (*Poa bergii* and *Poa schizantha*), quantitative and qualitative changes are detected among their populations. The morphological differentiation of the symbiont endophytic fungi and their association with *P. bergii* and *P. lanuginosa* species are evaluated through regression analyses; *Poa schizantha* was not associated with *Epichloë*. Intermediate characteristics between *P. lanuginosa* and *P. bergii* suggest events of recent speciation and hybrid formation as an adaptive and evolutionary process in coastal dunes.

**Key words:** coastal dunes, dioecy, endophyte, grasses, speciation.

### **Systematics and evolution of the Neotropical woody bamboos (Poaceae: Bambusoideae: Bambuseae)**

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Bambusoideae (bamboos), comprising 1,680+ species, are classified into three tribes: Arundinarieae (temperate woody bamboos, 581 species), Bambuseae (tropical woody bamboos, 976 species) and Olyreae (herbaceous bamboos, 123 species). Arundinarieae is primarily of eastern Asia and has no native species in the American tropics. Bambuseae consists of two major clades, the Paleotropical (PWB, 554 species) and the Neotropical woody bamboos (NWB, 422 species), and is distributed broadly in the tropics and subtropics with a few taxa in subtemperate regions, and extensive diversity in montane systems. Except for 1 genus endemic to New Guinea, and 1 species occurring in both the Neotropics and Africa, the rest of the Olyreae is restricted to the Neotropics. Recent molecular analyses using both plastid and nuclear DNA sequence data continue to support the topology of Chusqueinae (Arthrostylidiinae + Guaduinae) within the NWB, but suggest some generic realignments. In the usual absence of flowering material, complete vegetative collections are essential for discovery and identification; new species continue to be described. Brazil has the greatest diversity of NWB, but the Andes, Panama/Costa Rica, Mexico and the West Indies also harbor significant diversity and endemism. In the Neotropics, *Chusquea*, *Guadua* and *Merostachys* still require significant taxonomic attention.

**Key words:** Arthrostylidiinae, bamboo classification, Chusqueinae, Guaduinae, New World woody bamboos.

### **A 250 plastome phylogeny of the grass family (Poaceae)**

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Plastome phylogenomic studies have contributed to advances in systematics, but in grasses these studies have been taxonomically restricted. Here we present a family-wide study of 250 grass plastomes (179 genera, 44 tribes). Plastome assemblies represented over 28.7 Mbases. Phylogenetic signal was characterized in 14 partitions: (1) complete plastomes; (2) protein coding and (3) noncoding regions; and (4) three loci widely used in grass phylogenetics. Each partition was further refined to alternatively include or exclude positively selected codons and gaps introduced by the alignment. All 76 protein coding loci were found to be predominantly under purifying selection, but specific codons in 65 loci showed evidence of positive selection. Widely used loci of prior studies had high numbers of positively selected codons. Our analyses fully supported the backbone topology for BOP taxa. Among the 14 analyses, 82 of 309 resolved clades were maximally supported. Analyses of new plastomes agreed with current classifications. Five (of seven) partitions from which alignment gaps were removed retrieved Panicoideae as sister to other PACMAD subfamilies incongruent with topologies recovered from partitions with alignment gaps. This suggests that alignment ambiguities in these uncertain regions introduced false signal. Our study indicates how different data partitions affect phylogenomic inferences.

**Key words:** partition, phylogenomics, plastome, Poaceae, selection.

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### **A phylogeny of Poeae chloroplast group 1 including genera in the Agrostidinae, Anthoxanthinae, Aveninae, Brizinae, Calothecinae, Echinopogoninae, Phalaridinae, and Torreyochloinae (Poaceae: Pooideae)**

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A phylogeny based on analysis of four molecular DNA sequence markers (ITS, *rpl32-trnL*, *rps16* intron, and *rps16-trnK*; 4477 total aligned sequences) was made on members of Poeae chloroplast group 1 including genera in the Agrostidinae, Anthoxanthinae, Aveninae, Brizinae, Calothecinae, Echinopogoninae, Phalaridinae, and Torreyochloinae. Our sample included 1341 accessions (190 additional accessions were unsuccessful due to poor material) representing 523 species selected from herbarium specimens (@80%) and silica field-collected material (@20%). *Trisetum* s.l. is polyphyletic and splinters into seven clades: *Acrospelion* (*Trisetum* sect. *Acrospelion*), Calothecinae (*Trisetum bulbosum*, *T. juergensii*, and *T. brasiliense*), *Grapphephorum*, *Koeleria*, *Peyritschia*, *Siberotrisetum* gen. nov. (*Trisetum* sect. *Sibirica*), and *Tristeum* s.s. Species of *Deyeuxia* are subsumed within *Calamagrostis*, *Cinnagrostis* (South American *Calamagrostis*), and *Deschampsia*

(*Deyeuxia* sect. *Stylagrostis*). Calothecinae includes *Chascolytrum* s.l., *Paramochloa* gen. nov. (*Calamagrostis effusa*), and Echinopogoninae includes *Dichelachne*, *Echinopogon*, *Greeneochloa* gen nov. (*Calamagrostis coarctata* and *C. tweedii*), and *Relchela*. *Lachnagrostis* and *Polypogon* are reticulate genera where species of each genus align as clades within *Calamagrostis* (ITS signal) and within *Agrostis* (plastid signal). Species of *Ammophila* are reticulate aligning within *Calamagrostis* (plastid signal) and *Agrostis* (ITS signal). Species of *Podagrostis* are reticulate aligning within *Calamagrostis* (ITS signal) and as sister to *Agrostis* (plastid signal).

**Key words:** classification, ITS and plastid DNA sequences, phylogeny, Poaceae, systematics.

## **Taxonomy, phylogeny and biogeography of the temperate woody bamboos (Bambusoideae: Arundinarieae)**

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The Bambusoideae (Poaceae) consists of three monophyletic tribes: Arundinarieae, Bambuseae, and Olyreae. With more than 500 described species from 31 genera mostly endemic to East Asia, Arundinarieae, i.e., the temperate woody bamboos, exhibits a wide range of morphological diversity. The taxonomy of Arundinarieae has long been controversial. After nearly two decades of molecular phylogenetic studies, twelve clades based on plastid DNA sequences have been recognized which deviated markedly from the morphology-based classification, and many genera were resolved as polyphyletic. Recently, we sampled more than 400 species from all of the recognized clades and genera of the tribe around the world for the plastid genome and ddRAD sequencing. In the ddRAD phylogeny, the majority of the tribe falls into two clades, a pachymorph one and a leptomorph one, along with three basal ones, and the monophyly of several genera was corroborated. However, highly conflicting topologies were obtained between nuclear and plastid phylogenies. We discuss the origin, divergence times, species diversification, and reticulate evolution of the temperate woody bamboos in the context of the newly reconstructed phylogenetic framework. In addition, we propose to establish two new genera, *Hsuehochloa* and *Ravenochloa*.

**Key words:** Arundinarieae, diversification, East Asia, evolution, phylogeny.

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## **Systematics and evolution of herbaceous bamboos (Bambusoideae: Olyreae)**

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The tribe Olyreae is the grass lineage that includes the herbaceous bamboos, often with culms weakly lignified and unisexual dimorphic spikelets. Members of Olyreae typically inhabit the understory of tropical forests, with several rare and/or poorly known species, mostly endangered. Olyreae is strongly supported as monophyletic and sister to the tropical woody bamboos (tribe Bambuseae). It includes 22 genera, of which 17 are found in Brazil. Based on field work and

multidisciplinary studies, we have improved knowledge about its diversity, systematics and evolution. Three subtribes are accepted, with Buergersiochloinae as sister to the Olyrinae + Parianinae clade. In Parianinae, *Parianella* is sister to *Pariana* + *Eremitis*. Olyrinae includes 18 genera, several of them of complex delimitation, grouped in four main lineages which are being analyzed using plastid and nuclear sequences, as well as macro- and micromorphological features. We have recovered the monophyly of some genera (i.e. *Raddia*, *Cryptochloa* and *Diandrolyra*), whereas *Sucrea*, *Parodiolyra* and *Olyra* are not monophyletic and their generic status is being reassessed. Our forthcoming efforts involve understanding past and present biogeographic patterns and evolutionary processes in this group.

**Key words:** molecular phylogenetics, Neotropical grasses, Olyreae, Olyrinae, Parianinae.

**Funded by:** CNPq, CAPES, FAPESB

## Grass Systematics, Evolution and Development - The PACMAD Clade

### Genomics of *Steinchisma* (Panicoideae: Paspaleae)

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Over forty years ago researchers recognized the diversity of photosynthetic types among monocot species and their utility in understanding photosynthetic pathway evolution. However, early work was impeded by a lack of genomic technologies and an accurate taxonomic framework. Since then, genomic technologies have greatly advanced and a well-resolved phylogeny of the Paspaleae subtribe Otachyriinae has been completed. The subtribe Otachyriinae includes C<sub>3</sub> and C<sub>3</sub>-C<sub>4</sub> species in the genus *Steinchisma*, as well as sister C<sub>3</sub> and C<sub>4</sub> genera. We used long-read sequencing technologies to sequence and assemble the genome of *Steinchisma hians*, a C<sub>3</sub>-C<sub>4</sub> intermediate species. This high quality genome assembly provides an anchor for genomic studies in this clade. We have also successfully recreated the interspecies cross between *S. hians* and the C<sub>3</sub> species *Steinchisma laxum* that was previously reported in the literature. With the updated phylogeny we are also attempting several new crosses that will bring together different photosynthetic types in interspecific hybrids. The combined genomic resources and unique hybrid germplasm are powerful resources for understanding the genomic changes underlying photosynthetic evolution.

**Key words:** C<sub>3</sub>-C<sub>4</sub> intermediate, Panicoideae, Paspaleae, photosynthesis, *Steinchisma*.

### Grass inflorescence evolution

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Morphology of the grass inflorescence is known to be extremely variable among species, fascinating in its development and incredibly intriguing in its underlying genetics. Inflorescence forms displayed by grasses lead to a general assumption that their evolution is random. When grass inflorescence morphology was analyzed comparatively on a species phylogenetic framework, we have discovered that despite appearing extremely diverse at first sight, they followed common evolutionary trends. Currently, in our laboratory we are dedicated to investigating the molecular

bases that promoted such macroevolutionary changes in the inflorescences of grasses. In particular, we focused on studying molecular mechanisms that determine the final fate of the apical and axillary meristems of the inflorescences. This talk will discuss some advances made in the study of genes (most of them, transcription factors) that played a key role in the evolution of grass inflorescence forms.

**Key words:** genetics, grasses, inflorescence, macroevolution, morphology.

## **The evolution of transcription factor protein-protein interactions and flower development in the grasses**

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The evolution of gene regulation is central in the evolution of plant and animal form. Interactions between transcription factor proteins are of profound importance in determining gene expression patterns. This is particularly true of the floral MADS-box transcription factors, which likely function as part of tetrameric protein complexes. Current models predict that the precise composition of these MADS-box tetramers determines downstream gene expression patterns and, in turn, floral organ identity. Arising from this model of MADS-box function is the hypothesis that shifting MADS-box protein-protein interactions affect downstream gene regulation, and thus drive evolutionary change. To test this hypothesis, we have developed an experimental system in *Zea mays* (maize) where we can manipulate MADS-box protein-protein interactions in an evolutionary context, and assess consequent impacts on global gene expression patterns and floral development. We have found widespread transcriptional changes downstream of altered protein-protein interactions. In contrast, floral morphology is subtly affected. Our results have implications for understanding flower development and evolution in the grasses, and for understanding the mechanisms driving the evolution of gene regulation more broadly.

**Key words:** evo-devo, genetics, flower development, maize, transcription factors.

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## **The use of low-copy nuclear genes in the delimitation of genera and species in the Andropogoneae (Panicoideae)**

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Andropogoneae is an economically and ecologically important group of grasses, which includes some of the world's most important crops such as sugarcane, maize, and sorghum, as well as many dominant species in both tropical and temperate grassland vegetation formations. Polyploidy and reticulate evolution are common in Andropogoneae, making taxonomic delimitation of its genera

and species difficult. Due to the high variability of the sequences and their ability for identifying hybrids, low-copy nuclear genes have proven to be great markers for reconstructing the phylogeny of Andropogoneae and circumscribing its genera and species. Phylogenetic trees inferred from nuclear genes are useful to understand the evolutionary relationships of polyploid taxa and identify allopolyploidization events because they produce characteristic double-labeled tree topologies in which the polyploid species appear twice. In such trees, allopolyploids can be recognized even in the absence of chromosome counts. Recently published papers based on low-copy nuclear genes have successfully delimited genera in several Andropogoneae lineages, including *Eriochrysis*, *Saccharum*, and *Sorghum*. Taxonomic circumscriptions of species complexes have also been elucidated. A recent study also showed that at least one third of Andropogoneae species resulted from allopolyploidy, with a remarkably high number of independent allopolyploidization events.

**Key words:** allopolyploidy, molecular cloning, reticulate evolution, species complex, taxonomy.

### **Diversity and evolution of rachilla appendages in the core panicoids (Poaceae - Panicoideae)**

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Panicoideae is the second largest lineage within Poaceae with 3,200+ species. Among its 12 tribes, Paniceae, Paspaleae, and Andropogoneae form a clade known as the core panicoids. The rachilla internode below the upper antherium may be elongated in some genera of this clade, comprising a stipe, whereas in others it may also be expanded into appendages of various shapes. This feature of uncertain role may have high taxonomic significance, as in *Ichnanthus*. Here we put together the results previously obtained in taxonomic, phylogenetic, and macro- and micromorphological studies on taxa with rachilla appendages, exploring the diversity and evolution of this feature within the core panicoids. The molecular phylogenies have contradicted the traditional classifications, resulting in the proposition of new genera and reestablishment or synonymization of others. The appendages may be of homogeneous or heterogeneous morphology (as in *Hilddaea* and *Panicum* sect. *Rudgeana*, respectively) and have multiple independent origins within the core panicoids. Oils found in the appendages of some species suggest myrmecochory, but data on dispersal is lacking. Ontogeny of the appendages is also unknown, and their phylogenetic distribution and influence on the diversification of certain groups need to be better explored, as well as possible coevolution with ants.

**Key words:** fruit dispersal, grasses, independent evolution, molecular phylogeny, myrmecochory.

### **Generic realignments in Paniceae and Paspaleae (Panicoideae)**

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Included in the PACMAD clade of the family, tribes Paniceae and Paspaleae are, together with the Andropogoneae, the largest tribes of the subfamily Panicoideae, with more than 122 genera and nearly 2000 species. Paniceae and Paspaleae include a huge morphological, cytological and physiological diversity represented by different inflorescence types, several basic chromosome numbers, and at least four major photosynthetic pathways. The  $x = 10$  Paspaleae is sister to the

Andropogoneae–Arundinelleae s.s. clade ( $x = 10$ ), while the combined  $x = 10$  clade is sister to the  $x = 9$  clade that contains the remaining genera of Paniceae. Within tribe Paspaleae, we here review relationships, and new alignments, in subtribes Paspalineae, Otachyriinae, and Arthropogoninae. On the other hand, new results are discussed in incertae sedis genera of tribe Paniceae, and in subtribes Dichantheliinae, Melinidinae, Panicinae, and Cenchrinae. Finally, a new classification of *Panicum* s. str., highlighting its morphological characters, is presented; species to be excluded from the genus are discussed. Relationships with incertae sedis genera, and those classified in the Boivinellinae, Melinidineae, and Cenchrinae are also considered.

**Key words:** Paniceae, Panicum, Paspaleae, systematics, taxonomy.

## Growing knowledge on monocot vegetative anatomy

### The Phoenix of the cerrado: anatomical explanation of the rapid response of *Bulbostylis paradoxa* to fire

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Cerrado is the richest tropical savanna in plant species and fire is a natural disturbance, being an important factor in the evolution and ecology of plant species. Thus, the aim of this study was to evaluate the rapid flowering of *Bulbostylis paradoxa* species after fire, evaluating anatomical and physiological traits in order to understand flowers emerging from bud development, as well as to determine types of reserves and means of mobilization. We used fire experiments established in Central Brazil. We marked 10 individuals in each area (burned and control) and sampled three individuals for anatomical studies, and three for physiological ones. We repeated the sampling after 24 and 48 hs, and 7 and 45 days. We detected the first inflorescences 30 hours after fire. Some individuals in burned plots had up to 20 inflorescences, whilst the ones in control areas did not have any inflorescences. One week after fire, inflorescences were fully developed and 45 days after fire, we could already observe seeds being dispersed. The anatomical analysis of the stem structure showed the presence of sclereids at covering, including leaf bases. The cortical and vascular regions include the outstanding presence of parenchyma containing starch grains, probably related to the reserves for flowering.

**Key words:** anatomy, cerrado, Cyperaceae, fire, resprouting.

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### Leaf structure in Alismatales with an emphasis on 3D vasculature and squamulae intravaginales

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The core group of the basal monocot order Alismatales includes rather diverse aquatic and semi-aquatic plants. Only a few morphological features are constantly present in all core Alismatales (though it is unclear if they can be regarded as synapomorphies). Among them is the occurrence of so-called squamulae intravaginales attached to stem just above the leaf axil. Novel data on the structure and development of squamulae intravaginales confirm the idea of their secretory function and allow evaluation of possible homologies of the squamulae. Half-submerged members of the group possess well developed aerenchyma in all organs, including leaves. Such thick leaves or their petioles often have a three-dimensional vasculature with slender peripheral bundles. As in angiosperm succulents, the three-dimensional venation in thick leaves of Alismatales serves to reduce transport distances between main bundles and chlorenchyma. In both cases, the patterns of orientation of peripheral bundles (with inverted adaxial or abaxial bundles) are unstable in large clades. We believe that these slender bundles cannot be used for the identification of unifacial leaves. Comparative analyses of thick photosynthetic leaves and stems could help in distinguishing functional and morphogenetic factors governing patterns of three-dimensional vasculature.

**Key words:** Alismatales, anatomy, leaf, morphology, vasculature.

**Funded by:** The work is supported by RFBR (grant 18-04-00797).

### **Functional anatomy, biomechanics and development of the branch-stem-attachment of *Dracaena marginata* revealed using high-resolution MRI**

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Monocotyledons are unique concept generators for developing novel, bio-inspired fibre-composite structures. However, the utilization of nature's inventiveness requires methods which enable 3D to 4D *in vivo* insights into living and intact plants. Common imaging techniques are highly invasive and impede repetitive imaging during ontogenetic development or prevent stress-strain analyses. Novel methodological approaches using magnetic resonance imaging (MRI) are presented which reveal the load-adapted tissue arrangement and development of branch-stem-attachments of *Dracaena marginata*. MRI allows the comparison and contrast of various plant tissues without damaging the plant prior to or during image acquisition allowing for repetitive imaging of the same intact plant during long term experiments. *In vivo* insights into tissue deformations were gained by MRI analyses of a branch before, during and after mechanical loading. This enabled the identification of a load-adapted tissue arrangement within the branching of dragon trees. Additional ontogenetic experiments allowed us to follow the development of these tissues and to better understand the complexity of the branch ontogeny by discerning seven distinct ontogenetic stages. In summary, MRI is a promising tool for visualizing and analyzing growth, biomechanics and functional anatomy of monocots in general.

**Key words:** biomechanics, biomimetics, *Dracaena marginata*, MRI, ontogeny.

**Funded by:** Linnea Hesse thanks the Joachim Herz Stiftung for support.

### **A comparative study of shoot apical meristem and bud preformation in monocot and dicot species**



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Perennial plants resprout annually from renewal buds that hide shoot apical meristems (SAM). Even though the SAM plays a key role in plant development, we possess almost no information on the correlation between meristem parameters and individual plant organ sizes. We studied the relationship of SAM anatomical parameters with plant organ sizes. We compared late autumn buds of 24 Central European monocot species with 80 dicot herbaceous species. SAM parameters correlated with a number of size-related traits. The meristem size showed strong phylogenetic signal. However, no significant difference was found between studied monocot and dicot species except for overall lower variation in SAM parameters across the monocot species. We also examined preformation of leaves and flower initials in the late-autumn buds before winter rest in a large set of species. Bud preformation was fairly more common in monocot (34%) than dicot species (25%). This helps them to accelerate their development after winter rest and resprout and flower earlier (about 35 days). Our analyses demonstrate that SAMs provide a functional link between sizes and numbers of plant organs. SAM parameters determine the timing of growth with monocot species being more often able to accelerate their development due to bud preformation.

**Key words:** bud preformation, genome size, meristem size, phylogenetic analysis, plant organ size.

**Funded by:** This study was funded by the Czech Science Foundation (project no. 14-36079G, Centre of Excellence PLADIAS)

## Evolution of Kranz anatomy in Cyperaceae

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Cyperaceae has about 1300 species with C<sub>4</sub> photosynthesis, which is related to Kranz anatomy. In general, four different types of Kranz anatomy (chlorocyperoid, eleocharoid, fimbristylloid and rhynchosporoid) have been described in the family. These types differ basically in number of vascular bundle sheaths, continuity of the bundle sheath with large chloroplasts and chloroplast localization. Examining available data about leaf or culm anatomy, taxonomy, phylogeny, geographic distribution and habitat from C<sub>4</sub> Cyperaceae species, it was possible to infer the putative origin of Kranz anatomy in the family. Kranz anatomy emerged numerous times (possibly 5-6) in unrelated phylogenetic groups of Cyperaceae with convergence of the chlorocyperoid, eleocharoid and fimbristylloid types in certain groups. In addition, the anatomical types are not associated with specific environmental conditions because the Kranz species of *Abildgaardieae*, *Rhynchospora* and some *Cypereae* occur in similar xeric environments and exhibit different Kranz types. Also, there is no general relationship between Kranz anatomy and xeric environments, because many *Cypereae*

Kranz species are mesophytes and *Eleocharis* Kranz species are hydrophytes. So, the diverse origin of these Kranz species might result from different environmental pressures that promote the reduction of photorespiration.

**Key words:** anatomy, C<sub>4</sub> photosynthesis, ontogeny, phylogeny, ultrastructure.

**Funded by:** FAPESP (2008/09380-2)

How can anatomy contribute to understanding monocot evolutionary patterns?

## Floral anatomy and evolution in the Zingiberaceae and Costaceae

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The complex ovaries of the Zingiberales provide excellent material for the study of character evolution. Ovaries of the sister families Zingiberaceae and Costaceae can be an important source of taxonomic characters and may provide information for phylogeny determination. To explore these possibilities, taxa in the Zingiberaceae and Costaceae were selected for study based on their position in published phylogenies. Characters were defined by considering similar positions. The levels selected for analysis were below the locule, 1/4 of the way from the bottom to the top of the locule, in the middle of the locule, 3/4 of the way from the bottom to the top of the locule, and above the locule in the middle of the tissue that closes the ovary. Data from these sections was used to describe anatomical and morphological characters, including characters related to the structure of the locules, the placenta, placement of ovules, the type of trichomes, and several anatomical characters. The characters were plotted on a combined phylogeny of the Zingiberaceae and Costaceae constructed from published trees of these families. Though the majority of the characters turned out to be homoplasious or equivocal, several phylogenetically informative characters were discovered.

**Key words:** anatomy, character evolution, ovaries, phylogeny, Zingiberales.

## Floral development in Orchidaceae: evolutionary origin of the calyculus in Dendrobieae

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One gap in the understanding of orchid flowers is the origin and function of structures called calyculus scales (also calyculus, calycle, epicalyx, jugum). These scales, described for some genera of Epidendroideae – notably *Bulbophyllum* – are adnate externally to the ovary and perianth. To understand the origin and significance of these structures in Dendrobieae, we analyzed the floral development of *Bulbophyllum* (5 spp.), *Dendrobium* (4 spp. and 3 hybrids), *Liparis* (3 spp.) and *Stichorchis* (1 sp.) by scanning electron microscopy. The early floral development of all genera is similar, except for the lateral carpels of *Bulbophyllum*, which are congenitally fused. Although previously ignored, a calyculus develops in all studied genera, being more conspicuous in *Bulbophyllum*. The calyculus segments appear simultaneously, but earlier than previously described – after the perianth and before staminodia inception – and their position and developmental chronology do not support their interpretation as parts of an epicalyx. Instead, these segments seem to be related to the origin of the six-valvate capsular fruit, characteristic of the Epidendroideae

subfamily. We suggest that this fruit type is composed of three carpels with marginal placentation, as in Apostasioideae, but forming excrescences from the ovary or receptacle that later will originate the sterile valves.

**Key words:** capsule, *Dendrobium*, epicalyx, Epidendroideae, floral ontogeny.

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## **Anatomy of fleshy fruits in the monocots**

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An anatomical and developmental study of distantly related fleshy fruits in the monocots was undertaken to better understand the evolution of baccate fruits in the monocot clade as a whole. We studied 14 species with fleshy fruits spanning the Alismatales, Arecales, Asparagales, Commelinales, Dioscoreales, Liliales, and Poales to determine various mechanisms through which baccate fruits attain fleshiness at maturity. Flowers and fruits of various stages were collected, sectioned, stained, and examined using light microscopy and scanning electron microscopy. Three basic pathways for attaining fleshiness were identified within the species examined (true berries, with a uniform pericarp; typical drupes, with an endocarp differentiated by the presence of stony pyrenes; and specialized drupes, involving mesocarp and endocarp differentiated by stone pyrenes). Furthermore, developmental characters differentiating basic fruit types were identified. Fleshy fruits in the monocots do not develop through a single shared pathway, indicating that fleshiness has evolved multiple times within the clade.

**Key words:** berries, capsules, drupes, evolution, fruit development.

**Funded by:** Research and study funds were kindly provided by The New York Botanical Garden, Oberlin College Department of Biology, and the NSF grant From Acorus to Zingiber, Assembling the Phylogeny of the Monocots (DEB-082762).

## **Evolutionary history of the monocot gynoecium**

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Monocot gynoecia demonstrate a huge range of sizes and shapes, but their diversity is limited by morphogenetic correlations. The primary evolutionary achievement of monocots is septal nectaries. The nectariferous cells are located in the abaxial epidermis on the lateral and sometimes ventral sides of each carpel. The presence of septal nectaries co-occurs with postgenital carpel fusion (though in rare cases, carpels are free). Species with nectariferous areas on the lateral carpel flanks develop three separate nectaries. In cases of ventral nectariferous areas, a common triradiate nectary is formed. Internalization of the triradiate nectary can result from an invaginated receptacle or inferior ovary. Taxa lacking septal nectaries develop syncarpous gynoecia with congenitally united carpels. The morphogenetic basis for the strong positive correlation between septal nectaries and postgenital fusion is unclear. We speculate that de-differentiation of the epidermal cells, which occurs during postgenital fusion, could have preceded an evolutionary origin of septal nectaries. The presence/absence of septal nectaries is homoplastic in monocots. Maximum parsimony analysis

of the evolution of this character provides equivocal results, but Bayesian (rjMMC) analysis strongly favors a hypothesis that septal nectaries appeared once and were then lost many times during the course of monocot evolution.

**Key words:** development, evolution, flower, gynoecium, morphology.

**Funded by:** The work is supported by RFBR (grant 18-04-00797).

## Evolution of the inflorescences in monocots

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Branching pattern and development of inflorescences in Eriocaulaceae have been analysed. It could be shown that by different combinations of a very small set of basic branching and developmental patterns, highly complex inflorescences may result. While the traditional approach via an enrichment of the inflorescence allows and even requires intermediate forms, the fractioning of inflorescence meristems may lead to higher complexity without any intermediate stages. It could be shown that a large apex produces small leaves, while a small apex produces large leaves. This is a general pattern that applies for all cormophytes. These findings allow detection of the changes of meristem size during inflorescence formation even from a careful analysis of mature material. This may allow differentiation of inflorescences formed by condensation from those formed by meristem fragmentation. It can be shown that the homologies established applying a comparative approach are different if either the condensation or the fragmentation process is assumed.

**Key words:** inflorescences, trait evolution, meristem fractioning, ontogenetic abbreviation, pattern combination.

## III Symposium on Neotropical Araceae - Floristics, Morphology and Evolution

### Development and anatomy of reproductive organs of *Anthurium* (Araceae, Alismatales) and their implications for taxonomy

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The systematics of *Anthurium* is predominantly based on foliar morphological characters, with gaps regarding reproductive traits. Our recent studies, emphasizing floral aspects in representatives of different sections of the genus, have demonstrated the uniformity in many micromorphological and anatomical characteristics. Despite this uniformity, some characteristics such as the variation in the overlapping of outer tepals in the floral bud and in the sequence of exposure of the stamens, and the presence of osmophores in tepals are highlighted as mainly useful for specific characterization. It is worth emphasizing the importance of the carpellary vasculature in *Anthurium*. Although two distinct vascular patterns were previously reported, our data allow the addition of a third one, contributing to the characterization of the carpel of the genus in Araceae. More important than

highlighting the existence of these three patterns of carpellary vasculature in *Anthurium* is to emphasize the variation in terms of the origin of the dorsal and synlateral bundles. Carpels vascularized by synlateral bundles alone could be a plesiomorphy for *Anthurium* whereas the occurrence of dorsal bundles could be a derived character.

**Key words:** *Anthurium* sect. *Urospadix*, floral vasculature, micromorphology, ontogeny, osmophores.

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### **Taxonomy and evolution of *Philodendron***

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The evolutionary history of the “*Philodendron* clade” has been discussed in several recent papers, as well as the relationship among the three subgenera of *Philodendron* as independent lineages. The last common ancestor of *Philodendron* diversified from the Middle to the Late Miocene in the Amazon forest. *Philodendron* earliest divergence events would have taken place in a scenario of wetland expansion into western Central Amazonia, which fragmented the rainforest and formed extensive wetland. The Amazon was the exclusive biome occupied by *Philodendron* species during a period of 5.0–6.0 million years. Atlantic forest lineages of *P.* subg. *Meconostigma* and *P.* subg. *Philodendron* would have diverged from Amazonian ancestors. The results of the molecular phylogenetic studies by Loss-Oliveira et al. (2016) did not support the traditional division of *Philodendron* into three subgenera; on the other hand, the phylogenetic analysis by Vasconcelos (2015) recovered *P.* subgenus *Pteromischum* as monophyletic and sister to *P.* subg. *Philodendron*, as well as *P.* subg. *Meconostigma* as the first diverging lineage of the genus. Considering the recent papers on Araceae concerning the recognition of new genera based on molecular data, our results point to considering the subgenus *Meconostigma* as a distinct genus, resurrecting *Thaumatophyllum* Schott.

**Key words:** biogeography, diversity, flower, Philodendreae, phylogeny.

**Funded by:** CNPq, CAPES e FAPERJ

### **Repatriating Araceae taxonomic data from European collections to Brazil**

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Brazilian collections of Araceae were examined at the Royal Botanic Gardens Kew (K) and the Muséum National d'Histoire Naturelle, Paris (P) as part of Brazil's REFLORA project. The objective was to update determinations, verify specimens marked as types and label unmarked

types, to facilitate interpretation of these specimens via their images, now available online through the ReFlora Virtual Herbarium website. At Kew, 4010 specimens were examined, 202 redeterminations made and 15 types tagged with bibliographic references. In pre-1970 collections at K, there are 669 sheets of 26 genera, 196 species and 74 type sheets of Brazilian Araceae; most were received in three separate periods: 1821-1830, 1871-1890 and 1961-1970; the most important field collectors represented are W.J. Burchell (59 spms.), A.F.M. Glaziou (78), B.A. Krukoff (20), G.T. Prance (34), R. Spruce (20), E. Ule (32); important collections from cultivated material are from N.E. Brown (119) and A. Engler (48); Brown's specimens include drawings of K. Koch's destroyed Berlin types. In Paris, 499 specimens were examined, 386 determinations made and 36 types verified (20 isotypes, 1 isoepitype, 3 isoneotypes, 9 isosyntypes and 3 syntypes); new determinations included the genera *Anthurium* (233), *Caladium* (3), *Heteropsis* (8), *Monstera* (20), *Montrichardia* (6), *Philodendron* (92), *Spathiphyllum* (9), *Urospatha* (8), *Xanthosoma* (7).

**Key words:** Araceae, metadata, monocots, REFLORA, repatriation.

**Funded by:** Universidade Federal do Piauí - UFPI, Universidade Estadual de Feira de Santana - UEFS, Royal Botanic Gardens, Muséum National d'Histoire Naturelle, Paris and REFLORA for institutional and financial support (MCT/CNPq/FNDCT/MEC/CAPES/FAP's N° 56/2010).

### **Araceae from Minas Gerais, Brazil: Flora, endemism and conservation**

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Minas Gerais is the Brazilian state richest in species, both in general and in endemic species. Many areas have not been inventoried yet and recently three new species of *Anthurium* are being described. *A. narae* is known only from fragments of the Atlantic Forest in Carandaí, Minas Gerais state. From direct observations we estimate a total population size of ca. 100 individuals, forming small groups of about ten plants each. *A. caparaoense* is known only at localities in two municipalities of Minas Gerais state, in Caparaó National Park. From direct observations we estimate that it occurs in small populations, distributed in groups of 3–4 individuals. *A. erythrospadix* can be found in Espírito Santo and Minas Gerais states. From our direct observations we estimate a total population size of ca. 100 individuals, forming small groups of five plants each. Despite the intense human activity and the high level of forest fragmentation within its original area, new species are still being discovered in Brazil's Atlantic Forest, and this reinforces its global importance as a biodiversity hotspot and the constant need for its preservation in order that these and many other species do not disappear forever in the coming decades.

**Key words:** *Anthurium*, botanical collections, forest fragments, inventory of plants, new species.

**Funded by:** CNPq.

### **Heinrich Schott and the importance of Brazil for aroid systematics in the 19th century**

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The Austrian botanist H.W. Schott (1794-1865) founded modern Araceae taxonomy between 1832 and 1860. His formative experience, during which he encountered tropical Araceae for the first

time, was a four-year sojourn in Brazil (1817-1821). He rose to become Director of the Imperial Gardens of Austria and the unchallenged scientific authority of the Araceae. He assembled and cultivated an astonishing living collection of Araceae at the Schönbrunn Palace in Vienna and created an archive of nearly 4,000 pencil and coloured drawings of unparalleled accuracy and beauty which still inspire Araceae taxonomists. His most important scientific innovation was the analysis of aroid reproductive structures, the basis for a new delineation of aroid genera, presented fully in *Genera Aroidearum* (1858). Schott's comprehensive horticultural knowledge, Brazilian field experience and refined aesthetic sense made him an exemplary Humboldtian scientist. In 1859-1860, Archduke Ferdinand Maximilian of Austria made a journey to the Brazilian Atlantic Forest, where Court Gardener Franz Maly collected Araceae for Schott, later published as the magnificent *Aroideae Maximilianae* (1879). Schott's life work was the starting point for A. Engler's new evolutionary classification of the Araceae (1876), and his account of the Brazilian Araceae (*Flora Brasiliensis* 1878).

**Key words:** Aroideae Maximilianae, Austrian Expedition, Flora Brasiliensis, Genera Aroidearum, Schönbrunn Palace.

### III Symposium on Neotropical Araceae - Systematics and Evolution

#### **Just how diverse is the floral scent chemistry of *Philodendron*? A megadiverse Neotropical aroid genus and its association with pollinating scarab beetles**

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With almost 500 documented species, and twice as many estimated, *Philodendron* is one of the most successful genera of Neotropical aroids. From forests and wetlands to rock outcrops and open savannas, these evergreen herbs often represent a dominant element of the vegetation and their pollination is believed to be chiefly, if not exclusively, dependent on cyclocephaline scarab beetles (Melolonthidae, Cyclocephalini). Eight years after the floral scent composition of the first species was elucidated, the volatile organic compounds which mediate the association of *Philodendron* and their night-active pollen vectors are still only incipiently understood. Nonetheless, analyses of the exquisite floral fragrances of an ever-increasing number of species are unveiling a remarkable chemical diversity, often characterized by unique molecules assumed to be directly involved with the rapid radiation of the genus through selective pollinator attraction

**Key words:** *Cyclocephala*, *Erioscelis*, floral semiochemicals, nocturnal pollination, scent-oriented attraction.

#### **A new subfamily level phylogeny of Araceae using nuclear data from Anchored Phylogenomics**

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The monocot family Araceae has a large morphological and ecological diversity that has been under constant scrutiny, but phylogenetic relationships of some key taxa remain elusive. Using Anchored Phylogenomics we recovered an average of 358 nuclear regions for 25 taxa of Araceae with an average of 651 nucleotides per region. Additionally, we included 11 other taxa from Araceae plus *Acorus* available from public databases. The 37 samples represent 8 subfamilies, 38 of 44 clades from the last classification, and include the genera *Calla*, *Anubias*, *Calloopsis*, *Montrichardia*, *Philonotion*, *Zantedeschia* and *Pistia*. We used gene and species trees approaches and compared our results with previous plastome phylogenies. We found strong support for all the subfamilies, but differences in the position of Lasioideae. The genera *Calla*, *Anubias*, *Montrichardia* and *Calloopsis* are located in a transitional zone between bisexual and unisexual clades. Our hypothesis represents a new phylogenetic framework to study the role of floral, pollination, and ecological traits in aroid diversification.

**Key words:** Anchored Phylogenomics, Araceae, *Calla*, phylogeny, species tree.

### **Disentangling the evolutionary history of *Anthurium* (Araceae) using genomic scale data**

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The evolutionary history of the genus *Anthurium* Schott is characterized by a rapid and recent radiation of thousands of species, and therefore, it has been difficult to reconstruct fully supported phylogenetic relationships among them. In this study, we sequenced almost complete chloroplast genomes for 40 species of *Anthurium*, and compared the utility of several genomic regions for phylogenetic reconstructions in the genus. At least 20 chloroplast regions, the majority of them intergenic and intronic regions, could potentially be useful for phylogenetic reconstruction. However, at least 60 different chloroplast regions, including coding and non-coding, should be used to produce a phylogeny with enough support for all branches included. This is primarily due to the fact that a smaller number of phylogenetic informative characters are located in the chloroplast genome. Regarding the nuclear genome, a comparative study of two *Anthurium* transcriptomes identified hundreds of putatively orthologous, low-copy nuclear genes with sufficient phylogenetic information to resolve phylogenetic relationships in the genus. This set of target genes includes more than 3,500 exons (longer than 120 bps), comprising more than 900 nuclear genes (longer than 960 bps), for a total of almost two million protein coding bases available for phylogenetic analyses.

**Key words:** *Anthurium*, Araceae, chloroplast genomes, phylogenomics, transcriptomes.

### Linking macro- and microevolution in Bromeliaceae

#### **Linking macro- and microevolution in Bromeliaceae**

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Bromeliads are good models for understanding how the interplay among evolutionary processes such as gene flow, drift and selection influence species-level patterns of diversification (speciation and extinction) in Neotropics. In Bromeliaceae limited gene flow due to geographic isolation may



have influenced macroevolutionary patterns of diversity. Most species recognized in the family are endemic suggesting limited dispersal. Moreover, the few widespread species usually show morphological variation across their distributions, suggesting that intra-specific gene flow may not prevent divergence from drift or/and local adaptation. And finally, in several monophyletic bromeliad groups geographical proximity is a better indication of species relationships than morphological taxonomy. The same factors influencing population differentiation within a species are presumed to influence rates of species divergence over deep evolutionary time. However, geographic genetic structure does not always lead to speciation. Low effective population sizes may lead to high levels of local extinction, low efficacy of selection, and decreased speciation. Furthermore, recent studies suggest that inter-specific gene flow may promote diversification by reducing extinction rates via introgression of adaptive alleles. In this context, other forces such as population persistence, evolution of reproductive isolation and ecological divergence may also influence diversification at the species-level.

**Key words:** diversification rates, extinction, phylogenetics, population genetics, speciation.

**Funded by:** FAPESP, CAPES, CNPq

### **Bromeliaceae life history evolution and conservation implications**

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Bromeliad taxa differ in life history attributes, including lifetime attempts at sexual reproduction (a single vs. multiple times), and relative ability to produce asexual clones, or pups. The diversity of life history across the Bromeliaceae provides a rich model for comparative study, and how life history evolves and impacts conservation of various bromeliad taxa is unknown. The bromeliad research and horticultural communities also lack consistent understanding of life history terminology and a consistent life history type has not been ascribed to most taxa. Semelparity, or a single and subsequently lethal attempt at sexual reproduction via flowers with no pupping, has evolved repeatedly within the Bromeliaceae. The majority of taxa are iteroparous, producing inflorescences repeatedly across the lifespan of the genetic individual, but the number, timing, and anatomical origin of pups differ between taxa. The talk will overview models of life history evolution as applied to bromeliads. Ongoing experimental data from multiple exemplar taxa, and field data from semelparous and iteroparous *Tillandsia* taxa in Florida and semelparous *Puya raimondii* in Bolivia, will be discussed. Mathematical modeling utilizes these data to provide a framework to theoretically and quantitatively explore and test the evolutionary drivers of different life history strategies.

**Key words:** Bromeliaceae, evolution, life history, monocarp, semelparous.

### **Towards understanding the genomic substrate of diversification in *Tillandsia* subgenus *Tillandsia***

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The species-rich and ecologically diverse Bromeliaceae family provides an excellent system for studying adaptive radiation in Neotropical plants. The genus *Tillandsia* is the largest and most diverse within the family with over 600 species, extensive geographical range and a variety of ecological adaptations to different habitats such as epiphytism and various photosynthetic syndromes. Our research goal is to identify the genomic variability that provided the substrate for adaptive radiation within the enigmatic subgenus *Tillandsia* of genus *Tillandsia* using tree-, network, and coalescent-based approaches. To that end, we used whole-genome sequencing data from selected species within a taxonomic clade (Core Group I, also known as ‘The Mexican clade’) to construct phylogenomic trees and networks and infer species delimitation. To shed light on the genomic processes that gave rise to diversity within the clade, we performed tests for reticulation, introgression and post-speciation gene flow. We will present our first results, describe the genomic tools used for inference and outline future prospects towards understanding of genomic processes at micro- and macro- scales. This research project provides a unique case study for emerging research on the evolutionary genomic underpinnings of plant species radiations in the neotropics.

**Key words:** adaptive radiation, introgression, phylogenomics, reticulation, *Tillandsia*.

### **Circumscription, diversity, and evolution of *Tillandsia* subg. *Tillandsia* based on a multi-locus DNA phylogenetic approach.**

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*Tillandsia* subg. *Tillandsia* is the largest subgenus within the genus, comprising great morphological variability and ecological distribution. It is diagnosed by having exerted stamens and style, with naked petals. However, previous studies have shown that these characters are homoplastic. The aim of this study is to assess the phylogenetic relationships of *T.* subg. *Tillandsia*, representing its full geographical range and morphological variation. We used four DNA markers, two nuclear (*PhyC*, *PRK*) and two from the plastid genome (*ycf1* and *matK-trnK*), and indels. Bayesian inference, Maximum Parsimony and Bootstrap analyses were performed. The genus *Tillandsia* was supported as monophyletic (including *Viridantha* and *Pseudovriesea*) in a sister relationship with *Pseudalcantarea*. The *Tillandsia multicaulis* group is confirmed as not related to *T.* subg. *Tillandsia*. The remainder of the subgenus is paraphyletic. Three large groups can be recognized: the *T. secunda* and *T. paniculata* groups (mainly from South America and the Antilles), and a Mexican (mostly) clade, inside which several groups are recovered: *T. utriculata* s. l. clade; *T. circinnatoides* clade (xerophytic Mexican species); Southern Mexico Highlands clade; *Allardtia* clade, and finally a large unresolved clade, in which many representative species complexes are found, including *T. capitata*, *T. fasciculata*, *T. juncea* (*pro parte*) and *T. ionantha*.

**Key words:** biogeography, Neotropics, phylogeny, *Tillandsia*, Tillandsioideae.

## Insights about the evolutionary history of species of *Aechmea* subgenus *Ortgiesia* from southern Brazil

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The study of microevolutionary processes clarifies how speciation and extinction took place at the population level. A great opportunity to understand these processes is provided by studying closely related but geographically restricted taxa. We investigated the evolutionary history of seven species of *Aechmea* subgenus *Ortgiesia* endemic to southern Brazil. We combined data from chloroplast and nuclear markers and applied phylogeographical and population genetic approaches to develop the study. The origin of the seven species was dated to the late Pliocene, with most of the lineages diversifying in the early Pleistocene. The genetic structure found suggests the taxa had their geographical distribution fragmented during the Pleistocene, surviving in multiple refugia. This fragmentation may have contributed to the genetic differentiation observed for *A. blumenavii*, *A. calyculata*, and *A. kleinii*. For *A. calyculata*, the advance of grassland over Atlantic forest during the Pleistocene was an important barrier to gene flow among populations. The remaining four species showed less genetic structure, which was attributed to incomplete lineage sorting and hybridization. Indeed, hybridization was confirmed as occurring among *A. caudata*, *A. comata*, and *A. kertesziae* where they are sympatric. This group of seven species presents a complex evolutionary history with a speciation process with gene flow.

**Key words:** Atlantic rainforest, diversification, molecular markers, phylogeography, speciation.

## Whole-genome and transcriptome signatures of diversification in bromeliads: crossing the micro / macro divide

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Bromeliads (Bromeliaceae) represent an ecologically important and intensively studied adaptive radiation among Neotropical plants. Understanding patterns, drivers, and limits of plant diversification and radiation is greatly facilitated by analytical approaches from ecological and evolutionary genomics and related “-omics” - sciences, embedded within a systems biology framework. Bromeliads are well suited for this type of research, because they offer a great diversity of species (>3000), numerous interesting shifts in functionally important adaptive traits, and abundant genome and transcriptome resources, compared to most other Neotropical groups. We have addressed the genetic underpinnings of diversification in two different groups of Bromeliaceae within highly collaborative research efforts, based on combinations of whole genome and transcriptome sequencing and molecular phenotyping of selected taxa. This includes research to understand the genomic substrate of adaptive radiation in tillandsioid bromeliads (genus *Tillandsia* and related genera), and research to understand genomic patterns and drivers of diversification in the genus *Ananas*, which includes the cultivated pineapple. Selected results from this work will be

presented. We will place special emphasis on aspects of relevance for evolutionary biologists and systematists wishing to tackle patterns and processes across micro- and macro-evolutionary time scales.

**Key words:** adaptation, phylogenomics, population genomics, radiation, speciation.

**Funded by:** Swiss National Science Foundation (SNF) and University of Vienna

### **Biogeographic history and macroevolutionary patterns in the core-Tillandsioideae with a focus on *Vriesea***

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Bromeliads are an iconic group of Neotropical plants that represents a well-studied case of adaptive radiation triggered by the appearance of specific traits such as the tank-habit or CAM photosynthesis. The large diversity of Bromeliads combined with the low rate of molecular evolution has complicated phylogenetic studies and no species-level phylogeny is available for the largest genera. Because of this limitation, the macroevolutionary dynamic shaping bromeliad diversity at shallower levels such as subfamilies or genera remains unknown. Here, we use a large dataset of 122,664 SNPs obtained from genome skimming to reconstruct the first time-calibrated phylogeny of *Vriesea* with more than half of *Vriesea* species included. We analysed the dynamics of diversification in *Vriesea* to better understand the factors that contributed to the emergence of its species richness. We combined these diversification analyses with biogeographic reconstruction to place the macroevolutionary processes within the geographic context where these species occur. Our results highlight the opportunity offered by genome-wide sequencing approaches to provide informative data for robust phylogenetic inferences. These data are essential to enable the investigation of the macroevolutionary processes driving the evolution of large and complex plant groups such as the genus *Vriesea*.

**Key words:** Atlantic forest, biogeography, Bromeliads, diversification, genome skimming.

## Monocot phylogenomics I

### **Genome evolution in heterotrophic orchids**

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Heterotrophy evolved approximately 30 times within the orchids, likely due to their mode of parasitizing fungi at the initial stages of development. Thus, orchids are an ideal clade in which to study the genomic and physiological consequences of shifting from autotrophy to heterotrophy. In this symposium I will discuss: 1) the biology of orchids and evolution of heterotrophy, 2) the extensive findings on plastid genome evolution in heterotrophic orchids, and 3) the less extensive findings on the evolution of mitochondrial and nuclear genomes in these plants. I will use two well-

sampled case studies, the North American orchid genera *Corallorhiza* and *Hexalectris*, and place these in the broader context of the evolution of heterotrophy in plants. Plastomes of fully mycoheterotrophic orchids range from some of the smallest known in land plants (e.g., *Epipogium*, *Gastrodia*) to those barely differing from photosynthetic close relatives. Specifically, case studies in *Corallorhiza* and *Hexalectris* reveal that the number of photosynthetic losses in orchids is greater than previous estimates indicate, and that plastome degradation can be traced to the population level. Transcriptomes of mycoheterotrophic *Corallorhiza* indicate loss of photosynthesis, expansion of gene families involved in fungal host manipulation, and reduced expression of genes involved in genomic maintenance.

**Key words:** fungi, photosynthesis, plastid genome, pseudogene, transcriptome.

## **Phylogenomics and comparative organellar genomics of monocot mycoheterotrophs and their green relatives**

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Multiple lineages of monocots independently have switched to the “dark side” of plant life: in place of photosynthesis they rely on soil-associated fungal partners for some or all of their carbon budget. Mycoheterotrophy is particularly common in monocots, and independent lineages of “full mycoheterotrophs” (completely non-photosynthetic lineages) arose in five monocot orders: Asparagales, Dioscoreales, Liliales, Pandanales and Petrosaviales. The morphology of some mycoheterotroph lineages has become so highly modified that until recently systematists struggled to place them in higher-order phylogeny and classification. Mycoheterotrophy has had a considerable impact on organellar genome evolution, particularly the plastid (“chloroplast”) genome. This complicated molecular systematic studies, especially for lineages with substantial gene loss (photosynthetic and other genes) and massively elevated rates of evolution. The mitochondrial genomes of mycoheterotrophs also show evidence of rate elevation. Nevertheless, both organellar genomes allow robust placement of the major monocot mycoheterotroph lineages in monocot phylogeny, summarized here. We also review how loss of photosynthesis has impacted the molecular evolution of the plastid genome, in terms of gene content, genome structure, and the selective regime that different genes evolve under. Finally, we relate patterns of gene loss to recent models of plastome evolution in heterotrophic plants.

**Key words:** extreme phylogenomics, heterotrophs, mitochondrial genomes, non-photosynthetic monocots, plastome structure and evolution.

**Funded by:** NSERC (Natural Sciences and Engineering Research Council of Canada)

## **Inferring the phylogenetic history of Pontederiaceae based on a taxon-dense phylogenomic analysis**

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Pontederiaceae (Commelinales) are a small family of aquatic herbs with showy flowers, comprising ~six genera distributed mainly in the Neotropics. Previous phylogenetic studies involved a few plastid markers and nuclear genes and focused on the diversity of pollination and mating strategies in the family. They employed a subset of representative taxa and left several questions about the phylogenetic history of Pontederiaceae unresolved, including the position of the root of the family and details of intrafamilial relationships. Here, we use shotgun sequencing to recover 82 plastid genes for phylogenomic inference using ~37 of the ~42 species in the family. We confirm the monophyly of Pontederiaceae, *Pontederia* and *Monochoria*; however, *Eichhornia* is polyphyletic and divided among three to four clades, and *Heteranthera* is paraphyletic as the African *Scholleropsis* is phylogenetically nested within it. The position of *Hydrothrix gardneri* remains partly unresolved, as it is either retrieved as the sister group of *Heteranthera* or is nested within it. Plastome-based inferences divide Pontederiaceae into two major and well-supported clades, one including *E. crassipes*, *Monochoria*, the Azurea group of *Eichhornia* and *Pontederia*, and the other including *E. meyeri*, the Paniculata group of *Eichhornia* and *Heteranthera s.l.*

**Key words:** aquatic monocots, multi-gene analyses, next generation sequencing, phylogenetic inference, plastid phylogenomics.

### A nuclear phylogenomic view of monocot diversification

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Monocots are a monophyletic group within angiosperms that comprises 11 orders, 77 families (APG IV) and nearly 70,000 species. Whereas there have been great advances in understanding monocot phylogenetics in recent years, resolution of relationships among some orders and families have remained elusive. In order to better understand relationships among monocot families and orders, The Monocot Tree of Life consortium has generated transcriptome assemblies for 80 species and compiled genome and transcriptome sequences from over 120 additional species including representatives of all but three monocot families (Blandfordiaceae, Corsiaceae, and Maundiaceae). OrthoFinder clustering of protein coding genes from available monocot genomes resulted in identification of over 600 predominantly single copy genes that could be used for species tree estimation. Species tree analyses are ongoing, but preliminary analyses provide support for a Liliales + Asparagales clade as sister to the commelinids. Extensive gene tree discordance and poor resolution of relationships among major commelinid lineages (Arecales, Commelinales + Zingiberales, and Poales) suggests rapid radiation. Our comprehensive phylogenetic findings will be discussed along with their implications for understanding monocot trait and genome evolution.

**Key words:** evolution, genomics, phylogeny, systematics, transcriptomics.

### Evolution of hyperdiverse Dendrobieae in time and space – phylogenomic insights

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Tribe Dendrobieae (Epidendroideae) includes two of the five largest orchid genera, *Dendrobium* Sw. and *Bulbophyllum* Thouars, which comprise over 1,500 and 1,800 mostly epiphytic species, respectively. The high species diversity together with the extraordinary morphological and ecological diversity of both genera has rendered the understanding of evolutionary relationships, systematics, and spatio-temporal evolution of Dendrobieae challenging. With the advent of molecular systematics significant progress has been made over the past two decades, however phylogenetic inferences have mainly relied on few molecular markers yielding limited resolution and support within this hyperdiverse orchid group. For this study, plastome data for 252 samples was generated via shotgun high-throughput sequencing, representing 90% and 48% of the sections in *Dendrobium* and *Bulbophyllum*, respectively. Phylogenetic tree inferences were carried out in RAxML and MrBayes based on 75 plastid protein-coding regions. Divergence time estimations were conducted using fossil and secondary calibration points in BEAST. Ancestral range estimations were carried out based on the BEAST chronograms applying the dispersal-extinction-cladogenesis model. This phylogenomic study yielded highly resolved and well-supported phylogenetic reconstructions allowing novel insights into higher-level evolutionary relationships and systematics in Dendrobieae and their evolution in time and space.

**Key words:** *Bulbophyllum*, *Dendrobium*, historical biogeography, Orchidaceae, phylogenomics.

## Comparative analysis of genome evolution across Monocots

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Our understanding of genomic diversity in monocots has increased greatly over the last five years. Though the first monocot genomes were members of the grass family and more whole genome sequences are found in Poaceae than any other monocot family, a wider breadth of monocot lineages now have whole genome sequences including Alismatales, Asparagales, Zingiberales, Arecales, and non-grass Poales. With large-scale transcriptome projects being completed by oneKP and the Monocot Tree of Life consortium, all monocot orders and most families now have genome-scale data available. Using a combination of whole genomic and transcriptomic data encompassing 74 of out 77 families (APG IV), we investigated the presence of whole genome duplication (WGD) across the monocots, identifying multiple instances deep in the monocot phylogeny. With this highly representative data set, we are able to phylogenomically place the *tau* event prior to the divergence of Petrosaviaceae from other monocots but after the divergence of Alismatales. Furthermore, we identify and place multiple events across orders. We assess the impact of WGD on genome composition looking at the GC profile of protein-coding sequences and gene family

composition. Finally, we discuss implications of this research on our understanding of plant genome evolution.

**Key words:** comparative genomics, genome evolution, phylogenomics, transcriptomics, whole genome duplication.

### **Monocot plastid phylogenomics, timeline, net rates of species diversification, the power of multi-gene analyses, and a functional model for the origin of monocots**

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We present the first plastome phylogeny encompassing all 77 monocot families, estimate branch support, and infer monocot-wide divergence times and rates of species diversification, based on 77 plastid genes across 545 monocots and 22 outgroups. Phylogenomic analyses shift the placement of 16 families relative to earlier studies, add seven families, date the divergence between monocots and eudicots+*Ceratophyllum* at 136 Mya, successfully place all mycoheterotrophic taxa examined, and support recognizing Taccaceae and Thismiaceae as separate families and Arecales and Dasypogonales as separate orders. Net species diversification underwent four significant, large-scale accelerations, each associated with specific ecological/morphological shifts. Branch ascertainment and support increase with gene number and branch length, and decrease with relative branch depth. An analysis of entire plastomes in Zingiberales shows how non-coding regions increase branch ascertainment and support. Our results provide the first well-supported monocot phylogeny and timeline spanning all families, and quantify the contribution of plastome-scale data to resolving short, deep branches. We outline a new functional model for the evolution of monocots and their diagnostic morphological traits from submersed aquatic ancestors, supported by convergent evolution of many of these traits in aquatic Hydatellaceae (Nymphaeales).

**Key words:** aquatic origin, diversification rate, functional model for the origin of monocots, monocot plastid phylogenomics, monocot syndrome, net rates of species diversification, the power of multi-gene analyses, timeline, Zingiberales.

### Monocot Phylogenomics II - new insights on genome evolution, diversification and biogeography

#### **From tree tops to the ground: reversals to terrestrial habit in *Galeandra* orchids (Epidendroideae: Catasetinae)**

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The colonization of the epiphytic niche of neotropical forest canopies played an important role in orchid's extraordinary diversification, with rare reversions to the terrestrial habit. To understand the evolutionary context of those reversals, we investigated the diversification of *Galeandra*, a Neotropical orchid genus which includes epiphytic and terrestrial species. We hypothesized that reversion to the terrestrial habit accompanied the expansion of savannas. To test this hypothesis, we generated a comprehensive time-calibrated phylogeny and employed comparative methods. We found that *Galeandra* originated towards the end of the Miocene in Amazonia. The terrestrial clade originated synchronously with the rise of dry vegetation biomes in the last 5 million years, suggesting that aridification dramatically impacted plant diversification and habits in the Neotropics. Shifts in habit impacted floral spur lengths and geographic range size, but not climatic niche. The longer spurs and narrower ranges characterize epiphytic species, which probably adapted to specialized long-tongued Euglossini bee pollinators inhabiting forested habits. The terrestrial species present variable floral spurs and wider distribution ranges, with evidence of self-pollination, suggesting the loss of specialized pollination system and concomitant range expansion. Our study highlights how climate change impacted habit evolution and associated traits such as mutualistic interactions with pollinators.

### **Phylogenomics of Australian (non-Orchidaceae) Asparagales**

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Asparagales comprise a third of monocots (14 families, 1,122 genera, ca. 36,205 species) and have the highest diversification rate among monocots. Despite the high diversity of “non-orchid” Asparagales in Australia (9 fam.; 48 gen.; ca. 327 spp.), phylogenetic studies to-date have included only limited sampling of native Australian taxa. The order has the greatest range of genome sizes, 0.3 – 82.2 pg, among angiosperms and has undergone massive genome and chromosome evolution. As a result of the lack of dense sampling of Australian taxa for phylogenetics little is known about the monophyly of genera and species diversification. Our study sought to: i) infer phylogenetic relationships, ii) estimate divergence times of major clades, and iii) investigate plastid genome evolution for Australian lineages. All native Australian genera are included in this study and are represented by 200 individuals. Genome skimming techniques and HTS were used to generate sequence data. Whole chloroplast genomes were de-novo assembled, mapped to reference, and aligned in Geneious. The maximum likelihood phylogeny supports monophyly of Asparagales subfamilies. Here we present improved understanding of relationships within Lomandroideae, Asphodelaceae, and of Boryaceae and Orchidaceae relationships. Survey of genome sizes showed heterogeneity among Australian lineages reflecting polyploidy in some taxa.

**Key words:** divergence times, genome size, Lomandroideae, plastid, polyploidy.

## **Anchored hybrid enrichment resolve a species complex derived from recent rapid diversifications**

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Most phylogenetic studies in the Orchidaceae are based on inferences from the sequencing of few molecular markers. However, these inferences usually do not solve recalcitrant nodes in phylogenies mainly in species complexes of recent and rapid diversifications. To tackle this problem, new sequencing techniques such as Anchored Hybrid Enrichment (AHE) allow the obtaining of hundreds of loci, thus increasing the amount of information analyzed. This technique has been evaluated in several plant groups yielding better resolution to recalcitrant nodes in phylogenies. In addition, these studies revealed high levels of discordance and conflicting topologies due to biological phenomena (i.e. hybridization, deep coalescence) or systematic/stochastic errors. In order to test the performance of the AHE datasets in a species complex of the genus *Lepanthes*, we conducted gene/species tree and network inferences together with coalescent-based methods. We obtained a fully resolved phylogeny but also found high discordance among individual gene trees and paraphyly in the grouping of haplotypes. This might indicate that ancient hybridization, polyploidy and/or incomplete lineage sorting may have contributed to speciation in *Lepanthes*. These analyses also revealed two undescribed species. Our study shows that only with a large number of phylogenetic markers it possible to disentangle cryptic taxa in species complexes and morphological traits evolving in parallel or convergently.

## **Changing tribal and generic concepts in Cyperaceae, new insights from phylogenomics**

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Despite recent advances in molecular phylogenetics, deep evolutionary relationships in Cyperaceae are not resolved. Reduction of floral morphology and complex inflorescences pose difficulties to unravel relationships based on morphology. One of the most phylogenetically informative structures

in Cyperaceae is embryos. The utility of embryo characters and types in Cyperaceae systematics has been reviewed in a molecular phylogenetic context using a DNA supermatrix incorporating sequences from five plastid (*matK*, *ndhF*, *rbcL*, *rps16*, *trnL-F*) and two nuclear ribosomal (ETS, ITS) regions. The phylogenetic hypothesis presented includes the most extensive sampling of Cyperaceae to date. Fourteen qualitative morphological embryo characters were coded, ancestral state reconstructions were performed, and the embryo of each sampled genus was classified in a typological system based on key morphological features. Embryo morphology provides a valuable source of independent data for Cyperaceae systematics that can be used to place species with unknown affinities, when molecular data is not available, or when results of analyses are inconclusive or conflicting. This work is now being compared with the first generic-level phylogenomic tree generated using the angiosperm-wide bait kit developed for Kew's PAFTOL programme. Together, these efforts will allow generating a new molecular-based classification for the family.

**Key words:** Cyperaceae, embryo, evolution, PAFTOL, phylogenomics.

**Funded by:** Plant and Fungal Trees of Life (<https://www.kew.org/science/who-we-are-and-what-we-do/strategic-outputs-2020/plant-and-fungal-trees-life>)

### **The Asphodelaceae tree of life: a phylogenomic evaluation of relationships among genera**

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The family Asphodelaceae comprises 41 genera and approximately 1,000 species in three subfamilies: the predominantly African Asphodeloideae; Eurasian and Australian Hemerocallidoideae; and the Australian Xanthorrhoeoideae. The family has undergone multiple nomenclatural changes in recent years, with the conservation of the name Asphodelaceae over Xanthorrhoeaceae, circumscription of multiple segregate genera among the alooid taxa in subfamily Asphodeloideae, and the recognition of *Chamaescilla* in subfamily Hemerocallidoideae, a genus previously included in Asparagaceae subfamily Lomandroideae. To assess generic relationships as they are currently circumscribed in Asphodelaceae, we generated genomic data for all 41 genera using a HybSeq approach with a universal angiosperm probe set applied in the Plant & Fungal Tree of Life (PAFTOL) project, targeting 353 low copy nuclear genes. We extracted the coding sequences and introns from high-throughput sequencing reads using the HybPiper pipeline and generated a species tree with Astral on our set of unrooted gene trees. The resulting tree provides phylogenetic evidence to support many accepted generic relationships within the three subfamilies, and critically evaluate those relationships that have hitherto remained unclear.

**Key words:** Asphodelaceae, PAFTOL, phylogenomics, systematics, taxonomy.

### **Phylogenomic analyses of hundreds of nuclear loci and plastomes yield new insights on orchid diversification**

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High-throughput sequencing methods have made it possible to access hundreds of loci for evolutionary studies in plants. Nevertheless, evolutionary relationships in the iconic and species-rich orchid family (c. 25,000 species) are still mostly inferred using sequence data from plastid loci and relatively limited taxon sampling. Thus, while such analyses have been used to provide the phylogenetic framework for understanding orchid macroevolutionary dynamics, they are based on limited data. Here, we have produced the most extensive phylogenomic framework for the orchid family to date. This was achieved by first sequencing 353 nuclear genes and partial plastid genomes (Angiosperms 353 universal probe set) in 410 orchid species. We then used this phylogenomic framework to constrain the species tree hypothesis inferred from a DNA sequence matrix made up of four plastid loci and one nuclear ribosomal marker sequenced for 2,500 orchid species and representing ~10% of orchid species diversity. Our combined multi-locus approach supports many of the previously recovered orchid relationships, but it also reveals distinct phylogenetic positions for several orchid genera compared to previous results. Our new phylogenomic framework unveiled the relationships of previously unplaced orchid clades, including insights on the origin and evolution of the orchid family.

**Key words:** bioinformatics, diversification, historical biogeography, next-generation sequence data, Orchidaceae.

## Completing the Plant Tree of Life

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The Plant and Fungal Trees of Life (PAFTOL) project at the Royal Botanic Gardens, Kew, uses high-throughput DNA sequencing technology to generate extensive new data for at least one species of every genus of plant and fungi. Here, we report on progress in the plant component of PAFTOL. We have established a targeted sequence capture (HybSeq) approach and designed a single probe (bait) kit that isolates up to 353 nuclear genes across all angiosperm families. Data obtained with this kit effectively resolve both deep and species-level relationships and are currently being evaluated as a “next generation” barcode. The kit is publicly available and is being adopted by numerous researchers. It is effective with degraded herbarium DNA from specimens up to ca. 200 years old. A refined bioinformatic pipeline is also in preparation. We have already sequenced at least one representative of every angiosperm family and we now aim to generate data for 25% of the 14,000 angiosperm genera within the coming year. Focused studies on families such as orchids, palms, and sedges are also underway. PAFTOL aspires to be highly open and collaborative, and researchers who share an interest in our project are warmly invited to get in touch.

**Key words:** angiosperms, museomics, next generation sequencing, phylogenomics, Tree of Life.

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Monocot mats on Gondwanan inselbergs: binding taxonomy, ecology and molecular aspects under a biogeographic view

### **Community ecology of monocot mats on Brazilian inselbergs and remarks on microsatellites in *Vellozia* species**

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Inselbergs are isolated outcrops of resistant rock that rise above the surrounding landscape as terrestrial islands. Monocot mats are the most conspicuous habitat for which it is possible to notice major differences in vegetation composition and structure throughout the tropics. Five families of angiosperms are typical mat-formers: Bromeliaceae, Cactaceae, Cyperaceae, Poaceae and Velloziaceae. It is commonly assumed that stochastic variation in colonization and establishment is a dominant driver of differences in plant assemblages developing on similar inselbergs. However, we found that climate is a dominant driver of variation in mat communities explaining more than 40% of variation between inselbergs in Southeastern Brazil. In these stable mats, climate results in strong species turnover with species replacement. With finer taxonomic resolution the relative importance of detectable deterministic processes increased, suggesting that genera contain species adapted to different climates. Studies on Neotropical inselberg-adapted species (e.g. bromeliads and vellozia), using microsatellite marker approaches, showed high population differentiation, high genetic diversity levels and strong phylogeographic structure in this naturally fragmented environment. However, based on our results, high isolation and low ongoing gene flow does not mean that there cannot be deterministic sorting of species along environmental gradients driven by rare historic dispersal events.

**Key words:** Atlantic Forest, beta diversity, community assembly, gene flow, rock outcrops.

**Funded by:** CNPq/DAAD-290155/2014-2

### **Metabolomics of Velloziaceae species and its evolutionary relationship with desiccation tolerance**

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Inselbergs are isolated rock outcrops that contain a peculiar flora, with high diversity and degree of endemism. Inselbergs are accompanied by extreme conditions and frequently exposed to severe water deficit to which very few species are adapted. Velloziaceae is one of the families found in Inselbergs and species of *Barbacenia* genus are examples of adapted plants to these environments. To deal with extreme situations strategies have been evolved, such as those that confer desiccation tolerance (DT). DT is the ability of a plant to cope with severe water loss and subsequent rehydration of their tissues, recovering their normal cell metabolism. To investigate this behavior in

the Brazilian flora, desiccation tolerant *Barbacenia* species collected from distinct areas were compared considering their morphological and anatomical aspects and were analyzed in a desiccation/rehydration experiment under controlled conditions to investigate physiological and biochemical features. Plants generally exhibit similar anatomical and morphological patterns, however differ in their biochemical and physiological strategies. Coordinated events such as photosynthetic rate control and non-enzymatic antioxidant system are two important differences among the DT species. As a conclusion, although belonging to the same genus, *Barbacenia* species show different strategies to survive in hostile conditions such as those found in inselbergs.

**Key words:** antioxidant system, *Barbacenia*, inselberg, osmoprotectors, resurrection plants.

**Funded by:** Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP 2012/16332-0 and 2012/23838-7)

## Genetic differentiation patterns of a desiccation-tolerant Velloziaceae on Malagasy inselbergs

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Only few plant species possess suitable sets of adaptive traits that enable them to survive irregular water supply or long drought periods. Here, desiccation-tolerant vascular plants like the Velloziaceae (Pandanales) are prominent. This monocot family forms a phytogeographical link among African, Malagasy and South American inselbergs and other rock outcrops, on which Velloziaceae species comprise important floral elements. Morphologically, Velloziaceae are often polymorphic at the intraspecific level, making the delimitation of taxa difficult. We hypothesize that isolated inselbergs could be a driver of population differentiation and ultimately speciation in Velloziaceae. Studies on the genus *Vellozia* in Brazil already supported this view by showing that rates of genetic exchange between inselbergs can be very low. So far little is known about the population structure of the Malagasy species of the genus *Xerophyta*. To evaluate the significance of inselbergs in terms of genetic differentiation patterns among these *Xerophyta* species, we developed versatile microsatellite DNA markers and used them to genotype a small set of *Xerophyta* populations from different inselbergs. Our preliminary results indicate low levels of genetic exchange, as expected. Our current research aims at elucidating the genetic diversity and population structure of *Xerophyta* species on Madagascar on a broader scale.

**Key words:** inselbergs, Madagascar, microsatellites, population genetics, *Xerophyta*.

## Diversity and endemism of monocots in Pre-Cambrian rock outcrops (inselbergs) of the Colombian Amazonia

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Pre-Cambrian inselbergs are insular and azonal habitats. Extreme edaphic conditions and the insularity of these sites have probably acted as powerful selective forces, generating a characteristic biota low alpha diversity, but with medium-high beta and gamma diversities, and a high endemism. The present study was undertaken as part of an inventory of Colombian plants. In Colombian

inselbergs the monocots are represented by 301 species, 131 genera and 22 families. The families with the highest number of species are Poaceae (100 species/45 genera/5 endemic species), Bromeliaceae (37/13/14), Cyperaceae (36/15/1), Xyridaceae (26/3/4), Eriocaulaceae (22/5/0), Rapateaceae (20/7/3) and Orchidaceae (18/15/1). The most speciose genera are *Xyris* (17 species), *Paspalum* (16), *Panicum* (15), *Axonopus* (13), *Navia* (11), *Syngonanthus* (11), *Cyperus* (8), and *Paepalanthus* (7). Thirty-eight genera and 49 species are endemic to inselbergs. Fifteen species are reported for the first time for Colombian flora (Cyperaceae, Eriocaulaceae, Poaceae, Xyridaceae), and two species are new to science (Poaceae, Xyridaceae). Biogeographical analyses support the hypothesis that the Colombian inselbergs belong to the Guayana Region rather than the Amazonian Region. Further knowledge of this habitat type is essential for conservation planning.  
**Key words:** Colombian Amazonia, Colombian Guayana, flora of Neotropical Pre-Cambrian rock outcrops, floristics of inselbergs, Neotropical monocots.

**Funded by:** Universidad Nacional de Colombia, Bogotá D. E., Colombia.

### Desiccation-tolerant vascular plants on inselbergs: a global perspective

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Granitic and gneissic inselbergs form old (i.e., millions of years) and isolated landscape elements that are particularly widespread in the tropics. They are characterized by harsh environmental conditions (e.g., high temperatures, prolonged droughts) and harbour a considerable number of specifically adapted plant species. Particularly prominent are desiccation-tolerant vascular plants that tolerate the loss of their tissue water over long periods (i.e., weeks or months). Ferns represent the largest group of “resurrection plants” (e.g., many Aspleniaceae and Pteridaceae). Within angiosperms monocots clearly outnumber dicots with Velloziaceae (more than 150 spp.) and Poaceae (e.g., *Microchloa*, *Oropetium*, *Tripogon*) being the largest families that contain desiccation-tolerant species. Inselbergs in Brazil and Madagascar are extraordinarily rich in desiccation-tolerant vascular plants.

**Key words:** Brazil, inselbergs, Madagascar, Poaceae, Velloziaceae.

### Monocots in society and tools to spread knowledge about monocots

#### How to get your paper accepted (or rejected) for publication: lessons from two editors

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Are you mystified by the scientific publication process? Two editors will share common mistakes made by authors and advice on how to increase your chances of publishing in international botanical journals. Topics covered will include how to prepare your work for publication before you write your manuscript, including reading the relevant literature and designing your research to address a significant question; how to choose a journal that matches the topic and scope of your research; how to use the Instructions to Authors and recent papers in your field as a model for how to structure your manuscript and figures; how write a manuscript for publication in a language other

than your own, including choosing an editorial service to review your manuscript before submission; how to avoid common language errors, including the misuse or over-use of terminology; and how to gain first-hand knowledge of the review process and of the quality of the manuscripts that are accepted for publication by volunteering to review for journals in which you hope to publish. You will leave with a better understanding of the publication process.

**Key words:** editors, journals, manuscripts, publication, research.

## **Visual learning: Plant identification and beyond**

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Learning to identify plants is extraordinarily difficult. This leads to what has been called plant blindness, the inability to see plants in the environment. Despite this difficulty, many botanists quickly and easily make identifications at a glance. They do this without the use of characters or keys. This talk will present a method for teaching instant recognition and overcoming plant blindness. This is accomplished using a cognitive psychology-based computer program. The program incorporates presentation of plant images in an active-learning format that was developed to stimulate expert-level visual recognition. In a controlled experiment the program resulted in up to a 25% improvement in exam scores. The software demonstrates how the use of interleaved examples, spaced repetition, and retrieval practice (SPRINT) can be used to train identification of complex and highly variable objects. The software is open source, and easily customizable. A four-minute introduction is at [https://youtu.be/lfoeldV-\\_94](https://youtu.be/lfoeldV-_94). A longer introduction, including instructions on how to add images is at <https://youtu.be/qMq0n8q8B8Q>. The source code and executables are at <https://github.com/Jasig/ImageQuiz/releases>. Related programs for teaching plant life cycles and plant structure terms will also be presented.

**Key words:** education, identification tools, plant identification, software, visual learning.

## **A worldwide phylogenetic classification of the Poaceae for the herbarium**

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The United States National Herbarium (US) in the National Museum of Natural History's Department of Botany houses a historic collection of approximately ½ million grass specimens of worldwide importance including 13,142 types (as of 22 May 2018). The grass collection at US is being reorganized using our new phylogenetic classification of 11,506 grass species in 770 genera, 12 subfamilies, 52 tribes, and 94 subtribes (Soreng et al. 2017; <http://onlinelibrary.wiley.com/doi/10.1111/jse.12262/full>). We assigned generic numbers from 1 to 770 based on their phylogenetic placement in our radial tree beginning with Anomochlooideae, Pharoideae, and Puelioideae, then the Oryzoideae, Bambusoideae, and Pooideae (BOP), and finally the Aristidoideae, Panicoideae, Arundinoideae, Micrairoideae, Danthonioideae, and Chloridoideae (PACMAD). Within each subfamily we ordered our genera alphabetically in their lowest rank (usually a subtribe). More recent changes in the subfamily placement and new genera are given a number placed in alphabetical sequence in their subtribe (or higher rank, if subtribe is unknown) and a letter (B, etc.). At US we divide our geographic units into 11 regions: United States and Canada, Mexico and Central America, West Indies, South America, Europe, Asia, Hawaii, Pacific Islands, Australia, Africa, and the Philippine Islands.



**Key words:** curation, Gramineae, grasses, herbarium, reorganization.

## Orchid Ecology and Conservation

### Why are deceptive orchids so phenotypically variable?

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An unusually high intraspecific continuous or discrete floral trait variability has been described within deceptive orchids, as opposed to rewarding ones. The reasons that can explain this pattern stimulated a long-standing debate. An appealing hypothesis is that phenotypically variable deceptive species would have a reproductive success advantage compared to those with a constant floral display because floral trait variability would decrease pollinator avoidance learning in dealing with nectarless flowers, hence increasing their visitation rate. Nonetheless, a review of the studies attempting to demonstrate this hypothesis showed non-significant results thus suggesting that the high phenotypic variability is not likely to enhance deceptive orchids' reproductive success but is more likely to be a consequence of relaxed selection by pollinators. Contrary to this idea, however, in recent years, several studies report on widespread directional selection mediated by pollinators in these deceptive orchids contrasting the idea of relaxed selection. The reasons that lay at the basis of the elevated phenotypic polymorphism of deceptive orchids are thus still to be completely discovered, however, a recent study showed significant differences in selection differentials across years in deceptive orchids suggesting that fluctuating selection can also contribute to the maintenance of phenotypic variation.

**Key words:** fluctuating selection, food-deceptive orchids, phenotypic polymorphism, pollinator behaviour, reproductive success.

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### Macro- and microevolutionary drivers of allopolyploid evolution in *Dactylorhiza* (Orchidaceae)

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Early-generation allopolyploids need to quickly accommodate divergent genomes into one nucleus with ecological consequences. *Dactylorhiza*, with its diverse array of often ecologically distinct polyploids, constitutes an excellent system to investigate allopolyploid evolution. With RADseq data we document the origins of more than ten independently formed *Dactylorhiza* allopolyploids. We bring examples of frequent gene flow in contact zones between related polyploids of different ages. This process enriches the genetic pool of these populations, but the patterns observed point to some genomic regions resilient to admixture. We further exemplify with RNAseq the molecular basis of ecological divergence between two of these independent, sibling allopolyploids, *D. majalis* and *D. traunsteineri*. Significant expression differences between these allopolyploids affect several

ecologically relevant genes. For example, genes in the photosynthesis metabolic pathway have been significantly upregulated in *D. traunsteineri*, which is adapted to northern environments. Finally, we conclude that the major transcriptomic divergence observed among the diploid parents became reconciled in different ways in the sibling *Dactylorhiza* polyploids, as a result of an interplay between stochastic genomic alterations and distinct selection pressures specific to their respective environments.

**Key words:** allopolyploidy, ecological differentiation, Orchidoideae, tetraploids, transcriptomic divergence.

## Research in support of orchid conservation and reintroduction

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With > 28,000 species, orchids are one of the largest families of angiosperms, and they are also one of the most threatened, in part due to their complex life history strategies. Threats include climate change and habitat destruction, and many orchids are also threatened by harvest for horticulture, food or medicine. In addition to being unsustainable, this trade is often illegal and/or undocumented. In this talk, I will provide examples of some current projects to illustrate approaches to orchid conservation. Focused approaches for the species at the highest risk will continue to be applied on a species-by-species basis, but the level of some of these threats now outstrips our abilities to combat them at a species level for all species in orchids (and other large groups). If we are to conserve orchids for the future, we will need to use approaches allowing us to address the threats for groups of species to complement the more focused methods.

**Key words:** conservation, illegal trade, Orchidaceae, reintroduction, threats.

## Ecology of floral perfumes in the Neotropical orchid genus *Catasetum*: does chemical composition predict pollinating orchid bee genera?

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The orchid genus *Catasetum* comprises about 170 fragrance-producing species pollinated by male euglossine bees, mainly of the genera *Euglossa* and *Eulaema*. Interestingly, species pollinated by *Eulaema* are rarely visited by *Euglossa*, and vice versa, and floral perfumes are assumed to be involved in this selective attraction. Recent data suggests that the evolution of floral perfumes in *Catasetum* is shaped by pre-existing sensory/behaviour biases of pollinators, however, experimental support is scarce. Here we tested whether scent traits (composition and daily fluctuation) differ between species pollinated by *Eulaema* and *Euglossa*. Comparative and multivariate analyses of chemical composition showed that floral perfumes of species pollinated by *Eulaema* and *Euglossa* differ significantly from each other. With respect to daily fluctuation in scent emission, we found that the peak of emission in species pollinated by *Eulaema* and *Euglossa* occur between 06:00h and

09:00h and between 09:00h and 12:00h, respectively. This pattern matches with the peak of foraging activity of *Eulaema* and *Euglossa* species in nature. We conclude that scent traits in *Catasetum* predict the genus of their pollinating bees, suggesting that the plants adapt to pre-existing sensory/behaviour biases of one of the euglossine genera.

**Key words:** euglossine bees, floral perfumes, Neotropics, Orchidaceae, pollination.

**Funded by:** CAPES, CNPq, FACEPE, DFG

### **The role of polyploidy and apomixis in diversification of *Zygopetalum mackayi* orchids**

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High elevation rocky complexes (HERCs) from eastern Brazil harbour a highly endemic and threatened flora. Yet, we know little about how polyploidy and apomixis affected diversification in HERCs. We gathered data on the reproductive biology, cytogeography, embryogenesis, and genetic diversity of the orchid *Zygopetalum mackayi*, a typical species from HERCs. *Z. mackayi* is a generalized food-deceptive species pollinated by large bees. It is also dependent on pollinators for reproduction. We identified three cytotypes. Cytotypes 8x and 16x are geographically structured and strongly associated with temperature seasonality and annual precipitation. The intermediate cytotype 12x is restricted to a secondary contact zone among 8x and 16x cytotypes. All cytotypes have meiotic irregularities, but they are more frequent in the intermediate cytotype. Facultative sporophytic apomixis is restricted to neopolyploids (12x and 16x). Paleopolyploids (8x) reproduce only sexually. Genetic variation within and among populations and cytotypes suggests apomixis is not important for the persistence of neopolyploids. Cytotype 12x likely acts as a triploid block. De novo production via crossing between 8x and 16x cytotypes is probably the primary means of 12x production. Studies are underway to evaluate how resistance to water stress and mycorrhizal associations is related to cytotype occurrence.

**Key words:** adventitious embryony, asexual reproduction, campos de altitude, campos rupestres, whole genome duplication.

**Funded by:** Coordenação de Aperfeiçoamento de Pessoal de Nível Superior; Fundação de Amparo a Pesquisa do Estado de São Paulo 2014/04426-5; Fundação de Amparo à Pesquisa do Estado de Minas Gerais (APQ-02096-14/PPM 00478-16); Fundação de Apoio ao Ensino, Pesquisa e Extensão (PRP/Unicamp)

### **How do chromosomes correlate with orchid distribution?**

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It is a central concept in niche ecology that species distributions are primarily determined by ecological and abiotic criteria. However, genomic constraints, like the amount of DNA (genome size - GS) and chromosome organization (chromosome number - 2n) have been poorly investigated

in an integrative approach that considers evolutive history and species ecological characteristics. To understand how these factors interact and how they influence plant geographical distribution, we focused on a large group of orchid which presents a wide geographic distribution and a large morphological, GS and 2n variation. Here we could detect that even with low frequency, the polyploids seem to have great influence in the number of different habitats colonized by species and in the occurrence of the exclusive epiphyte habitat (increase in 2n was negatively related with an increase in the number of habitat types and exclusive epiphyte habitat). The same was observed for GS variation: species presenting exclusively epiphytic habitat presented smaller genomes than non-epiphytic species or, at least, not exclusively. We can conclude that 2n and GS influence plant ecology and that the species geographic distribution is a product of the interaction between its GS and some abiotic characteristics.

**Key words:** ecology, genome size, Maxillariinae, phylogeny, polyploidy.

## Palms - The ecologically most diverse tropical plant family?

### Importance of scatter-hoarding rodents for seedling establishment of large-seeded palms

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Although rodents can also act as seed predators, seeds of certain palm species are exclusively removed and dispersed by rodents, suggesting palms may benefit from these interactions. Nevertheless, direct evidence that rodents do not predate all removed seeds and that seedlings are the result of seed caching by rodents is still scarce. We aimed to assess to what extent seedling establishment of three palm species (*Astrocaryum aculeatissimum*, *Astrocaryum sciophilum*, *Attalea humilis*) is favoured by seed caching by investigating naturally established seedlings in the Amazon and the Atlantic Forest. We found that nearly all seedlings of all three species established from seeds dispersed and not recovered by rodents (mainly located 10–15m away from parents), and 83%, 94% and 75% of seedlings of *A. aculeatissimum*, *A. sciophilum* and *A. humilis*, respectively, established from seeds buried in the soil. Results show that the studied palm species depend almost entirely on caching of seeds by rodents to establish seedlings, improving our understanding about the net outcome of seed caching for large-seeded palms. The high dependence on scatter-hoarding activity for regeneration also reinforces the evolutionary aspect of rodent-palm interactions and the vulnerability of large-seeded palms in scenarios of increasing defaunation.

**Key words:** Areaceae, Neotropics, scatter-hoarders, seed burial, seed dispersal.

**Funded by:** CAPES - Ciência sem Fronteiras and UMR 7179 CNRS-MNHN

### Effects of *primatization* on the population dynamic of a palm that is vulnerable to extinction

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The hypothesis that increasing the abundance of a predator could lead to a negative growth rate of a palm population was tested in this study. In order to test this hypothesis, individuals of *Euterpe edulis* were marked in 2005, and re-sampled from 2006 to 2017 in an isolated forest fragment of 2,600 ha in a fragmented landscape of Atlantic Forest (Rio de Janeiro, Brazil). The predator of *E.*

*edulis* palm-heart (Capuchin monkey, *Sapajus nigritus*) is native but its population is superabundant in the fragment. Lefkovitch matrices were built for each transition year. As a result of these twelve years of study a linear decrease of population size was found. There were 839 individuals with 60 adults in 2005 and 46 individuals with no adults in 2017. This decrease is mainly due to mortality caused by the predator preying upon palm-hearts, which ranged from 10 to 49 adult individuals dying per year. According to the last three lambdas, it is estimated that the palm population is decreasing 31%, 39% and 32% in each interval. If the monkey population remains without control in the area, the palm population will be extinct in the next few years in the studied area.

**Key words:** Arecaceae, *Euterpe edulis*, fragmentation, population ecology, predator superabundance.

**Funded by:** Fapesp, Faperj, CNPQ, CAPES

## Global patterns of palm abundance

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Palms (Arecaceae) are an iconic and diverse group that provides myriad ecosystem services in tropical forests. For example, palms account for 7 of the top 20 ‘hyperdominant’ species of Amazonia. Previous work has examined global patterns of palm diversity but we currently lack a quantitative global analysis of palm abundance patterns. Given the morphological and physiological differences between palms and dicot trees, the responses of these groups to environmental changes are likely to differ. As a result, a better understanding of palm dominance will help identify knowledge gaps and improve the predictive ability of global vegetation models. Using a dataset of >1,000 tropical forest plots, we quantified (1) relative abundance of arborescent palms versus dicot trees across different scales, and (2) how abundance of arborescent palms is related to abiotic variables. We show that arborescent palm abundance is low in the African and Asian tropics compared to Neotropics. We then examined spatial variation in palm abundance in light of evolutionary, biogeographical, and ecological hypotheses, and use statistical models to examine relationships between palm abundance and plot-level environmental covariates. We introduce some novel hypotheses regarding the dominance of the arborescent palm life form in the New World.

**Key words:** biogeography, diversity, dominance, life forms, tropics.

## Non-native palms as engineers of novel ecosystems in the Anthropocene: a global review

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Human activities are generating novel ecosystems without historical precedent, notably by moving species around the world. While the increasing emergence of novel ecosystems is widely recognized, there is still limited understanding of the involvement of many taxonomic groups in this process. We provide the first global synthesis for non-native palms. Palms are considered of high ecological importance as keystone species and are of tremendous value for humans, providing a

range of ecosystem services. Of the 2550 recognized palm species, 80 species are recorded naturalized and 27 species are classified invasive. Main recipient areas are Southern America, followed by the Pacific Islands, Africa and Tropical Asia. Subtropical regions boast a lower amount of non-native palms. Forests, wetlands and anthropogenic habitats are the most frequently colonized habitats. Non-native palms are mainly reported competing with the native flora and altering vegetation structure. They are also capable of affecting native fauna, altering water and fire regimes and in some cases initiating cascading effects, leading to novel ecosystems. As the majority of reports are based on anecdotal evidence, we recommend that more empirical studies explore the ecological role that palms have in generating novel ecosystems also under future global change scenarios.

**Key words:** invasion biology, naturalized, novel ecosystems, palms, plant invasions.

### **Local to regional ecology of the genus *Geonoma***

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With 68 species, the genus *Geonoma* (Arecaceae) is one of the largest, geographically most widespread, and most ecologically diverse Neotropical palm genera. Yet, little is known about the factors that determine the distribution of the diversity of the genus. We used a dataset of 11250 herbarium records and 750 vegetation plots to model the distribution of species richness at two spatial scales: A regional model predicting species richness in equal-area polygons of 7666 km<sup>2</sup> each, and a local model of species richness at 4 km<sup>2</sup>. We then identified the environmental parameters that affect species richness at these different scales. Next, we used the regional predictions as proxies of the regional species pool for the local assemblages, with the aim to understand the roles of regional versus local factors in determining local richness and community composition. We document an overriding role of regional-scale factors in determining local communities.

**Key words:** America, diversity, macroecology, palms, scales.

### **Phylogenomics, biogeography, and evolution in the American palm genus *Brahea***

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Slow rates of molecular evolution at low taxonomic levels hamper studies of relationships among species, biogeography, and evolution. An example is the genus *Brahea*, which is among the most poorly understood lineages of American palms and is characterized by a variety of growth forms and intermediate morphology. We generated ~400 kb of genomic data from all three genomes for 11 currently described *Brahea* species to provide support for relationships, reconstruct ancestral growth form, estimate ancestral geographic ranges, and test for niche equivalency among closely

related species with overlapping geographic regions. Relationships receive strong support, and conform to previous subgeneric assignments. Our phylogenetic hypothesis reveals trends in growth form including an increase in height in the *B. armata* clade, and independent evolution of dwarf forms in the *B. pimo* and *B. dulcis* clades. Ancestral range estimation reveals roles of dispersal and sympatric speciation. We find evidence of niche non-equivalency among species in northwestern Mexico, and between *B. berlandieri* and *B. dulcis*, which are synonymized under *B. dulcis*. Our findings have implications for the complex biogeographic history in Central America and Mexico, and advance our understanding of patterns of biodiversity in these ecologically and economically important palms.

**Key words:** Central America, diversification, growth forms, niche, phylogeny.

## A revision of Mexican *Chamaedorea* (Arecaceae)

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The palm family (Arecaceae) is represented in Mexico by 21 genera and about 100 species. The three genera with the largest number of species in Mexico, *Brahea*, *Chamaedorea* and *Sabal*, also have their greatest diversity in the country. Even though Mexico has been considered as an important center of diversification for *Chamaedorea*, no proper revision of the Mexican species of the genus has been done. Furthermore, its species are actively threatened by deforestation, loss of habitat and uncontrolled extraction. A detailed revision of the Mexican species of *Chamaedorea* was done, based mainly on herbarium specimens. 50 species were recognized in Mexican territory, of which 20 are endemic to the country. This work has resulted in the discovery of new species, corrected species distribution, and a taxonomic treatment for all 50 species. This is a first step towards a better understanding of *Chamaedorea* in Mexico, considering the need for future work that addresses its ecology and conservation, and the Mexican species importance in the evolution and biogeography of this most interesting Neotropical palm genus.

**Key words:** Arecaceae, *Chamaedorea*, Mexico, palms, taxonomy.

## Martius' pioneering work on palm diversity

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The celebrated German botanist Carl Friedrich Philipp von Martius (1794-1868) is widely recognized as one of the earliest and most important contributors to palm research. An extensive study of his original specimens and documents stored at the Munich herbarium (M), as well as his private collections deposited since 1870 in Meise (BR), reveals Martius to be a meticulous palm collector with a visionary appreciation of international networks of collaboration. An analysis of the taxonomic criteria employed for his benchmark publication, *Historia Naturalis Palmarum* (1823-1850), is provided, and the six palm groups that he proposed (Arecinae, Borassinae, Coryphinae, Cocoinae, Lepidocaryinae, Palmae Heteroclitae) are discussed in the context of current palm

classifications. Finally, an assessment of his contribution to our current knowledge of palm taxonomy (i.e., number of genera and species described), and of the Brazilian palm flora in particular, is offered.

**Key words:** Arecaceae, botanical history, Brazil, floristics, taxonomy.

**Funded by:** Conservatory and Botanic Gardens of Geneva, Switzerland

### Macro and micro perspectives on *Geonoma* evolution

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The development of next generation sequencing technologies now allows biologists to obtain tremendous amount of genomic data in a time and cost-effective manner. For the palm family (Arecaceae), genomics resources have recently been developed, providing the opportunity to study the processes shaping the evolution of this iconic tropical family at different geographic and time scales. *Geonoma*, the third largest palm genus in the Neotropics, presents a large amount of intraspecific phenotypic variation that, on one hand, complicates taxonomic delimitation but, on the other hand, provides an ideal opportunity to study the factors promoting divergence in palm populations. Using a recently designed target-capture bait kit, we sequenced 3988 genomic regions for more than 800 individuals of *Geonoma*. This data allowed us to study the macroevolutionary history of this group by inferring a time calibrated phylogeny and performing biogeographic and diversification analyses. In parallel, we investigated the microevolutionary dynamics of two species complexes with very different characteristics, the *G. interrupta*-*G. pinnatifrons* group and the *G. undata*-*G. orbignyana* group. Our analyses at different geographical and evolutionary time scales show that various factors acting at different scales have shaped the evolution of *Geonoma* diversity at the genetic and phenotypic levels.

**Key words:** Arecaceae, diversification, population genomics, species complexes, target-capture sequencing.

### Phylogenomics and biogeography of South-East Asian Areceae

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Palms are a prominent plant family of rainforests, and millions of humans depend on them daily as food, medicine, timber and other materials. Many palm species are endemic to small islands in the Pacific and Indian Oceans, which makes them a good model for comparative studies of the mechanisms of speciation in archipelagos. Such analyses were so far impaired by the lack of a well-resolved species-level phylogeny of the family, leading palm specialists to initiate an international cooperation to fill this gap. Our contribution to this effort is to use targeted DNA sequence capture to generate a genus-level phylogeny of all palms (181 genera), and a species-level phylogeny of tribe Areceae (ca. 700 species). Most data have now been generated, allowing us to initiate phylogeny reconstruction. These phylogenies are crucial to better understand palm biogeography



and evolution, as illustrated by current ongoing studies at Kew on the evolution of palm seed traits and genome size.

**Key words:** Areaceae, Areceae, phylogenomics, South-East Asia, target sequence capture.

## **Palm hydraulic strategies and climatic ranges**

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The monocot family Areaceae (palms) are morphologically, developmentally and functionally distinct from other large woody plants that dominate forests. It is uncertain how palms will respond to increasing drought as hydraulic studies are biased towards conifers and dicotyledonous angiosperms. Here we combine cutting-edge hydraulic studies (*in vivo* micro-CT X-ray and optical visualization of embolism) with macroecological analysis to understand the hydraulic strategies which allowed palms to colonize different climates. Our results show that differences in the onset of cavitation account for most of the difference in vulnerability to drought between the species studied. Embolism commences when most of the water has been exhausted from parenchyma tissue and subsequently progresses rapidly. Phenotypes that minimize water loss (smaller leaflet size) and, maximize water storage (larger stem diameter) and supply to evapotranspiration sites (larger guard-cell sizes) are more likely to be found in drier and warmer biomes. Palms show great ability to face drought either by being highly resistant to xylem embolism or by holding water in their tissues for a long period. Trade-offs in these two hydraulic strategies allowed palms to colonize different biomes on earth and may be key to understand their fate in changing climates.

**Key words:** Areaceae, drought resistance, synchrotron, succulence, xylem embolism vulnerability.

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## **Phylogenomics of palm subtribe Dyspidinae, a spectacular insular radiation from Madagascar**

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Madagascar is home to a spectacular palm radiation, which now for the first time has a comprehensive phylogenetic tree. In numbers, the palm flora of Madagascar resembles the palm floras of other major tropical islands, but it stands out in phylogenetic composition: 80% of the species belong to one genus, *Dypsis*, which has radiated extensively on the island. The mechanisms behind this radiation remain unknown, as no phylogenetic hypothesis has been available until now. Three smaller endemic genera (*Marojejyia*, *Masoala* and *Lemurophoenix*; 2 species each) are classified together with *Dypsis* to form the subtribe Dyspidinae, but the monophyly of this group

been challenged. Moreover, generic delimitation within Dypsidinae is notoriously difficult, and the monophyly of the currently accepted genera needs to be tested. To resolve the phylogenetic relationships of Dypsidinae, we have sampled >90% of the species of the subtribe in the wild or from herbarium specimens and sequenced 176 nuclear loci using target capture and high throughput sequencing. Here we present the first phylogenetic results of this study, discussing the recovered relationships mainly in relation to the systematics of the tribe, and briefly outline the macroevolutionary studies we are planning to test the drivers of this spectacular radiation.

**Key words:** Arecaceae, Areceae, *Dypsis*, systematics, target capture.

**Funded by:** This work was supported by the the European Union FP7-People programme (grant #327259), the National Geographic Society - Global Exploration Fund Northern Europe (grant GEFNE125-14), the International Palm Society, the Royal Horticultural Society, the Bentham-Moxon Trust, The Lord Faringdon Charitable Trust, and Pam Le Couteur.

## Recent advances in Bromelioideae systematics, taxonomy, and evolution

### Phylogeny of Central American Bromelioideae (Bromeliaceae)

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Most Central American members of Bromelioideae belong to clades that originated and diversified in South America, whereas others diversified in Central America. Molecular evidence suggests that the Central American Clade I (*sensu* Sass & Specht, 2010, hereafter the *Androlepis* Alliance, AA) a morphologically variable group that has been classified in four genera, comprises nine species. Cladistic analyses under MP and BI of nuclear (*g3pdh*, *rpb2*, *ETS*) and plastid (*trnL-F*) DNA sequences of 220 Bromelioideae taxa, were conducted to test the monophyly and composition of the clade. We also performed a more restricted analysis to test the monophyly of constituent species as currently circumscribed; in this analysis of nuclear and cpDNA (*g3pdh*, *agt1*, *rpl32* and *ycf1*) regions of 60 genotypes mostly belonging to the AA were included. We also evaluated the significance of some morphological characters, such as sexual expression and anther ornamentation, in the diversification of this group. We found the AA to comprise at least twelve species. Topologies from nuclear DNA show strong topological incongruence relative to topologies from plastid DNA. Furthermore, our finding of non-monophyly and morphological intermediacy of AA species suggests hybridization and subsequent plastid capture in the diversification of these species.

**Key words:** *Androlepis* Alliance, anther ornamentation, hybridization, plastid capture, sex expression.

### Floral morphology still underexploited: the example of the "Cryptanthoid complex" (Bromeliaceae: Bromelioideae)

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The huge morphological and ecological diversity in Bromeliaceae, together with the high degree of convergent and parallel evolution, and the current limits demonstrated for molecular approaches, despite all advances achieved in the last years, make the taxonomy of the family an exceptional challenge for the taxonomist today. The documentation of under-utilized leaf and floral morphological characters and the combination of geographical range, habitat, as well as leaf trichomes, calyx and corolla conformation, petal appendages, stigma, pollen, fruits and seeds, to name a few, pointed toward the need of a new circumscription of the genera of the *Cryptanthus* complex. It reveals that geographical range, habitat, and morphological characters, when accessed in detail and combined, played an important role to support genera and species alliances and renew the relevance of morphology based taxonomy in Bromeliaceae.

**Key words:** Bromelioideae, Cryptanthoid complex, genera alliances, morphology, under-utilized characters.

### **Limitation of molecular studies in Bromelioideae taxonomy**

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Molecular studies have had a profound impact on Bromelioideae taxonomy. For example, DNA data have confirmed that Bromelioideae, sensu Smith and Downs (1979), is monophyletic; consistently place *Bromelia*, *Ochagavia*, *Deinacanthon*, *Fascicularia*, and *Fernseea* near the base of the subfamily; and demonstrate the non-monophyletic status of several long-recognized genera such as *Aechmea*. Unfortunately, the slow evolving bromeliad genome provides a limited number of informative sites and is proving to be largely deficient in resolving species-level relationships, and thus, a robust and well-supported phylogeny for most of Bromelioideae. The problems of low nucleotide substitution rates are compounded by limited sample sizes for most genera, especially larger ones (e.g., *Cryptanthus*, *Hohenbergia*, *Neoregelia*, *Orthophytum*). Further magnifying these impediments for developing a well-supported phylogeny, and thus a Bromelioideae taxonomy that reflects phylogenetic relationships, is the widespread lack of extensive, detailed morpho-anatomical data for most species. Recommendations are presented for how Bromelioideae phylogenetic studies should be conducted to overcome the now recognized limitations.

**Key words:** Bromelioideae, phylogeny, sample-size, sequence-data, taxonomy.

### **Systematics and evolution of *Aechmea* subg. *Chevaliera***

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A better understanding of the phylogeny of *Aechmea* subgenus *Chevaliera* is hampered by the limited taxonomic sampling in molecular studies of Bromelioideae. In order to understand the evolution of *Aechmea* subgenus *Chevaliera*, we conducted a molecular phylogenetic study using three DNA regions (ETS, *matK* and *phyC*). We ran analyses of reconstruction of ancestral states of morphological characters, divergence time of lineages, reconstruction of ancestral area, reconstruction of ancestral habitat, and reconstruction of evolution in environmental space. Our sampling included 22 of the up to 30 species recorded in the subgenus. *Aechmea* subg. *Chevaliera* turned out to be clearly polyphyletic. Amazonian species previously included in *A.* subg. *Chevaliera* were found in separate clades of Bromelioideae phylogeny. Two distinct groups comprised the majority of species up to now grouped in *Chevaliera*. The first of which (*Aechmea multiflora* group) includes seven species; the second (*A. sphaerocephala* group) comprises 11 species. Both groups are endemics of Atlantic Forest. Morphological and evolutionary dynamics analyses shows that they share the same morphological and geographical spaces, but they had different dynamics of occupation of Atlantic Forest in the last three millions of years.

**Key words:** Atlantic Forest, biogeography, Bromelioideae, diversification, taxonomy.

### New results from the *Ochagavia-Fascicularia* group using genome skimming

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*Ochagavia* (4 species) and *Fascicularia* (1 species) form a well-supported clade of the early diverging Bromelioideae. The two genera are morphologically similar, but can be easily discerned on the basis of generative characters. Besides the species distributed on the Chilean mainland, the group includes *Ochagavia elegans*, endemic to the oceanic Isla Robinson Crusoe of the Chilean Juan Fernández Islands. The somewhat enigmatic species morphologically clearly is assigned to *Ochagavia*, but in previous molecular phylogenetic studies formed mostly a sister clade to the remainder of *Fascicularia* and *Ochagavia*. A genomic approach, including whole plastomes (ca 160k bp) and nuclear rDNA cistron (ca 6k bp) was used to analyse the evolution of this clade.

**Key words:** early-diverging Bromelioideae, Chile, Isla Robinson Crusoe, *Ochagavia elegans*, plastome.

### Genome size evolution in Bromelioideae

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The subfamily Bromelioideae is one of the most diverse groups among the Neotropical plant family Bromeliaceae. Previously, key innovations have been identified which account for the extraordinary radiation and species richness of this subfamily, especially in the so called “core” Bromelioideae. However, in order to extend our understanding of the evolutionary mechanisms involved, genomic processes underlying “fast” speciation (e.g., polyploidy, dysploidy) also need to be tested. Here, using PI and DAPI staining and flow cytometry we estimated genome size and GC-content of 268 plants covering 30 genera and 192 species sampled in order to cover the most recent phylogeny of the subfamily Bromelioideae. Published and newly collected data from the subfamily were analyzed and interpreted in a phylogenetic framework. The data suggest significant differences between the subfamilies. With respect to polyploidy, contrasting patterns were detected for “core” (“tank”) and “early diverging” (“tank-less”) Bromelioideae. The results exemplify that even in fast radiating plant groups diverse evolutionary mechanisms could act in closely related clades.

**Key words:** Bromeliaceae, C-value, flow cytometry, polyploidy, tank habit.

## Recent advances in the systematics of Bromeliaceae

### **Systematics of *Hechtia* (Hechtioideae): Insights in phylogenetics and plastome evolution in a non-model organism with Next Generation Sequencing**

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*Hechtia* (75 species), the sole genus in the subfamily Hechtioideae, represents an isolated lineage of bromeliads restricted to North America. This morphologically diverse group is greatly understudied despite having a number of interesting traits—including dioecy, ovary position, leaf characters, and growth pattern—that should prove interesting to study. Lacking any published molecular phylogeny, this study utilized Next Generation Sequencing (NGS) approaches to infer evolutionary relationships to begin testing evolutionary hypotheses. Advances in NGS and computing technologies have vastly improved our abilities to assess the evolutionary history among taxa, particularly in non-model organisms. The primary goal of this research is to reconstruct a highly resolved, well-supported phylogenomic hypothesis of the majority of species of *Hechtia* using complete plastomes (Genome Skimming) and nuclear data (RADseq) to clarify evolutionary history and enable future the investigation of evolutionary questions. Here, I present the results and discuss unexpected insights into the molecular evolution of the plastome including gene deletion and degradation. These results suggest closer scrutiny in other lineages of Bromeliaceae is warranted. The phylogenomic hypothesis from this research also provides a solid foundation to support future comparative analyses of morphological and functional traits, biogeography and ecology across the entire lineage.

**Key words:** Bromeliaceae, Next Generation Sequencing, phylogenetics, phylogenomics, plastome evolution.

## Recent advances in the systematics of Bromelioideae

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Progress in the molecular approaches in Bromelioideae systematics since Monocots V in New York is summed up. Numerous phylogenies have contributed to a better understanding of the evolution of this subfamily, but still major questions remain. The insufficient resolution of the backbone of the Bromelioideae phylogeny, often contrasting phylogenetic signals of the nuclear and plastid genome, and insufficient species sampling are still major weaknesses of the molecular phylogenies. Recent results in Bromelioideae research based on the synthesis of molecular and (reassessed) non-molecular data are presented in the separate symposium "Recent advances in the Bromelioideae Systematics, Taxonomy and Evolution".

**Key words:** contrasting phylogenetic signals, evolution, genus concept, molecular systematics, phylogeny.

## Preliminary results on the evolution of Bromeliaceae in the northern Andes

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Phylogenetic data have increasingly revealed that the northern Andes are one of the most prolific hotspots of plant diversity because of its recent and rapid uplift. The specific mechanisms that explain this phenomenon, however, still need further study due to the lack of robust species-level phylogenetic hypotheses. In this context, our study seeks to infer robust phylogenies of four lineages of Bromeliaceae that diversified almost exclusively in the northern Andes in order to identify the main drivers of their diversification. The first case corresponds to the high altitude radiation of *Puya*, which likely diversified in an archipelago-like pattern. Here, páramos act like islands, where their confined species exhibit high endemism and contrasting phenotypes, probably as a strategy to fill different niches. The second case corresponds to a clade of Core Bromelioideae that diversified in the eastern Andes. The diversification of this lineage was probably modulated by the evolution of structural defenses against herbivores and a close association with ants. The last case is the evolution of *Ronnbergia* and the *Sodirola* clade of *Guzmania* in the hyperhumid foothills of the western Andes. These radiations were probably influenced by the evolution of facultative epiphytism and the drastic modification of the rosettes.

**Key words:** Bromeliaceae, Core Bromelioideae, *Guzmania*, northern Andes, *Puya*.

## Recent advances in Pitcairnioideae systematics

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Bromeliaceae subfamily Pitcairnioideae comprises the five genera *Pitcairnia* (~400 species), *Fosterella* (31 species), *Deuterocohnia* (18 species), *Dyckia* (168 species) and *Encholirium* (29 species). In the last decade, several studies aimed to elucidate phylogenetic relationships and spatio-temporal evolutionary patterns within the subfamily and its genera. Most investigations relied on plastid DNA data, but a few nuclear loci were also analyzed. Pitcairnioideae usually came out monophyletic in plastid-based trees, whereas *Puya* (Puyoideae) groups within subfamily Pitcairnioideae in some nuclear phylogenies and makes it paraphyletic. Recent plastid phylogenies provided evidence that the mostly mesophytic genus *Pitcairnia* is monophyletic and sister to the remainder of the subfamily. *Pitcairnia* is however deeply divided into two major distinct plastid lineages. The likewise mesophytic genus *Fosterella* is monophyletic in all studies and sister to a well-supported “xeric clade” consisting of *Deuterocohnia*, *Dyckia* and *Encholirium*. *Fosterella* probably originated about 10 mya in seasonally dry forests. The Andean genus *Deuterocohnia* is paraphyletic in all plastid phylogenies but not in nuclear trees, suggesting an ancient chloroplast introgression event. *Encholirium* is likewise paraphyletic, with monophyletic *Dyckia* arising from within the genus. Dated plastid phylogenies provided evidence that *Dyckia* experienced a recent radiation that started ~2.5 mya.

**Key words:** Bromeliaceae, monophyly of genera, phylogeny, Pitcairnioideae, plastid DNA.

### **Taxonomic revision of Bromeliaceae subfam. Tillandsioideae based on a multi-locus DNA sequence phylogeny and morphology - a review**

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Bromeliaceae subfam. Tillandsioideae is a well-defined, monophyletic taxonomic group. Several studies within the last 30 years since the last monograph was published have altered the internal classification based on new morphological and molecular DNA data. It has been shown that traditional generic concepts and morphological characters used to delimit taxa were in the need of a careful revision, especially within the most species-rich genera *Tillandsia* and *Vriesea*. Hence, a taxonomic revision of Bromeliaceae subfam. Tillandsioideae was undertaken aiming at inclusive taxonomic coverage, multi-locus DNA sequence data from plastid and nuclear loci, and morphological assessments. This enabled the circumscription of monophyletic units using

synapomorphic combinations of diagnostic morphological characters. As shown already earlier, stigma morphology has proven to be highly indicative. Two new subtribes, twelve additional genera and three subgenera were established to render taxonomic units monophyletic and morphologically well circumscribed. The four tribes previously proposed were confirmed, with the exception of *Mezobromelia*, according to its type species, not being part of Tillandsioideae. This work will be discussed in a broader context of recently published studies, advances using NGS sequencing technologies and in the light of future taxonomic treatments (e.g., lumping vs. splitting and mono- vs. paraphyletic taxa).

**Key words:** Bromeliaceae subfam. Tillandsioideae, synapomorphic combination of diagnostic morphological characters, taxonomy, *Tillandsia*, *Vriesea*.

## Molecular phylogenetics of core-Tillandsioideae with emphasis on *Vriesea* using NGS

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*Vriesea* is one of the largest genera of the Tillandsioideae subfamily still needing systematic and phylogenetic investigations. Here, we present a molecular phylogeny of the core-Tillandsioideae using a genome skimming approach based on a sampling of eleven genera and fifty three percent of known species of *Vriesea*. This approach allows us to recover nearly the whole chloroplast genome and a total of 122,664 SNPs for the nuclear, chloroplast and mitochondrial genomes. We performed phylogenetic reconstruction using both maximum likelihood and coalescent-based methods. In all phylogenetic inferences the support values were high for inter-generic relationships and low within the *Vriesea* s.str. clade, but some putative groups are recognized. Incongruent topologies were recovered between nuclear data and chloroplast and mitochondrial data. In particular, the phylogenetic tree based on nuclear SNPs does not support the existence of tribe Vrieseae as it was described in the last revision. Instead it shows that the group of eastern Brazilian lineages is sister to a clade composed by tribe Tillandsieae and subtribe Cipuropsidinae with high support. Our findings bring new insights on the systematics of *Vriesea* s.str. and reinforce the importance of choosing an appropriate sequencing approach to deal with evolutionary questions in Tillandsioideae.

**Key words:** Atlantic forest, genome skimming, Neotropics, species tree, systematics.

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## *Setaria* as a model system for monocot development and biotechnology

### Using *Setaria* as a model system to dissect vascular development in monocots

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Most of the lack of knowledge about C<sub>4</sub> photosynthesis is due to the absence, until now, of an appropriate model to study C<sub>4</sub> plants. In this context, *Setaria viridis* was proposed as model plant for C<sub>4</sub> Panicoideae. The first step of this study was to evaluate the best normalization genes for expression analysis in *S. viridis* through qPCR. The expression stability of fifteen candidate genes was evaluated, allowing the identification of the best reference genes for qPCR studies. Due to vascular bundle's role as the organizing center of Kranz anatomy, the identification of regulatory pathways that control vascular development in a C<sub>4</sub> plant was the aim of this work. For that, known markers of initial vascular development, characterized in *A. thaliana* (AtHB8 and AtPIN1), had their orthologues identified in *S. viridis*. Their expression pattern was confirmed through qPCR and *in situ* hybridization. In addition, CRISPR/Cas9 mutants targeting HB8 and PIN1b were created for a more accurate characterization of initial vascular differentiation in *S. viridis*. In addition to experimental efforts to characterize vascular-related genes in *S. viridis*, an *in silico* approach was also used to characterize the bHLH transcription factor family, known to be involved in early step of vascular development.

**Key words:** bHLH, C<sub>4</sub> photosynthesis, C<sub>4</sub> photosynthesis, CRISPR/Cas9, phylogeny.

**Funded by:** CNPq, CAPES, FAPERJ and Newton Foundation.

### **Respiratory metabolism in *Setaria* biomass production**

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Some species of *Setaria* (*S. viridis* and *S. italica*, Poaceae) are prominent candidates as model systems to investigate C<sub>4</sub> metabolism and bioenergy traits. Their small genome, fast life cycle and the development of new transformation protocols for these species make them special targets to access the functional genomics of C<sub>4</sub> plants. In order to better understand the carbon accumulation in C<sub>4</sub> plants, with special attention to growth and biomass accumulation, we investigate the importance of cell respiratory metabolism in *Setaria* species. For this purpose we used the RNAi approach in which the mitochondrial fumarase and malate dehydrogenase (MDH) genes were independently downregulated. Physiological parameters of T1 generation were analyzed and no substantial changes in its photosynthetic rates were observed. However, some transgenic events showed delay in growth, presenting short plants compared to wild type. These results indicate that decreased fumarase and MDH expression may influence growth, but it seems to be not a limiting factor for C<sub>4</sub> photosynthesis at ambient CO<sub>2</sub> partial pressure, making these plants interesting targets for exploring the role of TCA cycle enzymes in C<sub>4</sub> plants targeting changes in the use and storage of carbon.

**Key words:** biomass, C<sub>4</sub> metabolism, fumarase, malate dehydrogenase, transgenics.

**Funded by:** Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP 2012/23838-7)

### ***Setaria viridis*: a C<sub>4</sub> model plant to study the regulation of carbon partitioning and growth**

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Photosynthesis lies at the heart of plant metabolism and the incorporated carbon (C) must be carefully directed toward cell wall propagation, survival and biomass accumulation. The apparent simple pattern of C assimilation, storage and utilization during the diel cycle is dependent on a complex and flexible network essential to keep C homeostasis. C<sub>4</sub> plants possess high photosynthetic capacity, increased nitrogen and water use efficiencies and are capable of accumulating biomass at impressive rates. Our group aims to dissect the role of the "Target of Rapamycin" (TOR) kinase, a master regulator of growth in eukaryotes that affects a plethora of metabolic pathways in plants. In this talk, our efforts in understanding the role of TOR in orchestrating C partitioning in photosynthetic organisms (using *Arabidopsis* and the emerging model *Setaria viridis*) will be presented, which has a profound impact not only on plant yield but also on the generation of fuels and chemicals.

**Key words:** carbon, growth, metabolism, *Setaria viridis*, target of rapamycin.

**Funded by:** FAPESP, Max Planck Society

## Systematics, evolution, and biogeography of Orchidaceae

### Pleurothallidinae, are we there yet?

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With over 5200 accepted species today, subtribe Pleurothallidinae is possibly the most species-rich of all orchids. DNA data has proven to be a powerful tool in placing the diverse species of Pleurothallidinae in a less subjective phylogenetic context. Nevertheless, floral similarity due to convergent evolution and floral dissimilarity as a consequence of adaptations to particular pollination systems renders many traditionally used morphological features uninformative. Identifying morphological traits, either floral or vegetative, that can consistently diagnose diverse species groups is still challenging, but there are several diagnostic features that can be very helpful in placing species. With the availability of molecular data from more than one thousand species of Pleurothallidinae, it is now possible to assess the phylogenetic relationships amongst the vast majority of them, reducing most of the discussion to the interpretations of inclusiveness of generic circumscriptions by diverse authors. These disagreements are likely to persist as they are a consequence of different interpretations of the same evidence rather than the weight or quality of it. What will hopefully prevail is a consistent use of the adequate phylogenetic framework, therefore how species are related to each other, rather than in what rank they are placed.

**Key words:** convergence, generic circumscription, Orchidaceae, Neotropics, species-rich.

### Pushing the limits: assessing the performance of nrITS for phylogenetic reconstruction and molecular dating of orchids

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The Internal Transcribed Spacer region of nrDNA is the most used DNA region for plant phylogenetic reconstruction. Many orchid phylogenies have been presented including only ITS, or with large ITS phylogenies coupled with reduced combined matrices, due to the fact that ITS generally provides reasonable levels of variation. Some questions were placed on ITS when presented as the only source of phylogenetic data, and there are reports of conflict between nuclear and plastid data. Despite this, there are no assessments of the impact using only ITS data for phylogenetic reconstruction and molecular dating. In this study we evaluate the use of only ITS instead of combined matrices, assessing whether combination of ITS with plastid or other regions indicates topological conflicts that exceed the random expectations due to differences in variation levels. We also assess how ITS performs in estimating molecular dating in relation to combined matrices. The assessment was carried out with matrices from subtribes Pleurothallidinae, Laeliinae, Cymbidieae and Cypripedioideae. The results indicated that the use of secondary structure modelling of ITS1 and ITS2 for alignment and modelling allow the use of ITS over broad phylogenetic distances within Orchidaceae. For molecular dating, ITS performs reasonably well in constrained matrices.

**Key words:** Internal Transcribed Spacer, modelling, Orchidaceae, phylogenetic conflict, RNA secondary structure.

**Funded by:** Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq)

### **Morphological diversity of pleurothallid roots**

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For orchids, when we combine the anatomical characters of roots it is possible to delimit taxa and point out synapomorphies, even if we consider homoplastic features, like those of the velamen. Different phylogenetic studies enable us to interpret the state and the evolutionary direction of these characters. Neotropical micro-orchids of the subtribe Pleurothallidinae, which encompass 20% of the family's species (~ 4000), are not all supposed to possess similar roots. We observed that the velamen-exodermis complex of their roots varies morphologically which, together with the cortex and vascular cylinder features, are useful to identify genera. Considering the phylogenetic uncertainties of the group, the root anatomy can add in the characterization of both suprageneric and infrageneric groups and can help in the delimitation of complex genera. Despite the difficulty in obtaining samples (plants are often rare, tiny and difficult to grow), a large effort is necessary to define limits in root morphology within diversified groups, such as Pleurothallidinae.

**Key words:** Pleurothallidinae, root anatomy, tilosome, velamen-exodermis complex, velamen stripes.

### **Evolution of Australia's rich endemic orchid flora in time and space – phylogenomic insights**

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The Australian orchid flora comprises over 1,300 orchid species exhibiting high levels of endemism with around 90% of species occurring nowhere else. Several orchid lineages underwent extensive diversification on the Australian continent, in particular within tribe Diurideae and subtribe Pterostylidinae – the latter two accounting for over 75% of the country's orchid diversity. However, the spatio-temporal evolution of the Australian orchid flora is still poorly understood. Plastome data for 353 samples was generated via shotgun high-throughput sequencing, representing all genera and major intrageneric lineages within the eleven subtribes in Diurideae and 45% of the species diversity within Pterostylidinae. Phylogenetic tree inferences were carried out in RAxML and MrBayes based on 77 plastid protein coding regions. Divergence time estimations were conducted using fossil and secondary calibration points in BEAST. Key morphological and ecological traits were coded and character state changes traced along the phylogenies under the MK1 model using maximum likelihood. Ancestral range estimations were carried out based on the BEAST chronograms and the dispersal extinction cladogenesis model. This phylogenomic study yielded highly resolved and well supported phylogenetic reconstructions allowing novel insights into the evolution of key morphological and ecological traits and the assembly of the Australian orchid flora.

**Key words:** Diurideae, historical biogeography, Orchidaceae, phylogenomics, Pterostylidinae.

The next generation of research on the evolution of the Crassulacean acid metabolism: integrating physiology, ecology, and genomics

### **RuBisCO activity and the efficiency of photosynthesis in the C<sub>3</sub>-CAM bromeliad *Guzmania monostachia***

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Plants that perform the Crassulacean acid metabolism deal successfully with environments with intermittent availability of water and nutrients, which has been at least in part associated with higher water- and nitrogen-use efficiencies. Limitations of water and nutrients can impair photosynthesis, through the reduction of RuBisCO and plant carbon balance. In this context, we conducted a controlled experiment with the epiphytic C<sub>3</sub>-CAM bromeliad *Guzmania monostachia* to investigate how the combined water and nutritional deficits affect the RuBisCO activity and its activation state (RAS), and the efficiency of photosynthesis during the transition from C<sub>3</sub> to CAM. We observed that, besides the increment in CAM activity, bromeliads submitted to both water and nutritional deficits showed higher RAS compared to C<sub>3</sub> bromeliads and, surprisingly, the quantum efficiency of photosynthesis increased. Additionally, glucose, fructose, and starch contents were kept while sucrose concentration increased even with both deficit impositions. These results combined with high RAS strongly suggest an increment in the efficiency of RuBisCO functioning. Our results reinforce the ability of epiphytic plants to deal with stressful habitats, in which higher efficiency of

RuBisCO during the transition to CAM maintain photosynthesis and their carbon balance, which ultimately favored their evolution in the epiphytic environment.

**Key words:** CAM pathway, *Guzmania*, photosynthesis, photosynthetic quantum yield, RuBisCO activation state.

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## Evolution of Crassulacean acid metabolism across the Agavoideae

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The evolution of Crassulacean acid metabolism, or CAM photosynthesis, has occurred at least 35 times across multiple lineages. CAM plants open their stomata at night to acquire carbon; the incoming CO<sub>2</sub> is temporarily stored as malic acid in the vacuole until the daytime, when stomata close and the malic acid is decarboxylated in the cytosol. CAM plants are thought to be adapted to water stress due to their carbon concentrating physiology and nocturnal stomatal opening, both of which promote increased water use efficiency. How CAM evolved from C<sub>3</sub> ancestors remains unknown, but evidence points to similar patterns of evolution in independent lineages: morphological characteristics evolve first, but the fine tuning of the biochemical reactions is a larger hurdle that only some lineages cross. Using the Agavoideae (Asparagaceae), a group that includes *Agave* and *Yucca*, we investigated physiological and genomic characteristics in closely related C<sub>3</sub> and CAM species. We find that CAM anatomy is prevalent in many species regardless of photosynthetic phenotype. Gene expression patterns reveals that low level temporal expression of CAM genes is found in some C<sub>3</sub> species, as well, indicating that the Agavoideae may have been pre-adapted for the evolution of full CAM both physiologically and genomically.

**Key words:** CAM, photosynthesis, physiology, transcriptomics, *Yucca*.

## Evolution and comparative ecophysiology of Crassulacean acid metabolism in tropical orchids

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Physiological diversification is an evolutionary mechanism with the potential to promote species radiations. We studied evolutionary patterns of C<sub>3</sub> and Crassulacean Acid Metabolism (CAM) photosynthetic pathways in tropical orchids to better understand interspecific variation. CAM is a water-conserving mode of photosynthesis present in water-limited environments. To investigate functional diversification related to degree of CAM expression in orchids, we used comparative analysis of RNA sequencing from closely related Oncidiinae species with CAM and C<sub>3</sub> photosynthesis to identify genes that follow time-dependent patterns associated with the CAM pathway. We also conducted analyses with species accessions of carbon isotopic composition, titratable acidity, character state reconstruction, and phylogenetic trait analysis of the occurrence and degree of CAM in the orchid family. We demonstrate plasticity of CAM using 24h gas

exchange under well-watered conditions and drought stress, and explore the mechanisms that are responsible for physiological changes in plant function. Anatomical and physiological traits that are important drivers in CAM evolution are also discussed. This project is part of a Dimensions of Biodiversity team effort aimed at understanding the evolution of CAM in plants.

**Key words:** Crassulacean Acid Metabolism, epiphytes, isotopes, Oncidiinae, Orchidaceae.

**Funded by:** National Science Foundation

### **Exploring CAM evolution in *Tillandsia* (Bromeliaceae) with phylogenomics, transcriptomics, and metabolite profiling**

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The Bromeliaceae (bromeliad; pineapple) family represents a highly diverse and enigmatic adaptive radiation of the New World Tropics. Diversification and radiation in this group was apparently facilitated by several 'key innovations' or adaptive trait shifts, one of which is the transition from C<sub>3</sub> to CAM photosynthesis. We have used a whole genome phylogenomic approach complemented by differential gene expression analysis (RNA-seq) and molecular phenotyping to address the patterns and mechanisms of C<sub>3</sub> / CAM evolution in the extremely species-rich bromeliad genus *Tillandsia* and related taxa. In this talk, we will focus on three main aspects: (i) genome-wide, branch-specific scans for adaptive protein evolution, (ii) genome-wide analyses of convergent gene family expansion, (iii) transcriptome-wide patterns of gene expression differences for selected taxa sampled from the CAM / C<sub>3</sub> continuum. Based on the results, we will highlight genes and pathways of potential interest for the evolution of CAM in tillandsioid bromeliads, and we will discuss the potential role of correlated trait evolution in producing patterns seen in genomic and transcriptomic data.

**Key words:** CAM, phylogenomics, radiation, speciation, transcriptomics.

**Funded by:** Swiss National Science Foundation (SNF), University of Vienna

### **CAM regulation by nitric oxide in the epiphytic bromeliad *Guzmania monostachia***

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*Guzmania monostachia* is a tank bromeliad capable of up-regulating CAM in response to water deficit. Nitric oxide (NO) is a molecule involved in many stress responses in plants, including CAM upregulation, but its mechanisms of action remain largely unknown. We investigated if NO would be capable of modulating the activity of key CAM enzymes (PEPC and MDH) and/or activating PEPC gene transcription. We showed that detached leaves exposed to a water shortage by 30% polyethylene glycol solution up-regulated CAM and increased NO emission in the apical part of the leaf along with a reduction in the amount of nitrosylated proteins, when compared to control leaves. By exposing protein extracts of *G. monostachia* to S-nitrosoglutathione, we found that nitrosylation decreased PEPC activity, while MDH activity remained unchanged. Finally, we exposed the plants to 50 ppm of NO during 5 days and found an increase in all parameters related to CAM when

compared to the control. We conclude that NO may have a dual role in the signaling of CAM in *G. monostachia*: post-translationally NO acts as a negative regulator for PEPC activity, but it is also capable of somehow increasing CAM when exogenously applied.

**Key words:** CAM, *Guzmania monostachia*, nitric oxide, tank bromeliad, water shortage.

**Funded by:** Fundação de Amparo à pesquisa do Estado de São Paulo; Coordenação de Aperfeiçoamento de Pessoal de Nível Superior; Conselho Nacional de Desenvolvimento Científico e Tecnológico

## Comparative genomics of CAM photosynthesis

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The altered carbon assimilation pathway of crassulacean acid metabolism (CAM) results in up to six times greater water-use efficiency than C<sub>3</sub> photosynthesis, making it an excellent pathway for engineering crop plants with improved drought tolerance. Central to the success of this approach is a deep understanding of the underlying genetic mechanisms controlling CAM photosynthesis. We surveyed detailed temporal and spatial transcriptomic and metabolomic changes in the obligate CAM plant pineapple (*Ananas comosus*) and facultative CAM plant *Sedum album*. Pineapple has a normal number of CAM pathway genes compared to grasses and other monocots, but CAM isoforms are enriched with cis-regulatory elements associated with regulation by circadian clock genes. This suggests CAM in pineapple evolved through the regulatory neofunctionalization of preexisting genes and not through the acquisition of neofunctionalized genes via whole-genome or tandem gene duplication. *Sedum album* shows strong CAM induction under progressive drought stress with strong co-expression of drought and CAM pathways genes. *Sedum* uses the same orthologs of CAM pathway genes as the model CAM plant *Kalanchoe* supporting parallel evolution. Together these resources provide targets for functional validation and increase our understanding of this complex trait evolution.

**Key words:** *Ananas*, CAM, comparative genomics, evolution, pineapple.

## Understanding Amaryllidaceae evolution using different approaches

### Revealing the evolutionary history of tribe Leucocoryneae (Allioideae) based on molecular, morphological and cytogenetic data.

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The subfamily Allioideae (Amaryllidaceae) consists of four monophyletic tribes: Allieae, Tulbaghieae, Gillieasiaeae, and Leucocoryneae. The Leucocoryneae encompass 6 South American genera with ca. 100 species and includes potentially medicinal, ornamental species, and weeds, either locally or worldwide distributed. This study is focused on an exhaustive sampling of *Beauverdia*, *Ipheion*, *Nothoscordum*, and *Tristagma*. Morphological and cytogenetic characters were explored through phylogenetics, phylogenomics and phylogeographic approaches using ca. 250 specimens. The findings support that the high rate of diversification within the tribe

Leucocoryneae is accompanied by a slow morphological variation and can be observed as changes in genome size. These changes are a product of chromosomal rearrangements such as Robertsonian translocations or polyploidization and might be acting as reproductive barriers, being the major triggers of speciation within the tribe. Finally, *Nothoscordum* + *Beauverdia* resulted in a monophyletic group with conflictive phylogenetic signals, suggesting reticulate evolution, probably due to hybridization events. Also, a hypothesis of divergence and diversification of *Ipheion* is proposed

**Key words:** Amaryllidaceae, chromosomal rearrangements, reticulation, speciation, South America.

## **Resolving the phylogeny of the Andean tribes Clinantheae and Eucharideae (Amaryllidaceae) with next generation sequencing data**

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The Andean tetraploid clade of the American Amaryllidaceae (subfamily Amaryllioideae) consists of four tribes: Clinantheae, Eucharideae, Eustephieae and Hymenocallideae. nrDNA ITS and several plastid loci resolve Eustephieae as sister to the other three tribes. We obtained sequences of 10 low copy nuclear genes and partial plastomes for the Eucharideae. More recently, we completed a genome scan (sequence capture) of Clinantheae, Eucharideae, Hymenocallideae, with several Eustephieae as outgroup. We present preliminary results of analyses of part of this large data set. In the Eucharideae, the Peruvian endemic *Rauhia* is the first branch, followed by the mostly Ecuadorean *Phaedranassa*. The rest of the tribe consists of a monophyletic *Eucrosia*, with the exception of *E.dodsonii*, a monophyletic *Stenomesson*, but the genera *Eucharis*, *Caliphruria*, *Urceolina*, and the monotypic *Plagliolirion* do not resolve as monophyletic. The generic radiation of Eucharideae appears to have been facilitated by the Huancabamba depression of northern Peru. Clinantheae consists of two monophyletic groups: *Pamianthe*, and *Clinanthus*. A clear floral character state transformation series can be seen within extant species of *Clinanthus*. We anticipate that when all of our data has been fully analyzed, a fully resolved phylogeny of both tribes will emerge that will lead to a stable classification.

**Key words:** geophytes, Huancabamba depression, phylogenomics, plastome, sequence capture.

**Funded by:** Some of this work has been partially supported by the National Science Foundation (USA).

## **Recent advances in the systematics of the Brazilian endemic tribe Griffinieae (Amaryllidaceae)**

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The tribe Griffinieae was proposed by Ravenna in 1974, including only the genus *Griffinia*, historically of uncertain classification. In 2000 molecular phylogenetic analyses revealed that *Griffinia* with the monospecific genus *Worsleya* constitute a clade. In the same year Ravenna



described and added to the tribe *Cearanthes*, another monospecific genus whose phylogenetic position, however, has not yet been determined. Currently there are 15 recognized species of *Griffinia* divided into two subgenera. Although preliminary analyses indicate that the species of *Griffinia* constitute a monophyletic group, the genus presents numerous taxonomic problems related to difficult delimitation of its taxa. In our on-going studies we have included accessions covering all three genera and virtually all extant species of *Griffinia*. Next generation sequence capture has yielded coding regions from at least 500 low copy nuclear genes and 70-90% of the entire plastome for phylogenetic reconstruction. Previously a hypothesis based on the nuclear marker ITS was elaborated. The taxonomic revision of the tribe is also in progress by the authors. Our preliminary results are presented and future prospects discussed.

**Key words:** *Cearanthes*, *Griffinia*, molecular phylogeny, Next-Generation Sequencing, taxonomy.

**Funded by:** ACR received financial support through a scholarship from PDSE/CAPES (process nº 419 88881.132085/2016-01).

### Using leaf anatomy to help solve problems of species delimitation

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The only Amaryllidaceae tribe endemic to Brazil, Griffinieae has its main center of diversity in the Atlantic Forest with the majority of the species considered threatened with extinction. *Griffinia* Ker Gawl. is the most representative genus and historically of uncertain classification; it is divided into two subgenera, *Griffinia* and *Hyline*, with significant ecological and morphological differences. *Griffinia* has about fifteen species, mainly understory plants of the Atlantic Forest, and presents some taxonomic complexes, and *Hyline* has two recognized species. Together with two monotypic genera, *Worsleya* Traub and *Cearanthes* Ravenna, they constitute the tribe Griffinieae. Besides the common leaf structure with a single-layered epidermis, mesophyll composed of chlorenchyma, aerenchyma and collateral vascular bundles, the species present some structural singularities. Our study aimed to distinguish the nineteen species by leaf anatomical structures to help solving some delimitation problems. Transverse and longitudinal sections were obtained from samples of fully expanded and mature leaves, which were fixed and submitted to usual histological techniques. The outer periclinal wall thickness of epidermal cells, leaf margin shape, and structure of the leaf midrib are promising features and we also discuss potential taxonomic importance of other anatomical data.

**Key words:** Amaryllidaceae, Atlantic forest, Griffinieae, plant anatomy, taxonomy.

**Funded by:** Capes and CNPQ

### Phylogenetic analysis of the Strumariinae (Amaryllidaceae) sheds light on generic concepts in the subtribe

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Previous molecular-based studies of *Nerine* and related genera (subtribe Strumariinae) suggested that either *Nerine* as currently defined is not monophyletic or that genera defined by distinctive

floral morphology are embedded within *Nerine*. These studies were based on a limited sampling of taxa. The present study, based on 57 samples representing 30 taxa from four genera, using four cpDNA regions (*ndhF*, *rbcL*, *trnK*, *trnL-F*) provides a more detailed survey of the subtribe. Our results confirm that while species belonging to *Hessea* and to *Strumaria* subgenus *Gemmaria* form discrete clades, these are embedded in the genus *Nerine* as currently delimited, rendering the latter polyphyletic. The remaining genera (*Strumaria* subgenus *Strumaria*, *Brunsvigia*, and *Namaquanula*) form discrete clades but their position with regard to *Nerine* is not fully resolved. The implications for defining the genera in the subtribe are discussed in the light of the apparent lack of congruence between morphology, cytology, and the phylogeny based on cpDNA. Incongruences include chromosome number, floral symmetry, anther attachment, and the presence or absence of cataphyll.

**Key words:** Amaryllidaceae, *Hessea*, incongruence, *Nerine*, *Strumaria*.

### **Population genetics of *Narcissus*: a case study of the *Narcissus minor* group**

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*Narcissus* is a genus of well-known and widely grown garden plants in temperate regions. The prevalence of natural hybridisation adds to the taxonomic complexity of the genus. In this study, we focus on the *Narcissus minor* group (part of section *Pseudonarcissus*) to report natural levels of variation and population structure across its native range in northern Iberia. Section *Pseudonarcissus* has been used extensively to breed garden daffodils and understanding levels of variation in the wild may be key to understanding the variation within cultivars. A geographic structure to populations that is incongruent with the current taxonomic treatment is revealed by cpDNA sequences of *matK*, *ndhF*, and *ycf1*. More than 600 individuals collected from 54 populations across the geographic range of the species were surveyed with eleven nuclear microsatellite markers revealing low levels of diversity and interbreeding amongst populations. There is geographic clustering of populations based on genetic data. These clusters do not match morphological taxa. The apparent conflict between morphology and genetics is being further investigated using whole plastid sequence approaches.

**Key words:** cpDNA, microsatellites, morphology, *Narcissus minor* group, taxonomy.

**Funded by:** Royal Horticultural Society

### **Whole chloroplast genome analysis in *Narcissus* and its implications for our understanding of the evolution of the genus**

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Despite the economic importance of daffodils their taxonomy remains unresolved, and the delimitation of taxa can be problematic in many sections. Current estimates of number of species in the genus vary from 30 to 80. Recent research sequencing a small number of plastid and nuclear

genes reveals a mismatch between the species concept defined by morphology, the clades recovered from plastid DNA analysis, and the clades recovered from nuclear DNA analysis. Here we present 10 new complete plastid genomes for *Narcissus*, as a first step in quantifying levels of difference across the genus. Variation among these genomes is compared with other published genomes in Amaryllidaceae. We find patterns in plastid variation that broadly follow the established cpDNA phylogeny, however there are some notable differences in genomes among even quite closely related species. We report on the patterns of variation found, the limits of resolution that plastid DNA might offer for a genus-wide study, and provide a list of genes ranked by levels of variability. **Key words:** Amaryllidaceae, genome, *Narcissus*, phylogeny, plastomes.

### Lessons from flowering time: the case of Amaryllidaceae sympatric species

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Natural hybridization plays an important role in the evolution of Amaryllidaceae. Species within this family display a large amount of homoplastic characters in their flowers suggesting pollinator-mediated adaptations. To understand natural hybridization, it is important to look at closely related species that occur in sympatry and share pollinators. For heterospecific pollen transference to occur, species have to flower at the same time. Learning about the species flowering strategies can help us to understand how coexistence patterns affect the phylogeny of the group. We used three species (*Habranthus gracilifolius*, *H. tubispathus* and *Zephyranthes mesochloa*) that co-occur in southern Brazil to check for natural hybridization chances. All species are visited by the same groups of insects. They flower only in the Brazilian summer, displaying two short and massive flowering peaks each. Despite the great amount of flowers produced by each species, flowering overlap between them does not differ from the expected by chance. This may mean that, even if they are pollinated by the same visitors, without significant flowering aggregation there is less chance for heterospecific pollen transference between these species and, consequently, the probability of hybridization. We discuss how, even with weak post-pollination barriers, phenological patterns can influence species delimitation.

**Key words:** Amaryllidaceae, floral visitors, flowering phenology, *Habranthus*, *Zephyranthes*.

### Complex population histories of *Phaedranassa* species endemic to Ecuador

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Plant speciation process in the Andean biodiversity hotspot is still understudied. *Phaedranassa* (Amaryllidaceae) is a small genus of eleven species in the northern Andes of Ecuador and Colombia and Costa Rica. Eight of the species are found in Ecuador and seven of them are endemic to the country. We investigated the population genetics of *Phaedranassa* in Ecuador with the aim of understanding the speciation patterns of the genus in its center of diversity. Using microsatellite data, we identified a variety of scenarios of divergence in the genus. Bayesian clustering resolved

genetic differentiation among *Phaedranassa* species, even across relatively small distances. Genetic differentiation was not evenly distributed across the genus. We found natural hybridization, genetic loss influenced by proximity of cities, the effect of historical volcanism and in one case emerging genetic differentiation among two species. Our results are an example of the complexity of speciation patterns in Andean plants.

**Key words:** Amaryllidaceae, Andes, Ecuador, population genetics, speciation.

**Funded by:** Universidad Tecnológica Indoamérica

## Zingiberales I - Evolution

### On the Evolution of the Amaryllidaceae Inflorescences and Some Topics of Models of Morphological Evolution

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The directionality of morphological evolution is a key question to evolutionary and developmental biology. A phylogenetic framework is necessary to contextualize developmental genetic data, however models of trait evolution are not commonly used in Evo-Devo studies. Here we use the inflorescence of Amaryllidaceae as a case study to investigate the directionality of morphological evolution. Inflorescences are reproductive branches comprising stem, leaf and floral tissue. Morphological studies indicate that the Amaryllidaceae inflorescence is a bostryx, a special case of a cymose inflorescence whereby the axis terminates and branching occurs laterally. We use model-based approaches to infer ancestral states and test this morphology-based hypothesis of inflorescence development in a phylogenetic framework. Sequences from Genbank were used to generate a multiple sequence alignment for XX species and to infer a phylogeny for Amaryllidaceae and closely related Asphodelaceae, Asparagaceae and Xantharaceae. The phylogeny was time calibrated using three independent fossils placed at nodes. Character states were scored from the literature using the Endress framework of inflorescence classification. Our models support the inference of raceme-derived ancestor for Amaryllidaceae – whereby branching occurs apically rather than laterally. Our work calls for a reinterpretation of the morphology of Amaryllidaceae inflorescence and the use of developmental genetics to clarify expression patterns and their influence on overall form. This study highlights the power of model based approaches and phylogenetic tree thinking to 1) understand patterns of morphological evolution, and 2) generate testable hypotheses for the evolution of morphology. Additionally, a discussion on the generation and use of morphological models is presented.

**Key words:** Inflorescence, Evo-Devo, Phylogenetic Comparative Methods

### The fossil record of Zingiberales

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Zingiberales are an ecologically and economically important group of (sub)tropical plants, comprising eight families and ~2,500 species today, with a fossil record extending back to the early Late Cretaceous from the Northern Hemisphere and South America. Leaves, stems, fruits, and seeds

have all been attributed to Zingiberales, but no pollen. A novel phenotypic framework for reinterpreting fossils was constructed, using microCT (industrial and synchrotron-based) data of fruits and seeds, and leaf architecture. Phylogenetic analysis confirms *Ensete oregonense* belongs in Musaceae, but all other described fruits/seeds were found to be crown Zingiberaceae, and fossil pyrenes of Heliconiaceae are now recognized. Vegetative parts have fewer characters than seeds for robust placement at the genus or species level, but allowed confirmation of familial affinities. Many vegetative fossils were found to be insufficiently preserved to accept them in Zingiberales, but fossil leaves are confirmed for Zingiberaceae and Musaceae, and some morphotypes belonging to Costaceae or Marantaceae. Zingiberaceae are best represented in the fossil record, while Strelitziaceae, Cannaceae, and Lowiaceae have no reliable fossil record. Robust results confirming affiliations and phylogenetic positions of fossil taxa are critical for reconstructing the evolutionary and biogeographic history of the clade.

**Key words:** anatomy, microCT, morphology, paleobotany, *Spirematospermum*.

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### **Speciation and morphological evolution in spiral gingers (Costaceae)**

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Pollinators can drive adaptive divergence in floral traits and contribute to the maintenance of reproductive isolation among closely related species. A rapid radiation emerged within the spiral gingers' evolutionary history 10 million years ago, following an event of long distance dispersal from Africa to Tropical America. In the resulting Neotropical *Costus* clade, ornithophilous morphology repeatedly evolved from melittophilous flowers. Here, a macroevolutionary approach was chosen to investigate evolutionary correlations between flower and inflorescence traits potentially related to pollination. Nine inflorescence and flower characters were assigned to Costaceae species based on taxonomic literature and herbarium specimen analysis. By correlating binary characters states on Costaceae phylogenies, we detected that labellum margin integrity and external bract colors are significantly correlated to pollinator shifts. The modular evolution of few morphological flower attributes selected by pollinator animals could have contributed to the rapid radiation documented for Neotropical *Costus*.

**Key words:** biogeography, flower morphology, macroevolution, pollination, Zingiberales.

## Zingiberales II - Diversity

### **Explaining the differences in African and Neotropical species richness by comparing diversification rates in *Renealmia* (Zingiberaceae)**

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The well-known high species richness of the tropical forests is not uniform through its different regions; Africa is species-poor when compared to Southeast Asia and the Neotropical region. One

of the hypotheses for differences between the richness in the Neotropics and Africa points to the importance of recent speciation in the Neotropics. Andean-centred taxa probably diversified in response to the opportunities for speciation offered by the final uplift of the tropical Andes (during the past *c.* 25 million years [Ma] to the present, with higher rates on the past 10 Ma to the present). We test this hypothesis in the genus *Renealmia* L.f. (Zingiberaceae), an Andean centred lineage (*c.* 68 Neotropical spp.) that also occurs in Africa (*c.* 17 spp.). We did a taxonomic account of the Colombian species (*c.* 37; the country with the most species) and designed a new approach for obtaining nuclear phylogenetic markers for estimating species-level phylogenies using transcriptomes for recent diversifications, which could be applied to samples from herbarium specimens. Finally, we estimated and compared diversification rates of the Neotropical and African lineages using dated phylogenies based on the trees estimated.

**Key words:** *amphi-Atlantic distribution, Andes, diversification rates, Renealmia, Zingiberaceae.*

### **Diversification dynamics of Marantaceae in the Amazon: the role of landscape transformation and functional strategies**

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An intriguing evolutionary question is why certain lineages are more diverse than others and what drives diversification dynamics. Amazonia harbours the greatest diversity of plants in the world, but such enormous diversity is not evenly distributed between clades. Using seven clades of Marantaceae, we demonstrate that the fast-slow growth functional trade-off (measured by leaf traits) is the major determinant of widely observed distinct diversification rates. Species richness in each lineage was well predicted by leaf traits. Clades with acquisitive strategy were often associated with highly productive habitats – nutrient-rich soils and bottomlands – and their origin and diversification dynamics match past geological events mediated by Andean uplift. Alternatively, diversification rates of conservative clades were not affected by any paleo events. Our findings indicate that past geochemical and physical transformation of landscape in the Miocene driven by Andean uplift provided opportunities to the evolution of clades with fast grow strategies. This functional strategy is often characterized by short generation times and high metabolic rates, which may have boosted the rate of molecular evolution and consequently the diversification in Amazonian forests. Intrinsic traits of species proved to be a crucial aspect to reveal the complexity history of diversification in tropical forests.

**Key words:** *diversification rates, functional traits, Marantaceae, soil, tropical forest.*

**Funded by:** PPBio, CNPq, BDFFP, FAPEAM/FAPESP, Danish National Research Council, Academy of Finland

### **Functional and biogeographical patterns of Zingiberales in the Amazon**

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Our studies of Zingiberales across the Amazon have shown that species distribution patterns are linked to soil and water gradients both at local and large scales. At local scales, and landscapes with low variation in soil fertility, water drainage given by topography is the main determinant of species distribution. At larger scales, soil fertility becomes more important determinant of composition. Moving forward to understand how these environmental filters structure ginger communities, we analysed functional traits of species in 451 plots across the Amazon. We expected that high productivity habitats (rich soils, bottomlands, wet zones) would promote functional convergence. However, these habitats are often associated with high levels of forest disturbance that could also promote divergence in functional composition. Among communities, functional composition mostly converged following the expectation of classical acquisitive-conservative functional trade-off, with higher specific leaf area, lower seed size and plant height on nutrient-rich soils and wet bottomlands. Contrary to expectations, average community plant height and seed size diverged more in drier regions. Within communities, functional variation was higher in bottomlands and nutrient poor soils. Among traits, LMA (leaf mass per area) was under convergence pressure and height and seed size were under higher divergence pressure.

**Key words:** Amazon forest, biogeography, community composition, functional structure, species distribution.

**Funded by:** CNPq; FAPEAM; CAPES; MCTI.

### **Phylogeny and taxonomy of the extra-Amazonian species of *Goepertia* (Marantaceae)**

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*Goepertia* Nees is a neotropical genus of Marantaceae, resurrected to encompass ca. 250 species formerly under *Calathea*. 95 are species occurring in Brazil; of these, 70 are distributed outside the Amazon, i.e., Atlantic Forest (AF), Cerrado (Brazilian savanna) and Caatinga (semi-arid vegetation). A comprehensive multi-locus phylogeny including 50% of the extra-Amazonian species diversity revealed that all these species belong to the Breviscapus clade, despite what was expected following the previous phylogeny of *Goepertia* and the infrageneric morphological-based classifications of *Calathea*. The Breviscapus clade is morphologically heterogeneous, gathering species from *Calathea* Sect. Monotiche, Sect. Breviscapus Benth. (Sér. Nudiscapae and Rhizanthae in Schumann, 1902), and Sér. Scapifoliae. Morphological characteristics classically applied to distinguish the infrageneric groups in *Calathea*, such as the absence of the outer staminode in the flower, the presence of a cauline leaf bearing the inflorescence, the inflorescence borne on a leafless separate shoot, and others, are apparently homoplastic and likely insufficient to circumscribe taxonomic groups. The infrageneric classification of *Goepertia* was not formally proposed and the question remains: are there synapomorphies to such a morphological heterogeneous group out there?

**Key words:** Atlantic forest, cerrado, *Goepertia*, infrageneric classification, phylogeny.

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### **Testing targeted gene enrichment strategies useful to recover a fully supported phylogeny in a rapid and recent plant radiation: the genus *Heliconia* (Heliconiaceae, Zingiberales)**

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In *Heliconia*, the only genus within the family Heliconiaceae (order Zingiberales), ca. 195 species are native to rainforests in the Neotropics, Asia and the South Pacific. Previous studies suggest that species diversification in the genus occurred extremely fast in just over a few million years, thus phylogenetic reconstructions within *Heliconia* have been challenging so far. The overall goal of this study was to test the usefulness of HybSeq techniques (i.e., sequencing targeted nuclear genes and “off-target” chloroplast genomes), to reconstruct a fully supported molecular phylogeny for *Heliconia*. First, we tested the hybridization efficiency of a set of target probes previously designed for the Zingiberaceae in a variety of *Heliconia* samples that are representatives of the taxonomic, geographic, and morphological range of the genus. Second, we tested different phylogenomic pipelines explicitly designed for analysis of HybSeq data. Our goal was to recover the highest possible number of exons and intron target sequences, as well as the most accurate alignments and supported phylogenetic reconstructions for the *Heliconia* species sampled. On average, we were able to recover half of the original target loci, for more than 800,000 bps of genomic information. In addition, almost complete chloroplast genomes were also recovered for all taxa.

**Key words:** *Heliconia*, HybSeq, phylogenomics, rapid diversification, target enrichment.



## Posters

### Morphology, development and cellular biology

#### Anatomical and reserve carbohydrate characterization of vegetative organs from Atlantic rainforest *Dioscorea* species (Dioscoreaceae)

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*Dioscorea*, the largest genus and the most geographically widespread taxon of Dioscoreaceae, has species-level taxonomic problems due to the morphological similarities and scarcity of samplings. Some species are used for human consumption, but they are a neglected and underutilized crop in Brazil. Here the anatomy of the vegetative organs of *Dioscorea delicata*, *D. tauriglossum* and *D. ovata*, Brazilian native species, were investigated using light and scanning electron microscopy to survey diagnostic characters to help their taxonomic delimitation. Reserve carbohydrates of the thickened underground organ were extracted and quantified biochemically according to specific methodologies. The anatomical features useful to delimit the three species are found in leaf (mesophyll type, cuticular ornamentation and sclerification degree of the interfascicular parenchyma), stem (shape, cuticular ornamentation, and trichomes), root (phenolic idioblasts and pericyclic stratification) and rhizophore - thickened underground stem (raphidic idioblasts and parenchyma rays). Appreciable amounts of starch were found in the rhizophores of *D. delicata* (49.25%), but not in *D. tauriglossum* (7.81%) and *D. ovata* (8.76%). Our findings contribute to showing that the three species are distinct as the structural features do not support the synonymization proposed to these species, and also to demonstrate the food potential of *D. delicata*.

**Key words:** *cará*, rhizophore, starch, structural analysis, taxonomy.

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#### Comparative floral micromorphology and anatomy of Thurniaceae (Poales)

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Thurniaceae comprise two genera: *Prionium*, with one species restricted to South Africa, and *Thurnia*, with three species that occur in Northern South America. The floral micromorphology and anatomy of *Prionium serratum* and *Thurnia sphaerocephala* were studied to characterize the family and evaluate their systematic importance. Although both species have trimerous flowers, we

observed dimerous ones in *T. sphaerocephala*, with inconstancy in the number of stamens. Both species studied share tepals with elongated epidermal cells with straight outer periclinal walls and subdermal supporting tissue. Stamens have basifixed tetrasporangiate anthers, with connective protrusion only in *P. serratum*. This latter has ovary with protrusions, two rows of ovules per locule with axile/parietal placentation; in *T. sphaerocephala* each locule has a single ovule with an appendage and axile placentation. Stigmatic surface differs among both species, presenting long papillae in *P. serratum* and short ones in *T. sphaerocephala*. The micromorphological and anatomical features here described characterize the species studied, but are not exclusive of Thurniaceae. This set of characteristics show more proximity between Thurniaceae and Juncaceae than with Cyperaceae.

**Key words:** cyperids, gynoeceum, merism, *Prionium*, *Thurnia*.

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### **Glandular punctations on leaf surfaces of *Anthurium* Schott (Araceae, Alismatales): ontogeny, anatomy and histochemistry**

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The presence or absence of glandular punctations on the leaf surfaces is a feature widely used in the specific characterization within *Anthurium*. Although relatively easy to verify in specimens in the field or in collections, their origin and function remain poorly understood. The aim of our study was to elucidate their ontogeny, anatomy and histochemistry sampling some representatives of *A. sect. Urospadix*, *A. sect. Pachyneurium*, and *A. sect. Tetraspermium*. Standard techniques of light microscopy and histochemistry were performed. Punctations were observed on the leaf surfaces of all species sampled, even in those described as lacking the character. In most species, punctations are inconspicuous and scattered, usually occurring on the abaxial surface. In transverse sections, punctations present three regions that are distinct by the cell arrangement (apical, central and basal regions) corroborating previous studies. The inception of the punctuation seems to be related to some stomatal complexes, differing in the accumulation of phenolic compounds in addition of mucilage or solely by crystals. Our data suggest that the presence/absence of gland punctations should be used with parsimony in the specific delimitation in *Anthurium*.

**Key words:** *A. sect. Pachyneurium*, *A. sect. Tetraspermium*, *A. sect. Urospadix*, epidermis, phenolic compounds.

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### **Anatomy of the leaf blade and scape of *Leiothrix* (Eriocaulaceae, Poales) and its contribution to systematics**

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Eriocaulaceae is one of the angiosperm families that occurs in Brazilian *campos rupestres*, comprising 1,400 species in 10 genera. Within the family, *Leiothrix* Ruhland comprises 47 species occurring in Brazil. Although *Leiothrix* is recognized as monophyletic, infrageneric relationships still need to be clarified. Thus, following standardized methods, an anatomical study of leaf blades and scapes was carried out in order to seek useful features for the systematics of *Leiothrix*. All the studied species exhibit leaf blades and scapes with thin cuticle, single-layered epidermal cells, stomata, and collateral vascular bundles surrounded by a double sheath. Stomata occur on the abaxial surface of the leaf blade; and, in the mesophyll, chlorenchyma occurs between each collateral vascular bundle, with bundle sheath extension (girders). Scapes exhibit an anatomical pattern comprising epidermis, cortex, and vascular cylinder. Leaf blade anatomical features such as thickness of epidermal cell walls, type of substomatic chamber, structure of leaf blade margin and hypodermis, and type of vascular bundle girders, plus scape shape in cross section, and rib and pericycle arrangement are useful in delimiting species. Anatomical features are useful in diagnosing species and also delimiting species within different subgenera.

**Key words:** anatomical structure, *campos rupestres*, evergreen plants, monocots, taxonomy.

**Funded by:** CAPES; CNPq

### **Morphometric studies of the C<sub>4</sub> genetic model *Setaria viridis*, complementing the BBCH scale of development**

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The universal phenological scale Bundesanstalt, Bundessortenamt und Chemische Industrie (BBCH) provides a methodological framework for identifying and interpreting phenotypic differences among mutant and/or natural ecotypes. During development of the A10.1 BBCH scale of *Setaria viridis*, a genetic model for C<sub>4</sub> monocotyledonous plants, measurements of morphological attributes of accessions A10.1 and Ast-1 were also performed. The following morphological attributes were measured: total leaf number, flag leaf number, length to the 9th sheath, final height of the main stem, length of internodes, total number of panicle branches, number of fruits in the main panicle, peduncle and main panicle length. In order to summarize information and investigate

morphological groupings, we used Principal Component Analysis. For comparisons between the accessions, the best indicators of plant behavior were leaf and panicle length, total number of leaves, and flag leaf number. The most homogeneous parameters of accession A10.1 (coefficient of variation < 15%) were total number of leaves, flag leaf number, length of the 1<sup>st</sup> and the last juvenile leaf, length of the 9th leaf, and panicle length. The evaluation of the morphology contributes to phenotypic characterization, increasing the possibilities of detecting phenotypic differences, contributing to the elucidation of the genetic functions.

**Key words:** A10.1, Ast-1, growth, morphology, PCA.

### **Floral anatomy and micromorphology of *Barbosella miersii* (Orchidaceae: Pleurothallidinae)**

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*Barbosella miersii* (Lindl.) Schltr., an orchid species endemic to the Atlantic Forest of southeastern Brazil is characterized by the smallest flower of the genus with translucent color and the lip is hinged to the tip of the column-foot, whereas in most species of *Barbosella* the lip is connected to the column-foot with a “ball-and-socket” articulation. The aim of this work was to describe the floral micromorphology and anatomy of *B. miersii* to identify secretory structures and investigate the relationship with attraction of pollinators. Fresh flowers were submitted to histochemical tests as lugol and neutral red reacting at the apex of sepals, petals and lip. The samples were analyzed using and light microscopy (LM) and scanning electron (SEM). Papillose-shaped epidermal cells with dense cytoplasmic content was found in the margin of sepals and petals. Stomata are present in the adaxial face of the sepals. Epidermal cells of the lip adaxial face has larger and dense content and conspicuous nuclei then epidermal cell of the abaxial face. The anatomy and micromorphology of the sepals, petals and lip suggest the presence of osmophores in the regions studied.

**Key words:** histochemical tests, orchids, osmophores, scanning electron microscopy (SEM), secretory cells.

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### **Comparative floral development of *Lachnocaulon* and *Tonina* (Eriocaulaceae) with considerations about perianth reduction in Paepalanthoideae**

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Eriocaulaceae generally have unisexual flowers arranged in bisexual inflorescences. Flowers in Paepalanthoideae are usually 4-whorled, but *Lachnocaulon* and *Tonina* form a clade characterized by the reduction of the corolla. *Lachnocaulon* is frequently described as lacking petals. *Tonina* is described as having petals reduced to lobes in pistillate flowers. We analyzed the floral development in both genera using scanning electron microscopy to better understand the evolution of the perianth reduction in Paepalanthoideae. For comparison purposes, we analyzed one species of *Actinocephalus* and one of *Paepalanthus*. In *Tonina*, petals arise from divisions of common petal-stamen primordia and develop normally in flowers of both sexes. However, they have reduced size in comparison to the petals of *Actinocephalus* and *Paepalanthus*. In *Lachnocaulon*, petals arise in some staminate flowers, but they appear later than in the other species, from the developing stamen. The highest degree of petal reduction in the pistillate flowers and the emergence of petals in some staminate flowers of *Lachnocaulon* suggest an evolutionary trend in petal reduction in these genera.  
**Key words:** floral evolution, merism, ontogeny, *Paepalanthus* sensu lato, Poales.

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### **Does leaf anatomy aid in species identification of *Butia* (Arecaceae)?**

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*Butia* (Becc.) Becc. is a Neotropical genus whose identification is based mostly on characters from external morphology, which are sometimes variable or inadequate for species differentiation. We aimed to verify if leaf anatomy of *Butia* species brings new characters suitable for species identification and if it corroborates the phylogenetic relationship within the genus. Moreover, we propose an anatomical key to assist in species identification. Pinnae were collected and subjected to the usual techniques for light and scanning electron microscopies. All species have an isobilateral mirrored mesophyll, amphistomatic leaves and secondary vascular bundles with sclerenchymatic sheath reinforcement connected to the hypodermis. Among the species studied, *B. marmorii* and *B. matogrossensis* showed exclusive characters. For the other species, up to five characters are sufficient for delimitation. Our anatomical key presents relevant characters that allow the identification of the recognised species of *Butia*. Reliable anatomical characters of easy observation, especially the raphides are valuable in species distinction. Leaf anatomy, already used to support new taxa in related genera such as *Allagoptera* and *Syagrus*, can also be useful to validate questionable *Butia* species and differentiate between similar species but do not reflect the proposed relationship between *Butia* species.

**Key words:** anatomical key, Attaleineae, Palmae, plant anatomy, taxonomy.

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## Origin and homology of complex structures in flowers of the *Ornithocephalus* clade (Oncidiinae, Orchidaceae)

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The Neotropical *Ornithocephalus* clade (OC), subtribe Oncidiinae (Orchidaceae), consists of small epiphytes comprising 12 genera and approximately 120 species, which may present complex floral structures. In order to investigate the origin of the tabula infrastigmatica and lateral appendages found at the base of the gynostemium of some species, we analyzed flowers at various developmental stages using scanning electron microscopy (SEM). We studied representatives of three genera: *Ornithocephalus* Hook. (2 spp.), *Phymatidium* Lindl. (2 spp.) and *Zygostates* Lindl. (2 spp.). In *Phymatidium*, the tabula infrastigmatica has a mixed origin: its central portion is derived from the lateral carpels whereas the lateral portions have staminodal origin. The lateral wings of *Phymatidium* derive from the gynostemium, arising in late development. The two *Zygostates* species investigated lack a tabula infrastigmatica, and the lateral appendages at the base of the gynostemium have staminodal origin. In *Ornithocephalus*, tabula infrastigmatica and staminodes are absent. The lip callosity present in all specimens studied differentiates later from labellar cells. Contrary to previous suggestions, the tabula infrastigmatica of the OC is not homologous to the lip callus and neither are the lateral appendages and lateral wings homologous structures.

**Key words:** floral ontogeny, gynostemium, SEM, staminodes, tabula infrastigmatica.

**Funded by:** DAAD (grant 57378183/91669890) for the first author and CAPES (grant 88881.065009/2014-01) and CNPQ (Proc. 308460/2017-0) for the third author.

## Functional characterization of C2H2-type zinc finger transcription factors involved in the development of grass inflorescence

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The grass family (Poaceae) includes over 12,000 species among which are outstanding cereals that constitute a very important part of human intake. The final form of a grass inflorescence is the result of a balance between the ability of the axillary meristems to form branches or terminate in spikelets. The *Cys2-His2-type (C2H2) zinc finger* transcription factors seem to play a central role at this stage of development. By comparison of *C2H2 zinc finger* peptide sequences available in databases and others generated in our laboratory, we observed partial conservation of the sequences towards the C-terminal. The aim of this work is to explore the functional divergence of these proteins in grasses. To begin the characterization, we investigated the expression preference of the coding genes

throughout the whole body of the plant (root, stem, leaf and inflorescence) in distinct grass species. In particular, such genes are preferentially expressed in inflorescences with developing branches. The expression decays at late stages of inflorescence development. In order to generate knowledge about their functions, plants of *Arabidopsis thaliana* that overexpressed these genes were characterized. The phenotype suggests that these proteins have differential functions in regulating meristem development, hormone biosynthesis and signaling.

**Key words:** development, evolution, grasses, meristem, morphology.

### **Intra-individual root plasticity in epiphytic *Anthurium* (Araceae)**

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The plasticity in the epiphytic root system is sparsely studied, particularly in the Araceae family. Within this concept lays the rectifier property, in which within the same root continuum its traits vary according to its surroundings. We examined the hypothesis that the aerial roots from *Anthurium scandens* (Araceae) would present morphological and physiological strategies in order to survive in a canopy environment. As a means to test this idea, we made morphological, anatomical and physiological comparisons (e.g., volume, succulence, RWC, velamen, exodermis, cortex width) within the same root between three different situations: ‘aerial’ (no contact with its host), ‘contact’ (parts connected to its host and to the atmosphere) and ‘substrate’ (encircled by substrate, growing below the hosts’ bark). The obtained data showed great water loss resistance (3% loss in 10 hours). In a total of 18 traits few showed significant differences (succulence, specific dry weight, cortex width and number of xylem vessel elements), while others exhibited minor differences between the three growth situations (e.g., xylem vessel diameter, velamen and exodermis height), which perhaps justifies its restricted shade canopy habits.

**Key words:** *Anthurium*, Araceae, epiphyte, plasticity, root.

### **Photomorphogenesis in *Epipremnum aureum* (Araceae)**

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Lianescent Araceae may exhibit an opposite morphology of sun and shade leaf pattern, that is, leaves exposed to high light irradiation (HL) and desiccant atmosphere are larger in area than leaves exposed to low light (LL). The induction process of leaf area expansion in *E. aureum* is associated with prop contact (thigmomorphogenesis), but the effect of light intensity exposure still unknown. In order to understand light effect in leaf expansion, we performed an experiment with six treatments (also considering the effects of grown axis and contact stimulus): (1) high light-horizontal, (2) high light-vertical-contact, (3) high light-vertical-no contact, (4) low light-horizontal, (5) low light-vertical-contact and (6) low light-vertical-no contact (n=10). Leaf morphological data was collected after ten months. High light exposure statistically proved to have an inductive role in

leaf area enlargement, regardless of contact, in all treatments. Therefore, leaf expansion is optimized when light is paired with vertical growth and contact, achieving leaves exceeding 1 m<sup>2</sup> in the treatment high light-vertical-contact. This evidence suggests light as a co-inducer of leaf expansion in *E. aureum*, a highly plastic and widespread aroid vine.

**Key words:** Araceae, golden pothos, leaf area, light foraging, vines.

### **Foliar anatomy of *Vriesea* (Vrieseae, Tillandsioideae, Bromeliaceae)**

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*Vriesea* Lindl., one of the four genera subordinated to the Vrieseinae subtribe, includes species with different substrates, traditionally divided between two sections. About 90% of *Vriesea* species occur in Brazil, having as a diversity center the Atlantic rainforest. Considering the great morphological, ecological and taxonomic diversity of the genus, we intend, through histochemical analyses and leaf anatomy from 24 selected species, to evaluate if leaf structure is related to the morphological groups, substrates or environments. The leaves are hypostomatic with absorptive trichomes. In cross section, epidermal cells with thickened walls, covered by cuticle and epicuticular wax were observed. The mechanical hypodermis is usually one-layered. Water-storage parenchyma occurs on both sides of the blade and chlorenchyma, on its median portion. Air lacunae are associated with diaphragms constituted by arm-cells. Collateral vascular bundles, arranged alternately with the air lacunae, are surrounded by a sheath of sclerified or parenchyma cells. Extravascular fibers are positioned below the adaxial mechanical hypodermis. Leaf structure is mainly xeromorphic, reflects ancestry and recent arrival in rainforests, and does not reflect species assemblages according to morphological groups, types of substrates or environments. This study provides consistent structural data that will clarify and characterize the leaf anatomy of Vrieseinae.

**Key words:** histochemistry, leaf, monocots, structure, Vrieseinae.

### **Taxonomic implications of pollen morphology in *Alcantarea* and *Vriesea* (Bromeliaceae, Tillandsioideae)**

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*Alcantarea* is related to *Vriesea*, and some species may be treated as parts of species complexes because intraspecific variation causes problems with species delimitation. This study aims to describe the pollen morphology of a broad sampling of *Alcantarea* (19 species, 43 specimens) and *Vriesea* (17 species, 31 specimens) to improve the morphological characterization of both genera. Pollen grains were acetolysed, measured, described, photographed and scanning electron micrographed. Statistical analyses were performed based on measurements of 25 pollen grains per sample. The results showed that the main pollen pattern of *Alcantarea* is similar to *Vriesea*, being



considered stenopalynous with amb elliptic (although some species had spheroidal pollen), monosulcate, sulcus with margin, reticulate ornamentation of the exine on the center area of pollen grain (except in *A. imperialis*, which displayed foveolate ornamentation) and apex of the equatorial axis with different ornamentation (microreticulate or psilate-perforate) from the center area of pollen grain. Nevertheless, secondary pollen characters provide useful tools for infra- and intergeneric palynotaxonomy, such as the calota ornamentation and sexine thickness, and contributed to separation of the majority of the species of *Alcantarea* from *Vriesea*, mainly due to the predominance of a microreticulate calota and the smaller dimension of the sexine of the latter.

**Key words:** Bromeliaceae, palynotaxonomy, pollen grains, Tillandsioideae, Vrieseae.

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### **Micromorphological study of the leaf epidermis of *Dendrocalamus asper* and *Dendrocalamus giganteus* (Poaceae: Bambusoideae)**

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*Dendrocalamus asper* (Schultz & Schultz f.) Backer ex K. Heyne (Bambusoideae: Poaceae), widely cultivated in Brazil, was in the past erroneously identified as *Dendrocalamus giganteus* Wall. ex Munro. The present study provides a comparison of the micromorphology of the leaf epidermis of the two species using light and scanning electron microscopy. For preparation of the paradermic sections leaf fragments were immersed in Franklin solution, and for analysis of scanning electron microscopy leaves were dehydrated in ascending ethanol series, subjected to critical point drying; mounted on aluminum stubs and metallized with gold. The results showed that both sides of the leaf lamina are divided into a costal and intercostal zone presenting characteristics in common: long cells, short cells, stomatal complexes, interstomatal cells, bicellular microhairs, papillae, dumb-bell silica cells and hooks. The most significant differences between the species were: presence of bifurcated papillae, cross-shaped silica bodies and unicellular longer and pointed macrohairs in *D. asper* and their absence in *D. giganteus*. In addition to these characters, in *D. giganteus* larger hooks and in greater quantity were observed, and they have prominent papillae at the base.

**Key words:** bamboo, bamboo taxonomy, leaf anatomy, surface view, ultrastructure.

**Funded by:** CAPES/CNPq

### **Ontogeny of unstratified and multiple epidermis, hypodermis and fiber strands in different tribes of Cyperaceae (Poales)**

In Cyperaceae, leaf blade characters are often employed in taxonomic and phylogenetic studies, especially those related to epidermis and hypodermis. However, in mature organs these tissues can be interpreted erroneously because they have different origins. So, to the correct denomination of epidermis and hypodermis, including fiber strands, ontogenetic studies are necessary. Thus, we studied the ontogeny of these tissues in 10 taxa belonging to eight tribes of Cyperaceae to determine the appropriate terminology for each tissue. For this, young rhizomes with vegetative apices were collected, fixed in FAA 50, stored in 70% ethanol and embedded in synthetic resin. Transverse serial sections of young leaves, located in a rhizome apex, were made using microtome. The occurrence of a multiple epidermis was verified in species of Abildgaardieae, Cypereae and Rhynchosporaeae, and the unstratified epidermis in Cariceae, Eleocharideae, Schoeneae and Sclerieae. A continuous hypodermis was observed only in Hypolytreae. The fiber strands originate from the protodermis in all species studied. Through the obtained data the number of species with multiple epidermis is amplified and the occurrence of hypodermis in Hypolytreae is confirmed. In addition, we emphasize that the subepidermal layers, denominated fiber strands, cannot be treated as hypodermis, since they derivate from the protodermis.

**Key words:** anatomy, development, homology, leaf, taxonomy.

**Funded by:** Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, CAPES, Brasil.

### **Effects of saflufenacil and clomazone herbicides on leaves of *Setaria viridis* C<sub>4</sub> model plants**

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*Setaria viridis* is a weed morphologically similar to most of the Panicoideae subfamily members. In addition, this species was proposed as a genetic model system for studies about C<sub>4</sub> photosynthetic metabolism. The aim of this study was to evaluate the morphological responses of the *S. viridis* C<sub>4</sub> model to saflufenacil and clomazone herbicides. Twenty-day-old plants grown in 0.4 liter pots were sprayed with five fractions of the commercial dose of saflufenacil and clomazone herbicide (98 and 1000 g ai ha<sup>-1</sup>, respectively). Leaf damages such as wilt and color alterations were evaluated at 2, 4, 8, 24, 48, 72 and 168 h after application (AA). Effects were observed as early as 24 h AA in both herbicides treatments. Saflufenacil effects were observed on old leaves presenting rounded spots and brownish color. At the end, plants were completely wilted and dried. Clomazone affected new

leaves, which became chlorotic. Latterly the plants exhibit wilted and completely colorless leaves. Higher doses of both herbicides had severe effects. *S. viridis* C4 model has shown potential for evaluating the effects of the tested herbicides.

**Key words:** genetic model, herbicide, morphology, Panicoideae, *Setaria viridis*.

### **Revealing dimorphic root anatomy of the underground and mycoheterotrophic orchid *Pogoniopsis schenckii* (Orchidaceae: Vanilloideae)**

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Orchids have great diversity and may be autotrophic and photosynthetic or mycoheterotrophic and totally fungal dependent for their nutrition. Mycoheterotrophic plants may have distinct morphology in the roots. The aim was to elucidate root system of *Pogoniopsis schenckii*. Roots of different individuals were fixed and usual histological techniques were carried out. *P. schenckii* has two root types, one with an acute apex and the other one with a rounded apex. None of the apices have a rootcap or velamen. The promeristem is open. Cortical parenchyma cells are isodiametric, cytoplasm is dense and nucleus prominent. Roots with a rounded apex have large amounts of starch accumulated in apical cells. This reserve of starch also occurs in roots with an acute apex, in smaller quantity and farther from the apex. Fungal hyphae were found in all roots, distributed in the epidermis and cortex, including cells that make starch reserves. The presence of two root types in *P. schenckii* may be related to different developmental stages. The roots that accumulate starch at the apex can be suppliers of carbon to ensure energy the reproductive phase of the plant.

**Key words:** achorophyllous plants, fungal dependent, Orchidaceae, starch, terrestrial orchid.

**Funded by:** Project funded by FAPESP 2015 / 26479-6. CAPES scholarship to first author.

### **Influence of herbicides on anatomical and ultrastructural traits of *Setaria viridis***

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*Setaria viridis* is being promoted as a model C<sub>4</sub> grass because it has a small genome, a short life cycle, small stature and transformation protocols are improving rapidly. Herbicides are commonly used in agriculture and most of the studies attempt to understand how herbicides influence plant physiology and metabolism. Our goal was to analyze the effects of herbicides on leaf anatomy and leaf surface by light and scanning electron microscopy. Leaf sections from the expanded leaves were immersed in a Karnovsky solution, post-fixed, dehydrated in a series of ethanol, then polymerized in Spurr resin. Leaf cross sections of control plants showed all cells completely preserved. In contrast, after paraquat application, mesophyll cells, bundle sheath cells and epidermal cells became completely withered. During surface analysis, a disorientation of epidermal cell caused by possible plasmolysis was observed. The shape of the stomata was narrowed, and the guard cells and subsidiary cells were fully retracted. Clomazone herbicide decreased the intercellular spaces and cell sizes in all leaf tissues. Flattening of the epidermal cells was observed. The guard cells and subsidiary cells showed pronounced wilting. Our preliminary results are a step toward a better understanding of how herbicide affects *S. viridis*.

**Key words:** anatomy, herbicides, morphology, *Setaria viridis*, ultrastructure.

### **Chemical composition and seed structure of *Hypoxis decumbens* (Hypoxidaceae)**

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Animal and seed interactions are directly related to the morphological structure and chemical compounds of plants. The presence of lipids in seeds of *Hypoxis decumbens* L. suggests that ants would be the dispersers of this species. Although, according to some reports, the dispersion may occur by water. Results from the Plant Anatomy Lab (IB/Unicamp) showed structures similar to oil droplets in the internal integument of *H. decumbens* seeds. The aim of this study was to investigate chemical composition and describe the seed structure of *H. decumbens*. Histochemical tests were carried out with cross sections of the seeds, using the following reagents: Nile blue, neutral red, Sudan IV and Sudan black (lipids), Nadi (terpenes), lugol (starch) and Xylidine Ponceau (proteins). Chemical analysis was performed with methanolic extract of mature seeds (1 g), by thin layer chromatography (TLC). Histochemical results showed lipids and terpenes into the internal and external integument. The endosperm showed proteins and no starch. TLC analysis revealed phenolic and terpenoid compounds. Our results elucidated the chemical compounds of *H. decumbens* seeds and, in addition, they allow a direction for further studies about seed dispersion of this species.

**Key words:** histochemistry, integument, lipids, phenolics, TLC.

**Funded by:** Capes and CNPQ

### **Vegetative anatomy of *Anathallis* (Orchidaceae: Pleurothallidinae)**

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*Anathallis* Barb. Rodr. includes 116 species subdivided into four complexes: *Anathallis* complex *Acuminatae*, *Alatae*, *Margaritifera* and *Panmorphia*. Vegetative morphology is similar between the species and anatomical features could be useful in taxonomic delimitation. Anatomy of the vegetative organs of 25 species was analyzed by light microscopy. The most important characters are: root velamen with 2-4 layers, epivelam with thickened internal anticlinal wall, presence of tilossomes, exodermis with U-shaped thickening, endodermis with O-shaped thickening, cortex varying 3-7 layers, 3-9 protoxylem poles, and sclerified pith present in all species. Ramicaul with one-layer epidermis, variable number of the layers in cortex, tracheoidal idioblasts present in some species, subepidermal layers with sclerified cells, vascular bundles variable 5 to more than 30 with variable distribution, and a concentric ring of sclerenchyma might be present. Leaf characters include: flat or semi-flat with a uniseriate epidermis, hypostomatic, 4-5 subsidiary cells, aquiferous parenchyma present with a variable number of layers, cylindrical or globose tracheoidal idioblasts present, leaf margin with or without fibers, with a variable number of vascular bundles from 3 to more than 10. *Anathallis* complex *Acuminatae* has more diagnostic characters than the others. This information could elucidate the species and complex relationships within this genus.

**Key words:** leaf anatomy, orchids, ramicaul anatomy, root anatomy, velamen.

### **Pollen wall development in *Juncus* (Juncaceae, Poales)**

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The cyperid families Juncaceae and Thurniaceae disperse their pollen grains in tetrads, an unusual feature in Poales. The aim of the present study was to describe pollen wall development in species of *Juncus*, in order to elucidate structural aspects that lead to tetrad cohesion. Anthers at different developmental stages were submitted to the usual techniques for both light and transmission electron microscopy. In all species studied a callose wall is deposited around each microsporocyte and degenerates before meiosis. The pollen wall is initiated right after the end of meiosis II with the primexine deposition around the tetrad of microspores. Sporopollenin is deposited on the primexine prior to cytokinesis and contributes to the formation of tectum and collumela. The tetrad inner walls consist only of intine. After cytokinesis, the exine is observed as a continuous layer surrounding the tetrad of microspores. The intine surrounds each pollen grain, but is also continuous around the tetrad; it is thin in the interapertural regions and thick in the apertural regions. The formation of pollen wall before cytokinesis differs from what occurs in Cyperaceae, a closely related family, in which the pollen grains are dispersed as pseudomonads, whose walls are formed after cytokinesis.

**Key words:** callose, cyperids, exine, intine, tetrads.

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## Morphopolinic diversity in dwarf species of *Dioscorea* (Dioscoreaceae)

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Within the genus *Dioscorea* (Dioscoreaceae), a group of small species is known as dwarf *Dioscorea*, presenting singular characteristics, such as the non-twining stem. Phylogenetic studies have shown that dwarf species have different lineages, appearing in 5 of the 10 large clades of the genus. Our target is the palynological study of dwarf *Dioscorea* and their taxonomic relevance. We studied pollen of 12 species of *Dioscorea*, 4 normal and 8 dwarf. The buds were obtained from exsiccatae. The pollen was acetolized for light microscopy and non-acetolysed for scanning electron microscopy analysis. Both normal and dwarf pollen grains were monads, heteropolar, ellipsoid, medium, small in 2 normal *D. acanthogene*, *D. campos-portoi* and 1 dwarf *D. humifusa*; disulcate in most species, monosulcate/disulcate in *D. anomala* and *D. humifusa*. In dwarf species, the sexine was striate in *D. besseriana* and *D. humifusa*, vermiculated in *D. itatiaiensis* and *D. perdicum*, microreticulate in *D. anomala*, rugulate in *D. sphaeroidea*, and reticulate in *D. pedalis* and *D. minima*. In normal species, pollen was mostly reticulate except in *D. grandiflora* (espiculate). We conclude that pollen morphology presents great taxonomic potential for detecting relationships between normal and dwarf species of *Dioscorea*, corroborating the assertion of several authors regarding the parallel origin of this habit and associated to survival, related adaptations.

**Key words:** *cará*, Dioscoreaceae, dwarf, palynology, yam.

## Morphological characterization of extrafloral nectaries and associated ants through phenological stages in *Epidendrum* (Orchidaceae) species from forest fragments of Northeastern Brazil

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Attracting ants by secreting extrafloral nectar is a common defense against herbivory in angiosperms, including several orchids. However, only a few of those have their extra-floral nectaries characterized. *Epidendrum* subg. *Amphyglottium* is reported to have such a strategy, but a description of their secretory structure is unavailable. This work aims to locate and describe ant attractive regions in *E. cinnabarinum*, *E. flexuosum*, and *E. macrocarpum* from Northeastern Brazil. Field observation was conducted monthly, during the 2017 austral spring and summer. Samples of continually ant-visited organs were also collected and lately analyzed under light and scanning electron microscopy. Extra-pollination nectaries occurred in floral bract insertion, sepal bases and fruit carpels. The presence of ants was general and changed organs according to phenological phase, but always guaranteed inflorescence patrolling. In all species, nectar-modified stomata occurred in all ant-attractive regions, with papillose, modified epidermis. In addition to these appendages, *Epidendrum flexuosum* exhibited specialized secretory trichomes. The lack of clear nectariferous

parenchyma indicates that direct phloem exudation may occur. As secreting structures are homologous and present in all species, ant attraction possibly was a key adaptation in the evolution of this subgenus, thus playing an important role in its diversification.

**Key words:** ants, *Epidendrum*, extrafloral nectaries, herbivory, orchids.

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### **Inflorescence axis of *Bulbophyllum* (Orchidaceae, Asparagales): structural characterization and taxonomic application to Neotropical sections**

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*Bulbophyllum* (Orchidaceae) is a pantropical genus and considered the second largest of angiosperms. Morphological and anatomical studies of its reproductive and vegetative organs are still incipient, but they have already demonstrated applicability of the character set in infrageneric and specific delimitations. The objective of our study was to verify anatomical characters of the inflorescence axis (scape and rachis) of representatives of the six Neotropical sections of *Bulbophyllum*, looking for an evaluation of anatomical evidence that can be used in the delimitation of the taxa studied. All species studied have a floral scape and rachis with great similarity and homogeneity in their anatomical characters, such as: uniseriate epidermis; stomata with suprastomatic chamber; sunken secretory trichomes; idioblasts with helical thickening or with raphides, present in both the cortex and the pith; continuous and uniseriate endodermis; and an atactostelic vascular cylinder formed by collateral vascular bundles. Our analysis also allowed the identification of two species with exclusive anatomical characteristics: *B. rupicolum* (*B. sect. Micranthae*), which has scapes with a papillose epidermis, and *B. weddellii* (*B. sect. Didactyle*), which has mixed idioblasts (with helical thickening and raphides).

**Key words:** Epidendroideae, epidermis, rachis, scape, trichomes.

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### **Facultative sporophytic apomixis in polyploid orchids of *Zygopetalum mackayi***

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*Zygopetalum mackayi* Hookr. is an apomictic species known by its multiple cytotypes (8x, 12x, 16x), which are geographically structured. The objective of this work was to clarify contradictions about the type of apomixis that occurs in this species. In addition, we examined whether the apomictic process occurs parallel to the sexual reproduction, and we verified the relationship between apomixis and polyembryony, and between apomixis and ploidy in *Z. mackayi*. Controlled self-pollinations were performed and histological analyses during seed maturation were conducted. We observed that the polyploid cytotypes are facultatively reproduced through the formation of adventitious embryo precursor cells (PEAs). These PEAs are differentiated from the nucellus in the micropylar region indicating the presence of sporophytic apomixis. The cytotype 8x has mostly monoembryonic seeds resulting from the sexual pathway and shows a high rate of abortion due to endogamy. However, there are polyembryonic seeds in all cytotypes. There are two distinct origins, one associated with multiple megaspores (all cytotypes) and the other associated with apomixis, parallel or not to the sexual embryo (polyploid cytotypes). Our results contribute to the growing database on the relationship between polyploidy and apomixis.

**Key words:** inbreeding depression, orchid, polyembryony, self-pollination, sporophytic apomictic.

## **Vegetative anatomy and floral glands of *Sansonia bradei* (Schltr.) Chiron**

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*Sansonia* is an early-divergent genus of Pleurothallidinae subtribe and comprises two endemic species of the Brazilian Atlantic Rainforest. Here, we describe the vegetative anatomy of *Sansonia bradei* and the occurrence of floral glands in this specie. Root: covered by a 2-3 stratified velamen of anastomosed thickened cells walls; tilosome absent; “∩” thickened exodermis cell walls; cortical parenchyma of ~6 layers, “O” thickened endodermis cell walls; ~6 protoxylem poles and sclerified pith. Rhizome: cortex with ~6 layers of starchy parenchyma; vascular bundles of atactostelic distribution; pith delimited by fibers. Ramicaul: semicircular with three major and two minor free vascular bundles surrounded by sclerenchyma. Leaf: covered by a thick cuticle; hypostomatic, 4-5 subsidiary cells, tetracytic or anisocytic stomata, heterogeneous mesophyll with bar-thickened; vascular bundles of two sizes intercalated. Flowers: ovary surface with six grooves and bicellular capitate colleters that release a mucilaginous exudate; labellum with osmophores restricted to the adaxial surface of the fimbriate apex; at the apex of coalescent lateral sepals, there are unicellular glandular trichomes covered by a smooth cuticle, dense protoplast with lipids drops and large vacuole. The vegetative anatomy is interpreted as related to the epiphytic habit and the floral glands as both protection and sapromyophilous pollination syndrome.

**Key words:** colleters, osmophores, Pleurothallidinae, ramicaul, velamen.

## **Characteristics and ovule types pre- and post-pollination in some Orchidaceae**

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Orchidaceae is a family with the highest number of ovules per pistil, but their morphological differentiation occurs only after pollination. The most frequent is the anatropous ovule, but there is little or no information about orthotropous and campylotropous ovules. In this research ovule traits of several Orchidaceae belonging to three subfamilies are studied and described before and after pollination: Apostasioideae (*Neuwiedia*), Cyripedioideae (*Cypripedium*, *Paphiopedilum*, *Phragmipedium*) and Epidendroideae (*Epipactis*, *Gongora*, *Huntleya*, *Keffersteinia*, *Masdevallia*, *Restrepia*, *Stanhopea*, *Stelis* and *Warczewiczella*). Ovarian sections were made with a freezing microtome and photographed with fluorescence, bright field MO and SEM. We observed two types of bitegmic ovules: orthotropous in *Cypripedium* and *Stanhopea*, and anatropous in the remaining studied genera. Before pollination, the ovule primordia adhering to the placenta are elongated and poorly differentiated, but in post-pollination the differentiation of the ovule begins with the division of placenta cells, forming a row of four to eight cells surrounded by an epidermis, including the archesporial cell in the upper part. In the primordial anatropous ovules, before the integuments are differentiated, the curvature of the apex row cells occurs. It defines the location of the chalaza and the micropyle position.

**Key words:** anatropous, Orchidaceae, orthotropous, ovules, post-pollination.

### Seed micromorphology of two species of *Xyris* (Xyridaceae)

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Xyridaceae has more than 420 spp. included within five genera. The largest genus is *Xyris* Gronov. ex L. with about 400 spp. and a pantropical distribution. Species identification is often problematic due to the high morphological similarity among the species of *Xyris*. Seed micromorphology has shown significant importance for taxonomy purposes in this group. This study aimed to analyze the seed micromorphology of two species deposited in the herbarium ESAL: *Xyris* cf. *glaucescens* Malme (689 Arruda, I.A.C. et al.) and *Xyris seubertii* L.A. Nilsson (678 Arruda, I.A.C. et al.). Mature seeds were coated with gold in an SCD 050 sputter coater. Observations were made using a Leo Evo 40 scanning electron microscope. Seeds of *Xyris* cf. *glaucescens* are distinguished by their oblong shape, truncate hilum, sulcate longitudinal ridges, smooth transversal ridges, inconspicuous longitudinal and transversal striations. Seeds of *Xyris seubertii* have an ellipsoid shape, truncate hilum, ridges absent, conspicuous longitudinal and transversal striations. The morphology of the hilum and transversal striations were the only traits shared by the studied species and *Xyris subsetigera* Malme, which belongs to the same clade.

**Key words:** *Nematopus*, Poales, seed coat, taxonomy, ultrastructure.

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### **Foliar epidermal micromorphology of representatives of *Eremitis*, *Pariana*, and *Parianella* (Olyreae, Bambusoideae, Poaceae)**

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Leaf epidermal microcharacters have been useful in the delimitation of representatives of Bambusoideae but analyses of microcharacters are scarce in Olyreae. As part of foliar micromorphological studies in the tribe, we present the patterns observed in the genera that compose the subtribe Parianinae (*Eremitis*, *Pariana* and *Parianella*), using scanning electron microscopy. The species of *Parianella* and most *Eremitis* are amphistomatic, while most *Pariana* are hypostomatic. On the abaxial surface, the stomatal complexes have two simple papillae on each subsidiary cell in *Parianella* and in some *Pariana*. Simple papillae are present on the abaxial surface of *Pariana* and *Parianella* and on the adaxial surface of most *Eremitis*. Some *Pariana* displayed papillae on the long cells forming a suprastomatic chamber. The species analyzed also possessed microhairs and prickly hairs. Silica bodies in the intercostal zone are crenate and in the costal zone they are axial-bilobate in *Parianella* and in some *Eremitis* and *Pariana*. Among the observed microcharacters, we conclude that the shape of the silica bodies in the costal zone, the presence of papillae on the subsidiary cells, on the long cells forming suprastomatic chambers and the papillae on the adaxial surface provide information useful for the taxonomic understanding of Parianinae.

**Key words:** herbaceous bamboos, microhairs, papillae, prickly hairs, silica bodies.

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### **Mucilage secretion in bromeliads: discovering horizons based on a study with *Aechmea blanchetiana* (Bromeliaceae)**

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Despite all the knowledge that has been produced regarding the bromeliads so far, secretory processes in this family remain widely understudied. This is especially true for mucilage secretory systems, for which few reports are available and even less information can be found on anatomical and secretory aspects of the mucilage source. Thus, we conducted several structural studies using

light and electron microscopy in the bromeliad *Aechmea blanchetiana* (Baker) L. B. Sm. to investigate structural and secretory aspects of possible mucilage-secreting glands. Our investigations bring us some novelties regarding secretion in Bromeliaceae, such as the first report of exogenous secretion by typical bromeliad scales in the inflorescence, and the presence of unique colleter-like glands at the leaf bases. Both structures are potentially related to the protection of young portions of the reproductive and vegetative axis. Our results indicate that mucilage has great importance in the success of *A. blanchetiana*, and we strongly believe that the presence and value of such processes extend much widely among bromeliads, as the literature suggests some additional examples. Therefore, we must consider mucilage secretion understudied and further investigations certainly should be embraced by bromeliad researchers.

**Key words:** Bromeliaceae, colleters, plant-environment interactions, polysaccharides, desiccation.

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### **Morphoanatomy of reproductive organs in species of *Typha* L. (Typhaceae)**

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A comparative morphological investigation of the reproductive organs in two species of *Typha* L. - *Typha domingensis* Pers., *T. latifolia* L. and their putative hybrid, *T. × provincialis* A. Camus is presented. Inflorescences at different developmental stages were collected from natural populations, desiccated, and submitted to morphological analysis. To visualize the internal structures, histological slides were produced, resulting from the fixation, dehydration, inclusion, cutting and mounting of permanent slides. Among the main morphological results are the neglected diagnostic features, both for species and for hybrids, such as the apex of the anthers, the morphology and maturation type of the staminate portion of the inflorescence, and the shape and position of the sterile pistillate flowers ("*carpodia*"). We analyzed the anatomy of anther, ovary, fruit, seed and sterile flowers and identified the presence of idioblasts with phenolic compounds in these different organs in all of the studied species. We confirm, for the first time under an anatomical analysis, the carpellary origin of *carpodia* and propose a taxonomic use for the morphological variations found. Finally, we discuss the evolution of the monocarpellary gynoeceium in *Typha*, comparing the modifications of the ancestral tricarpellary-trilocular condition in different families of Poales.

**Key words:** anatomy, *carpodia*, *carpodium*, Poales, *Typha*.

**Funded by:** CNPq/CAPES

### **Leaf anatomy of grasses (Poaceae) occurring in the campo rupestre of Chapada Diamantina, Bahia, Brazil**

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Members of Poaceae are abundant in campos rupestres along the Espinhaço Range, the main mountain range of Brazil. Plants are affected by distinct environmental filters in this region, but the influence of these factors on the leaf anatomy of those species has been poorly documented. We analyzed paradermal and transverse sections of the median portion of leaf blades in six species of Poaceae occurring in four areas in the Chapada Diamantina, the northern portion of the Espinhaço range, comparing these to samples of the same species occurring in the Atlantic Forest. They are members of Panicoideae and Chloridoideae subfamilies, and *Axonopus aureus*, *Echinolaena inflexa*, *Eragrostis petrensis*, *Renvoizea trinii* and *Renvoizea* sp. display expanded leaf blades, while *Andropogon selloanus* is V-shaped. Among other features analyzed, only the sinuosity of anticlinal walls and the presence of papillae on the epidermis significantly varied among species. We observed low variation in anatomic features among these species, even when we compared both environments evaluated, indicating that the pressures imposed by the environmental filters did not influence the anatomical variations of the studied grasses.

**Key words:** Chloridoideae, Espinhaço Range, Gramineae, micromorphology, Panicoideae.

**Funded by:** Capes, Flora da Bahia

### Floral anatomy and micromorphology in *Dryadella* (Orchidaceae, Pleurothallidinae)

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The delimitation of the species of *Dryadella* Luer is difficult due their high morphological similarity. The systematics of the genus has not been studied from a phylogenetic approach. Flower anatomy and micromorphology could provide more characters and also give information about floral biology. The flowers of eleven species of *Dryadella* from the Atlantic Forest and the Andes were examined using light microscopy and scanning electron microscopy. Most of the analyzed features are common in the genus such as oblong-shaped epidermal cells, striated cuticle, stomata in the upper third of sepals, sepals with crenate margins and three vascular bundles, reduced obdeltoided-shaped petals. The characters with differences between species are sepal shape, which varies from ovate with short tails to oval with long tails, the presence of papillae at the petal base in *D. summersi*, the lip with a dentate margin in *D. ataleiensis* and the papillae at the base of the column-foot in *D. lilliputiana* and *D. simula*. The morphology of epidermal cells of the sepals and lips indicate a secretory structure and future studies involving ultrastructural analyses are necessary for understanding the compound release mechanism and the occurrence or not of deceptive pollination.

**Key words:** floral biology, reproductive organs, scanning electron microscopy, secretory structure, trichomes.

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## Palynological features of species of *Anthurium* sect. *Urospadix* (Araceae, Alismatales)

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*Anthurium* sect. *Urospadix* Engler comprises two clades supported mainly by vegetative and biogeographic characters: clade 1, formed by Brazilian species, and clade 2, formed by species from western South America, Central America and the Caribbean. The aim of our study was the analysis of micromorphological and ultrastructural pollen characteristics of Brazilian species of *A.* sect. *Urospadix*. Standard techniques of both scanning and transmission electron microscopies, as well as of light microscopy, were used. All species studied present ellipsoid and aperturate pollen; exine varies from reticulate to microreticulate; exine sculpture is mostly spinescent, except in *A. augustinum*, *A. loefgrenii* and *A. miquelianum*, which show psilate walls; and crystals occur attached to the pollen surface of all species. Both shape and type of aperture here described for species of clade 1 are homogeneous characteristics already described for the genus. However, reticulate exine is an unusual feature in Araceae and is reported for some Brazilian species of *A.* sect. *Urospadix*. Variation in the shape of crystals may indicate an application in delimitation of species from clade 1, especially *A. gladiifolium* which presents spherical crystals.

**Key words:** *Anthurium* sect. *Pachyneurium*, aperture, crystal, exine, pollen.

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## Germination and protocorm development of the mycoheterotrophic orchid *Pogoniopsis schenckii* (Orchidaceae: Vanilloideae) by the fungal isolate of the genus *Clonostachys*

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During the germination of orchid seeds occurs the formation of the protocorm, a coniform structure. Orchid seeds need to be associated with mycorrhizal fungi for protocorm development. This fungi form pelotons inside living plant cells. However, little is known about the role of non-mycorrhizal fungi for germination in Orchidaceae. *Pogoniopsis schenckii* Cogn. is an endangered, aclorophylactic and mycoheterotrophic orchid. The objectives of this study were to promote symbiotic germination and to analyze the ultrastructure of the *P. schenckii* protocorm. For this, trials of symbiotic germination with fungus of the genus *Clonostachys*, previously isolated from the fruits of the species, were carried out. Anatomical ultrastructural analyses were performed with the samples obtained. Seed germination was observed at 20 days of the experiment, resulting in the slow initial development of protocorms. The protocorms analyzed presented living cells with intact cell walls and organelles. In addition, fungal hyphae could be observed throughout the protocorm cell, but there was no peloton formation by the fungal isolate. This is the first report of the germination of a myco-heterotrophic orchid species in the presence of non-mycorrhizal fungi.

**Key words:** *Clonostachys*, endophytic fungi, mycoheterotrophic plant, Orchidaceae, symbiotic germination.

**Funded by:** Project funded by FAPESP 2015 / 26479-6.

### **Micropropagation of *Epidendrum secundum* using *Kappaphycus alvarezii* (Rhodophyta) extract: anatomical features and free polyamines quantification**

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This study aimed to evaluate the effect of culture medium supplementation with different concentrations of *Kappaphycus alvarezii* extract (0, 50 and 100 mg L<sup>-1</sup>) during in vitro development of *Epidendrum secundum* Jacq. After 90 days of inoculation, anatomical preparation and free polyamine (PA) quantification were performed. Root apices from 0 and 50 mg L<sup>-1</sup> treatments showed the same cell differentiation pattern. In contrast, root apices from 100 mg L<sup>-1</sup> treatment showed alterations in the differentiation pattern, with hypertrophy and shape alterations in mesophyll cells and without fiber bundles. The leaf blade of the 0 and 100 mg L<sup>-1</sup> treatments showed a well-organized epidermis. However, the mesophyll showed large and poorly organized parenchyma cells without fiber bundles. In contrast, the 50 mg L<sup>-1</sup> treatment showed more compact mesophyll with well-organized cells and many fiber bundles, which is a typical feature of the species. Free PA quantification indicated the presence of spermidine and putrescine and a gradual decrease of putrescine on 50 and 100 mg L<sup>-1</sup> treatments. Thus, the results suggest that the use of *K. alvarezii* extract at 50 mg L<sup>-1</sup> had a biostimulating effect on *E. secundum*, positively affecting the development of plantlets.

**Key words:** biostimulant, morphoanatomy, macroalgae, orchids, plant tissue culture.

### **Evolution of the gynoecium in *Xyris* (Xyridaceae, Poales)**

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The floral anatomy and development of species belonging to all sections of *Xyris* (*Xyris*, *Nematopus* and *Pomatoxyris*) were comparatively studied to elucidate the ontogenetic processes involved in gynoecium variation and to understand its evolution in the genus. Placentation has been one of the key characters in recognizing the taxonomic sections of the genus. Most of the species examined

exhibit three peripheral groups of vascular bundles, and a central cylinder from which the carpellary bundles diverge. In the species of section *Pomatoxyris*, the ovary consists of a fertile, longer synascidiate zone and a sterile symplicate zone. In the species of section *Xyris* the ovary has a short synascidiate zone, whereas the symplicate zone is longer and fertile. In the ovary of the species of section *Nematopus*, the synascidiate zone is extremely short and sterile. Considering the phylogeny of *Xyris*, axile placentation is most likely the ancestral condition, from which parietal, free central and basal placentation have derived by shortening and sterilization of the synascidiate zone and elongation of the fertile symplicate zone during the evolution of the genus.

**Key words:** flower, ontogeny, ovary, placentation, vasculature.

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### **Palynotaxonomy of three species of *Aechmea* (Bromeliaceae – Bromelioideae): *Aechmea blumenavii*, *Aechmea calyculata* and *Aechmea gamosepala* from Atlantic Forest, Brazil**

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*Aechmea* is one of the Bromeliaceae genera that has the most disagreements regarding taxonomic treatments. Some local characteristics may influence morphological variation. The habit of the individuals, associated to climate factors, may contribute to pollen morphologic changes, mainly pollen size. The present work aims to describe the pollen morphology of three species of the *Aechmea* subgenus *Ortgiesia* of different localities: *A. blumenavii* Reitz (Canelinha, SC and Itapema, SC), *A. calyculata* (E. Morren) Baker (Três Coroas, RS, Maquiné, RS and Blumenau, SC) and *A. gamosepala* Wittm. (São Bento do Sul, SC, Joinville, SC and Cambará do Sul, RS). The pollen grains were acetolysed (ACLAC), measured, described, photographed with light and scanning electron microscopy. The measurements were treated statistically. The pollen grains were considered stenopalynous: monads, medium sized, with amb elliptic and 2-(3)-pores. Nevertheless, the exine is reticulate heterobrochate with simplecolumellate muri and rare perforations, with or without a microreticulum surrounding the rounded to polygonal lumina, presence or absence of granules within the lumina; or microreticulate exine (only *A. gamosepala*). Sexine thicker than the nexine. Pollen morphology of *A. gamosepala* was significantly different and should therefore be considered in the taxonomy of the group.

**Key words:** Atlantic Rainforest, pollen grains, pollen morphology, southern Brazil, subgenus *Ortgiesia*.

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### **Ornamental potential, floral morphology, phenology, and geographical distribution of *Syagrus cearensis* (Arecaceae), a rare palm from the north portion of Northeastern region,**

## Brazil

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Here we documented the ornamental potential of *Syagrus cearensis*, considering it as a good native species to be used in urban forestation of streets and gardens. The species flowers throughout the year and attracts stingless bees, such as the specie *Trigona spinipes*, and each flower has a short life span (~1 day). The distribution indicates its occurrence in humid areas, such as Atlantic Forest and in humid areas in the Caatinga domain, usually between 100-750 m above the sea level. The species is poorly represented in herbaria collections, and most records are from northeastern Brazil herbaria. As a monoecious plant, it has staminate and pistillate flowers in the same inflorescence. Also known as *coco-católé*, the species has an average size, it usually grows from 4 to 10 m tall, with 1 to 4 stems in the same plant. Although sporadically cultivated in Natal and Rio Grande do Norte, the species is commonly extracted from the wild affecting its conservation. Different flowers/buds were observed under scanning electron or light microscopy. Flowers are yellow-cream colored. Staminate flowers have three valvar, lanceolate and asymmetric petals, six free stamens, which bear stomata on the epidermis of the anthers. Maps, potential uses in landscape design, and flower morphology are discussed.

**Key words:** mapping, Monocotyledons, native species, palms, phytogeography.

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## Anatomical and histochemical analysis of roots in *Globba* species (Zingiberaceae)

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The genus *Globba* comprises over 100 species, which are divided into three subgenera and seven sections. The present work aims to characterize root anatomy and histochemistry of the species of *Globba*. Five species were obtained from the Botany Greenhouse. Root processing followed both light and scanning electron microscopy procedures. Root cross sections show a uniseriate epidermis. The cortex consists of an exodermis with thin and lightly suberized cell walls, parenchymatous cells, lacunas and a uniseriate endodermis. The vascular cylinder is bounded by one layer of cells – pericycle, surrounding the vascular tissues. Root tracheary elements contain two cell types: tracheids and vessel elements. Vessel elements have oblique scalariform perforation plates and scalariform parietal thickening. Oleiferous idioblasts were found to be spread throughout the cortex and vascular cylinder in the root of *G. curtisii*, *G. magnifica* and *G. sherwoodiana*, and phenolic cells in the root of all species, except for *G. schomburgkii*. Simple and envelope-layered starch grains occur in the cortex and pith of roots of all species studied. *Globba curtisii* and *G. schomburgkii* are part of the same section and are supported by these evaluated characters.



*Globba* species studied show uniformity in their root anatomy, except for the variation of metabolites.

**Key words:** Globbeae, metabolites, underground organs, vessel elements, Zingiberoideae.

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### **Seed structure and its relation to germination in Bromelioideae: a case study of *Neoregelia bahiana***

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*Neoregelia bahiana* is distributed throughout the *campo rupestre* vegetation in the Espinhaço mountain range, Brazil, and belongs to a species complex. Because of the high heterogeneity of the habitat, the processes of seed germination and seedling development are very important to *N. bahiana* establishment. To better understand such processes, the structure of the mature seed was analyzed with light, fluorescence, and scanning electron microscopy. The mature seed has a mucilaginous testa, which is rich in pectin (homogalacturonans) and cellulosic compounds. Internal to the testa, the exotegmen is tanniferous and consists of cells with cellulosic thickened anticlinal walls. Positive reaction to PAS in the exotegmen cell walls indicates that these cells can play a function in the seed imbibition. Internal to the seed coat there is an aleurone layer surrounding the starchy endosperm and the differentiated embryo. During the germination, the seed coat rupture occurs at the micropylar region, where the aleurone cells start to divide, protecting the developing embryonic axis. Pectins (rhamnogalacturonans) and proteins (arabinogalactans and extensins) were detected in the aleurone cells facing the micropyle during germination, indicating they are functional at the early stages of seedling development.

**Key words:** anatomy, Bromeliaceae, immunodetection, seed coat, seedling.

**Funded by:** CAPES - Coordenação de Aperfeiçoamento de Pessoal de Nível Superior and CNPq - Conselho Nacional de Desenvolvimento Científico e Tecnológico (#455510/2014-8).

### **Binding ecology to morphology through floral anatomy of *Alcantarea* and *Vriesea* (Bromeliaceae) plus a new species from Bahia, Brazil**

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Details on Bromeliaceae floral anatomy are still scarce, especially if compared to data available for vegetative parts. In this work we investigated the floral anatomy of two genera that are practically endemic to the Brazilian Atlantic forest: *Alcantarea* and *Vriesea*. From a systematic and taxonomic

view, it is known that floral anatomy, especially when focused on nectaries, can help to delimit genera or species and reveal new data that relate plants to different types of pollinators. Histochemical tests were conducted, and we also observed flowers and buds under scanning electron or light microscopy. Our hypothesis was that infra- and interocular nectaries are more developed in *Alcantarea* than in *Vriesea* and it was confirmed. *Alcantarea* species visited by moths or hummingbirds had less developed nectaries. We observed distinct morphologies for the nectaries as well as for the nectar releasing orifices. The great amount of mucilage produced in some *Alcantarea* species is attributed to channels of mucilage and pores identified at the base of the flowers, which reacted positively for mucilage tests. We further discuss on the position of the ovary comparing *Alcantarea* and *Vriesea*, presence of pedicel, and present a new species of *Alcantarea* from Bahia related to *A. nahoumii*.

**Key words:** anatomy, morphology, pollination, Tillandsioideae, Vrieseae.

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### **Occurrence of aerenchyma in monocotyledon roots**

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Aerenchyma is a fundamental tissue for adaptation of wetland plants to their normal habitats but it can also occur in plants from dry soils. This occurrence may be due to genetic information that is maintained in the plant in places that were previously waterlogged and then became dry, which can also be expressed in plants where the opposite occurs. The aim of the present study was to analyze the occurrence of aerenchyma in the cortex of monocotyledonous roots of dry environment. Root samples of *Aechmea aquilega*, *Anthurium affine*, *Dichorisandra* sp., *Epidendrum cinnabarinum*, *Epidendrum orchidiflorum*, *Eragrostis curvula*, *Hohenbergia caatingae*, *Philodendron acutatum* and *Rhynchospora holoschoenoides* were collected in the Environmental Protection Area of the Marituba do Peixe (Piaçabuçu municipality, Alagoas state, Brazil), which underwent a soil drying process in some regions. Aerenchyma was found in three species. In one species we observed rounded intercellular spaces and in two other species, tangential lysigenic spaces. As we observed, although only a few species presented aerenchyma, our results confirm what the literature states and our data may be useful for taxonomic and physiological studies on monocotyledons.

**Key words:** cortex, dry environment, intercellular spaces, plant anatomy, tangential lysigenic spaces.

### **Floral anatomy of *Thurnia sphaerocephala* (Thurniaceae) with emphasis on the vasculature**

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Thurniaceae comprise four species belonging to two genera. Only one species occurs in Brazil, *Thurnia sphaerocephala* (Rudge) Hook. f., growing in flooded habitats in the Amazon region. This work aimed to elucidate the floral vasculature of this species, comparing it with that of other cyperid species. Therefore, anthetic flowers were embedded in historesin and serially sectioned on a rotatory microtome for light microscopy analyses. The flowers are homochlamydeous, monoclinous, with two fertile stamen whorls. The tepals and stamens are free, and the gynoecium is superior and tricarpellate. The ovary consists of a fertile synascidiate zone, with three ovules inserted on axile placentae, and a sterile symplicate zone. At the receptacle a central vascular plexus from which the vascular traces of each floral part diverge is observed, as occurs in the flowers of Cyperaceae. Each tepal of *T. sphaerocephala* receives only one vascular trace, which is probably a result of reduction processes during the evolution of the cyperids.

**Key words:** cyperid, evolution, flower, Poales, tepals.

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### Initial development in *Cattleya* roots

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Orchidaceae roots are composed of a vascular cylinder, cortex and velamen. The cell wall thickenings, and their chemical constitution in the different tissues, are variable according to the environmental conditions and probably due to stimuli during the initial growth. We described the initial development of *Cattleya* roots, determining when velamen (key tissue for survival in the epiphytic environment) differentiation occurs. Freehand sections were made in *Cattleya nobilior*, *C. schilleriana*, *C. velutina* and *C. walkeriana* roots, as well as tests for lignin and suberin detection. The roots had an area between  $5.2 \pm 1.6 \text{ mm}^2$  (*C. nobilior*) and  $11.8 \pm 2.4 \text{ mm}^2$  (*C. velutina*). All roots showed a vascular cylinder (13 to 20 xylem/phloem strands), thickened endoderm and exoderm cells (suberin and/or lignin), and a velamen. The velamen occupied about 40% of the total root area (with 4-9 layers). The meristem was closed with bulging of the outer cortical initials into the inner sides of the rootcap, curving along the base of the columella, and with separated layers of the cortical and epidermal initials (as in other Orchidaceae). The protoderm cells divided even below the rootcap, finishing their differentiation about 1-1.5 cm from the root apex (where it assumed white color).

**Key words:** functional anatomy, Orchidaceae, root anatomy, root meristem, velamen.

### Presence of fungi on the indehiscent fruit of the mycoheterotrophic orchid *Pogoniopsis schenckii* (Orchidaceae)

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*Pogoniopsis schenckii* Cogn. is a mycoheterotrophic orchid endemic to Brazil. The species is autogamous, and the role of fungi in its seed development is unknown. The objective of this work was to evaluate the ontogenesis of the fruit, trying to understand how the mycoheterotrophy influences the development of the fruit in the species. Fruits at different stages of development were collected and followed the usual methodology for anatomical studies and analysis of transmission microscopy. Anticlinal divisions were found within the ovary, promoting the increase of the diameter of the fruit. The young fruit, cells of the mesocarp of the fertile and sterile valves show an increase of the cellular volume, indicating a potential factor responsible for the growth of the fruit. The mature fruit, the adjacent epicarp and mesocarp cells begin to collapse, always from the epicarp towards the endocarp. Fungal hyphae were observed in the inner layers of the mesocarp and in the placental region. The hyphae occur within the cells and are able to traverse the cell wall. These hyphae were found in a larger quantity in the mature fruit when the cells are collapsed, and are able to involve the seed before the fruit tipping in the soil.

**Key words:** anatomy, fungal hyphae, ontogeny, Orchidaceae, saprophytism.

**Funded by:** Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - CAPES Conselho Nacional de Desenvolvimento Científico e Tecnológico - CNPQ

### **Root cortex in wetland monocotyledon plants**

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Root cortex is a primary tissue derived from the ground meristem in the root apex. Wetland plants usually develop aerenchyma in this region as an adaptive strategy to this habitat. Some studies showed a relationship between families and the pattern of development of the aerenchyma. In this work we aimed to analyze the type of aerenchyma in monocotyledon wetland plants. In total, there are nine species from the families Cyperaceae, Poaceae and Xyridaceae that were collected at the *APA do Catolé e Fernão Velho (Satuba, AL, BR)*. In Cyperaceae, we observed a tangential lysigenous aerenchyma which develops through cellular disintegration from outside to inside in three species and from inside to outside in two species. In Poaceae, we found a radial lysigenous aerenchyma with cellular disintegration from outside to inside in one species. In Xyridaceae, one of the species exhibited tangential lysigenous aerenchyma which develops through cellular disintegration from inside to outside and the other one a radial lysigenous aerenchyma with cellular disintegration from outside to inside. Although the number of analyzed species was small, the results confirm the data found in the literature. The data herein shown may also be useful for taxonomic studies on monocotyledons.

**Key words:** aerenchyma, cellular disintegration, intercellular spaces, plant anatomy, waterlogged area.

## Taxonomic revision of the annual *Paspalum* species, Plicatula group (Poaceae)

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With 214 species, mostly assigned to informal groups, *Paspalum* L. is the largest Poaceae genus in Brazil. The delimitation of species in the Plicatula group is still uncertain, and disagreement persists over the circumscription of taxa and synonymy. Many Plicatula species have potential for fodder and recovery of degraded areas. Except for one, species of this group share a dark and glossy antherium, and the lower lemma with transverse wrinkles. This work reviews the taxonomy of the annual species of *Paspalum*, Plicatula group. Results derive from exhaustive collection work, live plant cultivation, chromosome counts, and examination of national and international herbarium collections. Of all names listed for the group, the following species are accepted: *Paspalum boscianum* Flügge, *P. centrale* Chase, *P. clipeum* G.H. Rua, Valls, Graciano-Ribeiro & R.C. Oliveira, *P. commutatum* Nees, *P. convexum* Humb. & Bonpl. ex Flügge, *P. crustarium* Swallen, *P. foveolatum* Steud., *P. macranthecium* Parodi, *P. melanospermum* Desv. ex Poir., *P. plowmanii* Morrone & Zuloaga, and *P. scrobiculatum* L. Brazil has the highest species richness for this group. The lectotypification of some old names and description of a new species are necessary. Significant advances were obtained in the taxonomy of the genus.

**Key words:** diversity, morphology, midwestern Brazil, nomenclature, synonymization.

**Funded by:** Financial support: FAP/DF Grants 0193.000.979/2015 and 0193.002051/2017. Capes.

## Pollen morphology of *Dioscorea* (Dioscoreaceae) from the Rodoanel Mário Covas, São Paulo, Brazil

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*Dioscorea* L. species are subdivided into sections and present taxonomic problems, mainly due to the morphological similarities and the difficulty in sampling the dioecious climbing species and also the fact that plants with pistillate and staminate flowers have often been described as different species. This study aims to describe the pollen morphology of nine species (27 specimens) of *Dioscorea* from north and south areas of Rodoanel Mario Covas (highway SP-21) to improve the morphological characterization of the genus. Pollen grains were acetolysed, measured, described, photographed and scanning electron micrographed. Statistical analyses were performed based on measurements of 25 pollen grains per sample. The results showed that the main pollen pattern of *Dioscorea* is stenopalynous, amb elliptic, small to medium size (the larger dimensions in *D.*

*dodecaneura* and the smaller in *D. amaranthoides*), disulcate, sulcus without margin. Nevertheless, the pollen wall ornamentation contributed to the separation of two species as well as the segregation of the other seven species in two groups: psilate-perforate [*D. altissima*], rugulate-perforate [*D. monadelphica*], microreticulate [*D. dodecaneura*, *D. multiflora*, *D. tauriglossum*] and striate [*D. amaranthoides*, *D. laxiflora*, *D. olfersiana*, *D. trilinguis*]. Thus, pollen morphology provides a useful tool for the infrageneric palynotaxonomy of *Dioscorea*.

**Key words:** Dioscoreales, grains, palynotaxonomy, taxonomy, SEM.

### **Pericarp development and anatomy in the monotypic genus *Talbotia* (Velloziaceae)**

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In recent phylogenies Velloziaceae is divided into three clades: the dubious *Acanthochlamys* (Tibet), African clade and South American clade. The monotypic genus *Talbotia* is basalmost in the African clade. So, the investigation of flower and fruit developmental anatomy in *Talbotia* is of importance for an understanding of morphogenesis. It is revealed that the epicarp originates from receptacle tissue and consists of epidermis and hypoderm cells with thickened walls, the mesocarp originates from carpel mesophyll and consists of 12-15 layers of thin-walled cells, and the endocarp originates from inner epidermis of carpel and is represented by one layer of longitudinally elongated sclereids with strongly thickened lignified walls. The lignification of endocarp cell walls starts in later developmental stages; it becomes the only lignified zone of fruit wall. There are 3 dorsal and 3 ventral double (peripheral and inner) vascular bundles and 6 lateral bundles in the fruit wall. The inner rings of dorsal and ventral bundles and the lateral bundles comprise the gynoecium vasculature, whereas peripheral rings of dorsal and ventral bundles make the tepal's vasculature. The structure of the coenocarpous indehiscent fruit of *Talbotia* allows it to be referred to the inferior pyrenarium of *Ilex* type with a lignified endocarp forming three pyrenes.

**Key words:** endocarp, epicarp, fruit, mesocarp, Velloziaceae.

### **Pollen morphology of subtribe Dichorisandrinae (Commelinaceae)**

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Dichorisandrinae is currently placed in subfamily Commelinoideae, tribe Tradescantieae, and includes five Neotropical genera (i.e., *Dichorisandra* J.C. Mikan, *Siderasis* Raf. *emend.* M. Pell. & Faden, *Geogenanthus* Ule, *Cochliostema* Lem., and *Plowmanianthus* Faden & C.R. Hardy). Morphological and molecular characters have yielded variable results and thus conflicting interpretations regarding the monophyly of the subtribe have been proposed. Aiming to contribute to the morphological characterization of subtribe Dichorisandrinae, palynological characters were investigated. This study was based on the analysis of pollen grains from fresh and/or dried herbarium samples. At present, only pollen grains of different species of *Dichorisandra* and *Siderasis* (*Cochliostema*, *Geogenanthus* and *Plowmanianthus* are still being studied) were acetolyzed, measured, qualitatively described, and photographed under light microscopy and scanning electron microscopy. Pollen grains from all species are heteropolar, monosulcate, oblate, ranging from small to medium in size, and are released as monads. *Siderasis* spp. (four studied species) presented fossulate to perforate exine ornamentation, while *Dichorisandra* spp. (18 species) presented microreticulate to rugulate interspersed with areolate exine. Results revealed distinct pollen morphologies for each studied species and indicated a substantial taxonomic potential at the generic and specific levels beyond classifications based on morphological variation. **Key words:** Atlantic Forest, Commelinales, Commelinoideae, palynology, taxonomy.

**Funded by:** CAPES

### **The long and winding road to Kranz anatomy**

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The Kranz syndrome is a specific set of leaf functional properties in plants using C<sub>4</sub> photosynthesis. This syndrome includes the existence of distinct compartments in the leaves: one more closely connected to the atmosphere, where the CO<sub>2</sub> is captured; and a larger one, where the Calvin cycle takes place. The current model of C<sub>4</sub> photosynthesis evolution from a C<sub>3</sub> ancestor proposes a series of gradual leaf anatomical changes followed by biochemical adaptation of the enzymatic machinery. This transition, which occurred several times in a wide range of angiosperm lineages, is particularly interesting in grasses. In this work, we performed qualitative and quantitative measures in leaf cross sections of distinct C<sub>3</sub>, C<sub>4</sub> and intermediates grasses to comparatively study anatomical changes that characterise each type of leaf. We determined that the grade of differentiation in photosynthetic structures increases towards the distal extreme of all leaf types analysed. We observed qualitative and quantitative differences among species in vascular bundle density, chloroplast distribution and bundle sheath size. Altogether, this analysis allows us to increase knowledge on the long and winding road that led to Kranz anatomy in grasses.

**Key words:** bundle-sheath, C<sub>4</sub> photosynthesis, grasses, evolution, leaf.

### **Aponogetons (Aponogetonaceae) in India and their embryo diversity**

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The genus *Aponogeton* L.f., with about 58 species, is distributed in tropical and subtropical regions of the world. It is represented by nine species viz. *A. appendiculatus*, *A. bruggenii*, *A. crispus*, *A. lakhonensis*, *A. natans*, *A. nateshii*, *A. satarensis*, *A. undulatus* and *A. wolfgangianus* in India. Among Indian species of *Aponogetons*, *A. appendiculatus*, *A. bruggenii*, *A. nateshii*, *A. satarensis* and *A. wolfgangianus* are endemic to India. Each of these species is of special interest from the point of view of phytogeography, reproductive biology and embryo morphology. *Aponogeton satarensis* is the only Indian dioecious species with a biforked spike whose close relative *A. decaryi* occurs in Madagascar. It is a relict narrow endemic restricted to higher plateaus in the Western Ghats. *Aponogeton bruggenii* is another narrow endemic with barriers of unknown nature in sexual reproduction and seldom sets seeds. It propagates through formation of daughter tubers on the mother tuber. *Aponogeton nateshii* is a unique in its embryo morphology. The embryo is globular and possesses 15-22 appendages all around the embryo. *Aponogeton wolfgangianus* is known from a single lake in Kerala. All the five species are narrow endemics. The present paper deals with species of *Aponogeton* in India, their morphology, cytology, embryo diversity and interrelationships. **Key words:** *Aponogeton*, cytology, embryo diversity, endemism, relationships.

### **Leaf anatomy in Tigridaeae reveals a diagnostic character for subtribes Cipurinae and Tigridiinae (Iridoideae: Iridaceae)**

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Tigridaeae is a monophyletic lineage subdivided into subtribes Cipurinae and Tigridiinae, based on cytogenetic, palynological and morphological characters. Recent phylogenetic studies demonstrated that the two subtribes are not monophyletic and the characters used for the separation are not homologous, thus Tigridaeae was divided into Clades A and B. The goals of this study were to analyze leaf anatomy of Tigridaeae and address, in a phylogenetic context, whether anatomical characters can be useful for identification of diagnostic characters for Cipurinae and Tigridiinae. Sequences from eight regions of plastid DNA were obtained from 60 species, and phylogenetic analyses were performed. Leaf anatomical analysis was conducted in 55 species and ancestral state reconstruction was evaluated for ten variable leaf anatomical characters. The species presented important variation in leaf outline ("terete", "plicate" or "foliate"); type of subdermal sclerenchyma (lignified or pectin-rich) and marginal vascular bundles (present or absent). The results revealed that characters related to the subdermal sclerenchyma could sustain a new circumscription of subtribes Cipurinae and Tigridiinae, where Cipurinae would include all genera present in Clade A and Tigridiinae include all genera present in Clade B.

**Key words:** anatomical characters, evolution of characters, leaf anatomy, pectin-rich sclerenchyma, subepidermal sclerenchyma.

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## Palynotaxonomy of *Orthophytum* and three related genera (Bromelioideae, Bromeliaceae)

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Phylogenetically, *Orthophytum* Beer is related to *Cryptanthus*, *Hoplocryptanthus*, *Lapanthus*, *Rokautskya* and *Sincoraea*. *Orthophytum* became a monophyletic group after the re-establishment of *Sincoraea*. This study aims to describe the pollen morphology of *Hoplocryptanthus* (two species), *Orthophytum* (18 species), *Rokautskya* (one species) and *Sincoraea* (six species) to improve the morphological characterization of the taxa. Pollen grains were acetolysed, measured, described, and photographed under light and scanning electron microscopes. The measurements were statistically analyzed. The results showed that the main pollen pattern of the genera is characterized by: the elliptic outline in polar view (although some species had spheroidal pollen), monosulcate aperture, diverse exine patterns [granulate-perforate in *Hoplocryptanthus*; areolate-perforate in *Rokautskya*; microreticulate or reticulate in most of the *Orthophytum* and *Sincoraea* taxa, except for *O. braunii*, *O. falconii*, and *O. leprosum* which displayed perforate ornamentation], as well as the presence or absence of an apex of equatorial axis (calota) with different ornamentation from the center area of pollen grain and the smaller thickness of the exine in most of the *Orthophytum* species. Pollen morphology of *Orthophytum*, *Hoplocryptanthus* and *Rokautskya* was significantly different, although similar in *Orthophytum* and *Sincoraea*, not being useful for the categorization of these two genera.

**Key words:** Atlantic rainforest, *Hoplocryptanthus*, rocky fields, *Rokautskya*, *Sincoraea*.

**Funded by:** This work was supported by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) [grant number PNADB 17/2009]. The authors are thankful to Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for providing a PhD degree scholarship [grant number 170851/2017-6] to the first author and for providing the fellowship of Productivity in research to the sixth author.

Economic botany, plant breeding and agriculture

## Genetic divergence among species of *Acrocomia* (Arecaceae) based on morphoagronomic descriptors

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Among the species of the genus *Acrocomia* Mart., *A. aculeata* and *A. totai* both stand out for their wide geographic distribution in the American continent, and their economic use, especially of their fruits, rich in oil and carbohydrates. Interest in pulp and almond oils for diverse purposes are increasing and commercial plantings can already be observed in Brazil, Paraguay and Costa Rica. However, due to the morphological similarities, they are commonly considered a single species - *A. aculeata*. Thus the present study aimed at characterizing the diversity using 31 morphological descriptors obtained from 30 individuals of three populations of *A. aculeata* in São Paulo and Minas Gerais and 30 individuals of three populations of *A. totai* in Mato Grosso do Sul, Brazil. The occurrence of intrapopulational variation, both intraspecific and interspecific (ANOVA –  $p < 0.005$ ), and strong structuring (UPGMA) with the formation of two groups corresponding to the species evaluated was verified. Therefore, it is proposed that the activities of collection of botanical material for use purposes, propagation and scientific studies consider the distinct taxonomic identity of these palms, not attributing the existing morphological variation only to genetic or environmental variability, but also to the fact that they are distinct species.

**Key words:** *bocaiúva*, *macaúba*, morphological diversity, palm tree, plant taxonomy.

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## Ecology and conservation

### Variation in seed germination traits and root growth of the threatened palm *Euterpe edulis* Mart.

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This study addressed temporal and spatial inter-population variation in seed germination and spatial variation in root growth of *Euterpe edulis*. Seeds were collected in Atlantic rain forest in Rio de Janeiro State during 2012, 2013 and 2014 in three populations located at different altitudinal ranges (0-100 m; 300-500 m; 1000-1200 m). For each harvest, seeds were germinated and submitted to different treatments of water availability ( $\Psi = 0\text{MPa}$ ,  $\Psi = -0.4\text{MPa}$ ,  $\Psi = -0.8\text{MPa}$  or flooding) under controlled conditions. Seeds harvested in 2014 were subjected to two more extreme water deficit treatments:  $\Psi = -1.0\text{MPa}$  and  $\Psi = -1.5\text{MPa}$ , and seedling roots were evaluated. Germination percentage, mean germination time and germination synchrony index were calculated and analyzed with General Linear Models. In the three years, populations showed high germination percentage (70%-100%), except for the flooding treatment, which had low seed germination percentage (0-60%). Seed germination time increased with the severity of water deficit. Synchrony index was more influenced by the harvesting time and for seeds collected in 2014 the lowest water potential induced asynchrony germination. For root growth, generally, low water potential treatments increased mean root length.

**Key words:** altitudinal gradient, germination, root growth, synchrony index, water deficit.

## **Reproductive phenology and floral biology of *Neoregelia ibitipocensis* (Bromeliaceae), an endemic cloud forest species of Serra da Mantiqueira, Minas Gerais, Brazil.**

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Bromeliaceae presents a high species richness and endemism in the Atlantic Forest, besides the diversity of floral attributes and pollination systems. The goals of this study were to investigate some reproductive aspects of *Neoregelia ibitipocensis* (Leme) Leme in the Ibitipoca State Park, MG. The population studied flowered from March to April and showed a phenology pattern of brief flowering. The flowers are odorless and have a white tubular corolla. Each inflorescence produces a mean of 12.8 flowers and opens from one to three flowers per day. The flower anthesis lasts for two days. The sugar concentration in the nectar was  $4.6\% \pm 2.1$  and the nectar volume produced was  $21.0 \mu\text{L} \pm 9.2$ . *Neoregelia ibitipocensis* presents the inflorescence inside the leaf rosette and flower anthesis can take place under the water accumulated in the tank. Further studies are being conducted to investigate the pollination biology and reproductive strategy of this species.

**Key words:** Bromeliaceae, nidular inflorescence, phenology, reproductive biology, Serra da Mantiqueira.

## **Reproductive biology of *Nidularium ferdinandocoburgii* (Bromeliaceae), in the Ibitipoca State Park, Minas Gerais, Brazil.**

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The Bromeliaceae are recognized by their endemism in the Atlantic Forest, with the highest number of threatened species in this biome, for which they are included as a priority in programs of conservation. The goals of the present work were to investigate some reproductive aspects of *Nidularium ferdinandocoburgii* Wawra, a species with restricted distribution to forested areas of the Serra de Ibitipoca and Serra de Araras, in the southeast of Brazil. The flowering and fruiting of the *N. ferdinandocoburgii* population in the Ibitipoca State Park is from October to January and from January to July, respectively. The species exhibits an intermediate (1-5 months) flowering phenology pattern, according to the literature. The flowers are odorless with tubular and purplish-blue corollas that develop immersed in the water accumulated within the leaf rosettes. The means of nectar volume and sugar concentration were  $29.9 \mu\text{L} \pm 19.5$  and  $29.6\% \pm 8.6$ , respectively. The mean number of flowers produced by each individual was 23 and anthesis lasts for two days. The fruit set under natural conditions was 91% and the mean number of seeds produced by each fruit was 230.1. Further studies related to pollination biology, breeding systems and reproductive success are being conducted for this species.

**Key words:** Atlantic Forest, Bromeliaceae, floral biology, nidular inflorescence, phenology.

## **Invasive Hydrocharitaceae in Rio de Janeiro state, Brazil**

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*Hydrilla verticillata* is the one of the most invasive plants in the Neotropics. Paleotropical in origin, it is able to colonize natural or artificial environments, as well as aggravating the silting of rivers and causing other types of impact. In Brazil, the species is a known biological invader of the Paraná hydrogeographic region. The present study reports the first record of *H. verticillata* in the Southeast Atlantic hydrogeographic region. This species was found fertile in an artificial reservoir near the Guapiaçu River, in the Atlantic Forest Domain. This reservoir is located in Três Picos State Park (Guapiaçu Ecologic Reserve, Rio de Janeiro, Brazil), where *H. verticillata* was found up to one meter of depth, in stagnant water, dominant or co-occurring with other aquatic plants. Two other similar species (submerged and bottom-rooted with linear and whorled leaves) occur in the region, *Apalanthe granatensis* and *Egeria densa*, occupying the same ecological niche of *H. verticillata*. The populations of these autochthonous plants can be negatively affected by the dissemination of *H. verticillata*. The possibility of the naturalization of this species in Brazil should be taken into consideration. Besides that, mechanical control and conscientious disposal is recommended to the managers of these Conservation Units.

**Key words:** allochthonous, aquatic macrophyte, Cachoeiras de Macacu, southeastern Brazil, weed.

**Funded by:** The study was funded by CNPq and supported by the National Museum of UFRJ.

## **Reproductive biology, pollination, and reproductive barriers in two Bromeliaceae of the Atlantic Forest in Rio Grande do Norte**

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Several bromeliads are sympatric, and interspecific (in nature) and intergeneric hybridization (in the greenhouse) can occur in most cases. To avoid that, strong reproductive barriers are needed to keep these species cohesive. This work aimed to describe the reproductive biology of two sympatric bromeliads in the Atlantic Forest of Rio Grande do Norte, to understand how reproductive barriers act, and the mechanisms that allow the cohesion of both. Investigated species were *Wittmackia patentissima* and *Hohenbergia ridleyi*, both Bromelioideae. We found that both have aromatized and diurnal flowers, a high rate of pollen germination *in vitro* and all flowers tested had receptivity in the stigma, the main floral visitors are bees, and basically all floral characteristics are shared, both flowering in July-September, without any phenological isolation. Pollination tests showed that both species are self-compatible with high rate of fruit production. In a hybridization crossing test, neither taxon produced seeds. Using fluorescence microscopy, we observed pollen tubes growing normally in all pollination tests. However, under hybridization, the pollen tube stops before reaching the ovary. It was possible to observe the irregular callose deposition along the style, indicating that there are post-pollination barriers that inhibit the formation of natural hybrids.

**Key words:** Bromelioideae, flowering phenology, *Hohenbergia*, pollination by bees, *Wittmackia*.

**Funded by:** Capes and NAPA-MEPA (Esalq/USP).

### **Pollen analysis and floral visitors in *Commelina benghalensis* (Commelinaceae)**

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*Commelina benghalensis* L. are pollen flowers, with three types of stamens: two long laterally located, with white anthers (LW), a central median (MY) and three central short (CY), all with yellow anthers. Morphological differences and the location of the stamens may indicate a division of labor between stamens for pollination (LW) and attraction (MY, CY). We analyzed the pollen types in each anther (by acetolysis) and the behavior of floral visitors during foraging (by frequency of visits during 15 hours). In all the anthers the pollen grains were the same, classified as monads, medium, prolate, elliptic, reniform, monocolpate, elongated with heteropolar colpi. Measurements in equatorial view ( $\mu\text{m}$ ): polar diameter ( $27.53 \pm 1.44$ ), equatorial diameter ( $18.49 \pm 1.54$ ) and relation between both diameters (1.55). In LW and MY we counted more than 100 grains, whereas in CY we saw a little more than 10 grains. The floral visitors were bees *Augochlorella* sp. (62.10%) and unidentified Diptera species, where two presented 10.8% of the visits and three presented 2.7% each. All the observed visitors came in contact with all anthers, including the shorter ones, occasionally or not, without distinguishing between them. Thus, from the data obtained, no functional separation of stamens was observed.

**Key words:** *Augochlorella*, Diptera, herbaceous, pollen, ruderal.

### **Spikelet phenology and germination of *Eleocharis laevigulumis* (Cyperaceae)**

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Aquatic macrophytes are important components of freshwater ecosystems, in which Cyperaceae is the most diverse family. Many Cyperaceae species form dense populations surrounding lake margins in the southern coast of Brazil, but little is known about their sexual reproductive strategies. In this study we describe the reproductive life cycle as well as seed vigor and germinability of *Eleocharis laevigulumis* R. Trevis. & Boldrini, an abundant emergent macrophyte in southern Brazil coastal wetlands. We described reproductive phenophases and estimated number of flowers and potentially viable fruits per inflorescence in a natural population. We also tested seed germinability and vigor between four months during one reproductive season. We found that spikelets are dichogamous and protogynous, and last, on average, 34 days (pre-anthesis, 1.7 d; anthesis, 6.9 d; fruit maturation, 22.3 d; fruit dispersion, 3.2 d). More than half of the flowers (62.2%) developed into fruits and only 5.5% of the seeds germinated. Germinability and vigor decreased over the reproductive season. Probably part of *E. laevigulumis* culms are originated from asexual reproduction or sexual reproduction of clones. We suggest that for restoration purposes, *E.*

*laeviglumis* propagation should be done by sowing seeds collected at the beginning of the reproductive season along with transplanting rhizomes.

**Key words:** aquatic macrophyte, coastal lake, germinability, seed vigor, sexual reproduction.

**Funded by:** Capes/CNPq

### **Phenology of a dominant emergent macrophyte, *Fuirena robusta* (Cyperaceae), in a coastal lake in southern Brazil**

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Aquatic macrophytes are an important component of freshwater communities, influencing production and ecosystem functioning. Within different macrophyte habits, emergent species, especially from Cyperaceae, constitute extensive communities in Brazilian coastal lakes, frequently dominating and surrounding internal lake margins. In spite of its importance, little is known about emergent macrophytes life cycle. This study aimed to evaluate the reproductive development and productivity, as well as the phenology of the emergent macrophyte species, *Fuirena robusta* Rottb. (Cyperaceae), typical to freshwater habitats in the southern Brazilian coast. We evaluated vegetative and reproductive phenology of a population occurring at the southern and northern margins of a coastal lake using activity and intensity indexes within a year in permanent quadrats. Flowering and fruiting culms were collected to estimate its production. The reproductive cycle is highly seasonal, occurring during the end of spring and throughout summer, while the vegetative cycle is non-seasonal. A slight phenological difference was found between the north and south margins, which we attributed to different wind conditions. Every reproductive culm lasted around two months and more than 90% of flowers developed into fruits. According to these results, we point to *F. robusta* as a possible candidate for restoration projects at coastal lakes in southern Brazil.

**Key words:** aquatic macrophyte, freshwater lake, phenophases, Poales, reproductive biology.

**Funded by:** Capes/CNPq

### **Monocots on quartzite rocky outcrops in the Atlantic Forest, Ouro Preto (MG, Brazil)**

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Monocots are well adapted to open habitats, such as the *campos rupestres* in southeastern Brazil. Environmental conditions of rocky outcrops influence plant species occurrence, leading to a pattern of islands of xeric communities arising in a matrix of mesophytic vegetation. Little is known about the influence of rock microhabitats on rupicolous flora spatial distribution. Here we evaluate the

floristic composition of monocots in *campo rupestre* within the Atlantic Forest (Ouro Preto, MG), comparing the richness and predominant life forms on different habitats of 9 distinct quartzite rocky outcrops, totaling 1,550 m<sup>2</sup>: top surface (total area: 1,000 m<sup>2</sup>), with exposed rock and crevices; lateral surface (450 m<sup>2</sup>), with major richness of microhabitats and soil and water retention; and base (100 m<sup>2</sup>), with sheltered microhabitats and sandy soil. We registered 38 species of 11 families, the richer families being Orchidaceae (11 species), Bromeliaceae (5) and Poaceae (5). Hemicryptophytes predominated in all surfaces, with 68% of frequency of occurrence. Top is the richest habitat, with 13 exclusive species. Base had 7 and lateral 5 exclusive species. The habitats shared 3 species, whilst top and lateral shared 10. Different surfaces may be under distinct anthropic impacts and need special management strategies addressing the rupicolous flora.

**Key words:** *campos rupestres*, floristics, microhabitats, monocotyledonous mats, rupicolous flora.

### **Mexican woody bamboo (Poaceae: Bambusoideae) richness and endemism: efforts for conservation**

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The Mexican woody bamboos comprise 52 native species, 36 of them endemic. The aims of this study were to identify biogeographical areas with the highest woody bamboo diversity, to locate areas of endemism, and to evaluate their conservation status. We gathered presence records of 52 Mexican woody bamboos, based on herbaria records and field work. The species richness was analyzed based on Mexican political divisions and a grid cell of 0.5° × 0.5°. Weighted endemism, corrected endemism, and areas of endemism were analyzed. Conservation status was evaluated using the extent of occurrence (EOO) and the area of occupancy (AOO). The highest richness areas were found in Chiapas and Veracruz. Weighted endemism identified an area in Veracruz, weighted corrected endemism found three areas in Durango, Oaxaca, and Veracruz. Finally, two disjunct areas of endemism were found in Oaxaca and Veracruz. EOO identified 16 critically endangered species, meanwhile, AOO revealed 14 species. Further, 14 species fell within the Endangered category based on the EOO analysis, meanwhile, AOO found 39 species with the same status. For conservation purposes, 15 species had both categories but none of them are protected by Mexican law or recognized by the IUCN.

**Key words:** Chiapas, critically endangered species, endangered species, Oaxaca, Veracruz.

**Funded by:** Laboratorio Nacional de Identificación y Caracterización Vegetal, Centro Universitario de Ciencias Biológicas y Agropecuarias, Universidad de Guadalajara

### **Seed germination and conservation of three endangered species of *Dyckia* (Bromeliaceae)**

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The genus *Dyckia* belongs to the Bromeliaceae family and at least 28 species are at risk, demanding actions for conservation. Among those, three species endemic to Brazil are on the edge of extinction, especially because their ornamental use has generated predatory exploitation of mature plants. Thus, this work aimed to evaluate the *in vitro* and *ex vitro* germination of seeds of three species of *Dyckia* [*D. fosteriana* L.B. Sm., *D. monticola* L.B. Sm. & Reitz and *D. ursina* L.B. Sm.] for conservation purposes. The seeds were cultivated *in vitro* in MS medium with 3% of sucrose, 0.7% agar and pH 5.8, while for *ex vitro* germination, the Vivato® substrate was used. The *in vitro* germination started at 15 days and presented better synchrony than in substrate (*ex vitro*), where germination started at 22 days after cultivation. 85% of germination was observed in *D. monticola*, 67% in *D. fosteriana* and 45% in *D. ursina*, compared with 35%, 45% and 15% from the *ex vitro*, respectively. Seed germination generated normal seedlings in both systems and can be useful to subsidize the conservation of these species, which are already conserved at Embrapa Cassava and Fruits.

**Key words:** *Dyckia fosteriana*, *Dyckia monticola*, *Dyckia ursina*, Bromeliad Genebank, ornamental plant.

**Funded by:** CAPES, PROCAD, PNPB, CAPES/Embrapa

## **Cryopreservation of pineapple shoot tips and the anatomical conditions of the starting material**

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The advances in biotechnology provide new options for genetic conservation of plants. Due to the cost-benefit of long-term conservation, cryopreservation in liquid nitrogen at -196°C is a technique that has been widely used. This work aimed to evaluate the anatomical conditions of the starting material and its correlation with the survival of the cryopreserved shoot tips. Four accessions, *Ananas comosus* (BGA-009); *A. bracteatus* (BGA-119); *A. paraguayensis* (BGA-376), *A. lucidus* (BGA-750) from the Active Germplasm Bank and two hybrids from the Genetic Breeding Program, FIB-ROX1 (*A. bracteatus* × *A. lucidus*) and FIB-ROX2 (*A. lucidus* × *A. bracteatus*) were introduced in vitro. Histological sections before freezing and the percentages of survival after freezing were obtained taking into account the different times of cultivation of the donor plants. There was significant interaction between genotypes and the time of culture. The accessions BGA-009 and BGA-119 showed the highest survival rates with 95% and 90% respectively for the 30-day culture time. Different results were obtained for each genotype showing the need for improvements in the standardization of the starting material, which would allow a good repeatability of the protocol.

**Key words:** Active Germplasm Bank of Pineapple, biotechnology, Bromeliaceae, conservation, microscopy.

**Funded by:** CNPq, FAPESB, CAPES, PROCAD, PNPB, CAPES/Embrapa



## Transition from anemophily to entomophily in *Urochloa plantaginea*: a case of ambophily in Poaceae

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*Urochloa plantaginea* (Link) R.D. Webster belongs to Poaceae, considered a typical anemophilous family. However, records of insects visiting flowers of this family suggest a readaptation to entomophily. Therefore, we described the floral biology and analyzed the pollination system and the role of pollen vectors of *U. plantaginea*. The studied species has anemophilous floral traits such as inconspicuous and naked flowers, a bifid and feathery stigma, long and flexible filaments exposing the anthers which have small pollen grains without ornamentation, a high pollen-ovule ratio and absence of floral scent. However, we also verified that this species has entomophilous traits, such as hermaphrodite flowers, exposing the male and female organs simultaneously during the anthesis, in addition to vibrant colors in its reproductive structure, with yellow anthers and purple stigmas, which are attractive for visiting insects. Six groups of floral visitors were recorded, including bees, flies and wasps. Experiments with fluorescent particles that simulate pollen grains in visitors' bodies and stigmas of flowers suggest that insects are potential pollinators. Anemophilous and entomophilous traits together characterize ambophily. Both pollen vectors, wind and insects, increase fruit set under different environmental conditions in *U. plantaginea*.

**Key words:** ambophily, anemophily, attractiveness, entomophily, floral signs.

**Funded by:** CNPq, Facepe and CAPES.

## Insights into *Cryptanthus zonatus* (Bromeliaceae) translocation

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Habitat losses are pushing *Cryptanthus zonatus* (Vis.) Vis. to the risk of extinction. Translocation is one of the potential conservation measures to avoid the decline of populations of *C. zonatus*. However, deep knowledge about better strategies of reintroduction is necessary before a successful translocation project. Here, we present results of an experimental translocation of *C. zonatus* using mature individuals. We collected 23 plants of *C. zonatus* from a wild population in Pernambuco, Brazil. These individuals were transplanted and cultivated in four plastic trays in a greenhouse of the Recife Botanical Garden. We monitored the phenology and shoot production of this *ex-situ* population from December 2017 to June 2018. All individuals were living until June. After three months the plants started vegetative growth. On June 20 transplanted individuals produced 84 shoots (mean = 4.2 shoots/plant). The density in each tray varied from seven to 31 shoots. Blooming started after four months and by June seven transplanted individuals had produced 95

flowers. We found an inverse relationship between the number of flowers produced and the mean of shoots produced in each tray. Our results provide elements for a protocol of reintroduction of *C. zonatus* and bring some insights about population dynamics of the species.

**Key words:** Atlantic Forest, Bromelioideae, conservation, ecology, reintroduction.

**Ecological niche modeling and implications for conservation of *Anomochloa marantoidea*, endemic to the Atlantic Forest, Brazil and belonging to the oldest grass subfamily (Anomochlooideae)**

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The monotypic genus *Anomochloa* is recognized as belonging to the oldest living lineage of Poaceae. For this reason, *A. marantoidea* is considered a crucial species to understand evolutionary patterns in this family. Although described in 1851, only two populations of the species were recorded until now, in a small area of the Atlantic forest in Southern Bahia. Few aspects about its ecology and biology are known, which makes it difficult to preserve. In the present work, niche modeling was used to access the potential distribution of the species, test for niche conservatism and verify its environmental requirements. A predictive model was also built to guide field work. Two new populations were found, both within a non-recognized indigenous reserve. The climatic niche is conserved, indicating high climatic restriction, which may be the main cause of this species's rarity. Tree cover and precipitation are the main environmental requirements influencing its distribution, which presents AOO 16 km<sup>2</sup> and EOO 12.244 km<sup>2</sup>. None of the four populations and only 35% of the suitable sites for the species are under protection. However, it is possible to invest collection efforts in these sites to locate unknown populations and/or use them to establish new ones.

**Key words:** Gramineae, IUCN, Maxent, rain forest, rare species.

**Funded by:** Conselho Nacional de Desenvolvimento Científico e Tecnológico (grants 484381/2010-5, 562349/2010-3, 563558/2010-5 and 401526/2014-3) and Fundação de Amparo à Pesquisa do Estado da Bahia (grant PNX0014/2009)

**Diversity of cultivatable endophytic fungi of the mycoheterotrophic orchid *Pogoniopsis schenckii* (Orchidaceae: Vanilloideae)**

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Endophytic microorganisms are those that inhabit the interior of healthy plant organs and tissues. Endophytic fungi are known to have properties of interest to plants as protection against pathogens. *Pogoniopsis schenckii* Cogn. is a rare, mycoheterotrophic and achlorophyllous orchid that develops under organic matter in dense forests. The objective of this study was to describe the cultivatable diversity of endophytic fungi obtained from different organs of *P. schenckii*, with the aim of understanding the role of the endophytic community and its interaction with the plant. For this, the fungi of the roots, floral stem and fruits of the species were isolated and identified through partial sequencing of the ITS region. As a result of the experiments, the genera *Xylaria*, *Coniochaeta* and *Trichoderma* were identified in the roots and the genera *Fusarium*, *Clonostachys* and *Colletotrichum* in both the floral stem and the fruits. Fungi of the genus *Trichoderma* have a strong potential for phytopathogen control and plant growth promotion. *Fusarium* has been reported as an inducer of the germination of different species of green orchids. All genera found belong to the phylum Ascomycota and are commonly isolated from orchids.

**Key words:** Ascomycota, endophytic fungi, molecular identification, mycoheterotrophic plant, Orchidaceae.

**Funded by:** Project funded by FAPESP 2015 / 26479-6. CNPq scholarship to first author.

### **Flower color polymorphism and preference of pollinators in *Deuterocohnia meiziana* (Bromeliaceae)**

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Understanding the relative importance of the genetic variation exhibited by natural populations in the face of natural selection and genetic drift remains a challenge for evolutionary biology. The flower color polymorphism within natural population is rare and has been described for a limited number of species. The objectives of this work were to describe the flower color polymorphism of *Deuterocohnia meiziana* Kuntze ex Mez in Brazil and to evaluate the influence of color on the preference of pollinators. We analyzed the color ratio along 15 transects (4 x10m) and analyzed the genetic structure (35) for one population with four microsatellite markers. We observed the frequency of pollinators in individuals with yellow (23), orange (27), and red (26) flowers during 10 min each. The population of *D. meiziana* presented 15.65% of yellow, 70.75% orange, and 13.60% red-flower individuals in a total of the 294 individuals analyzed. We did not observe genetic clustering of individuals of the same color. Individuals with different flower colors did not show variation in the number of flowers produced. The pollinators did not show preference for a specific color ( $p>0.05$ ), therefore they may not be responsible for the occurrence and maintenance of color polymorphism.

**Key words:** color ratio, floral display, genetic structure, natural population, pollinators.

**Funded by:** CNPq, CAPES, FUNDECT

## **Rock and leaves: can anatomical leaf traits inform environmental heterogeneity in inselberg vegetation?**

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Inselbergs, isolated rock outcrops that rise above the surrounding landscape, harbor a highly diverse flora. Differences in slope, water availability, soil depth and sun exposure result in the creation of microhabitats characterized by distinct resource availability, which are circumscribed by specific plant communities. However, we lack studies examining specific traits structuring communities in each microhabitat. We investigated the leaf anatomical structure of inselberg species by comparing the traits of plants occurring in two microhabitats with contrasting resource availability: monocot mat (MM; the most stressful) and shallow depression (SD; the less stressful). Based on the ‘fast-slow’ plant economics spectrum, we hypothesized that species in the contrasting microhabitats would show different arrays of leaf anatomical traits. We qualitatively characterized and measured leaf anatomical structures, and then compared the measured tissues between plants in SD and in MM, using ANOVA and ordination analyses. Our results show that most of the species growing in MM showed more conservative traits related to water retention and drought resistance. While species growing in SD showed a broader spectrum of anatomical strategies, including conservative and acquisitive traits. These findings reinforce the heterogeneous aspect of inselberg vegetation, which is under variable degree of environmental stress.

**Key words:** community assembly, monocots, plant economics spectrum, resource gradient, rock outcrops.

**Funded by:** CNPq/DAAD-290155/2014-2

## **Ecological specialization in species of Tigridaeae (Iridoideae: Iridaceae): interaction between oil-bees and oil-flowers predicts pollination system?**

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Flowers with elaiophores are associated with specialized oil-bees, due to morphological adaptation to gather oil and the possible role of these bees in mediating selection on floral traits. We investigated the role of oil-collecting bees in the pollination system of four Tigridaeae species that offer floral oil (*Cypella amplimaculata*, *Herbertia pulchella*, *Cipura formosa* and *Cipura* sp.) and assessed the level of ecological specialization among these species. We followed at least 37 flowers of each species for two years, totaling 274.5 observation hours to evaluate the frequency, behavior

and foraging time of two functional-groups: oil-bees and generalist bees. Our results revealed that *Cypella amplimaculata* and *Cipura* sp. were specialized for oil-bees, while *Cipura formosa* and *Herbertia pulchella* presented generalized pollination. The behavior of oil-bees to collect distinct resources, was determinant for effective pollination and was related to the position of the elaiophores in the flowers. Species of *Arhysocele* were the most important specialized pollinators. Halictidae and stingless bees pollinated flowers during pollen and/or nectar collection. The ecological specialization of flowers in this study revealed that the interaction of oil-collecting bees and flowers with elaiophores does not predict functional relationship in oil-pollination systems.

**Key words:** *Arhysocele*, elaiophores, generalist pollination, plant-insect interaction, specialized pollination.

**Funded by:** CAPES

### **Floral biology and breeding systems of four oil-flower species of Tigridaeae (Iridoideae: Iridaceae)**

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Reproductive strategies and floral adaptations of species are of special importance because of its direct effect on pollination. The focus of this study was to characterize breeding system, flower phenological stages, flower duration and stigma receptivity of four species of Tigridaeae, Iridaceae (*Cypella amplimaculata*, *Herbertia pulchella*, *Cipura formosa* and *Cipura* sp.) and evaluate inter- and/or intraspecific variation by surveying two natural populations of three of them. To determine the breeding system, we performed five pollination treatments: hand self-pollination, hand cross-pollination, emasculation, natural pollination and autonomous self-pollination. We studied floral biology following nine phenological stages in natural populations of the species, in 30 to 73 flowers, as well as the flowering phenology. The species are pollinator-dependent and breeding systems were distinct among species and between populations of *C. formosa*. *Herbertia pulchella* and *Cipura* sp. were self-incompatible, whereas *C. amplimaculata* and *C. formosa* were self-compatible. Flowers of these species lasted less than one day, varying from 5.5 to 13.5 hours, with flowering time of 2 to 3.5 months, depending on taxa. We assume that inter- and intraspecific variation of breeding systems and in duration of floral stages were possibly related to specialized pollination strategies.

**Key words:** anthesis, floral traits, mating system, phenology, reproductive biology.

**Funded by:** CAPES

### **Spatial distribution and vegetative propagation in *Syngonanthus caulescens* (Eriocaulaceae)**

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*Syngonanthus caulescens* (Poir.) Ruhland occurs throughout the Brazilian territory in large populations, growing in damp to relatively dry soils. To quantify its vegetative propagation, two populations (P1 and P2) were analyzed in São Francisco de Assis, RS, Brazil. P1 was considered as P1A and P1B because a stream separates it into two subpopulations. P1B was also analyzed for the spatial distribution pattern, with the data collected along the river bank in 6 plots of 2.4 m<sup>2</sup> each. For the vegetative propagation analysis all plants in four plots of 100 cm<sup>2</sup> were collected in each population and subpopulation. P1A presented the lowest number of individuals and clumps, and a higher number of flowering individuals. P1B presented equal number of flowering and vegetative individuals, while P2 showed a higher number of vegetative individuals. The average number of individuals per clump (5.41) did not differ among populations and subpopulations. The Morisita index indicated a slightly clumped distribution (I=1.188). The results suggest vegetative propagation as an important mechanism for the maintenance of populations in this species, thus allowing their establishment and permanence in microenvironments already colonized. In general, *S. caulescens*, even preferring soaked soils, adapts in different environmental conditions.

**Key words:** aquatic plants, clumped distribution, everlasting plants, Poales, tillering.

**Funded by:** Universidade Regional do Noroeste do Estado do Rio Grande do Sul (UNIJUÍ); Programa de Educação Tutorial (PET Biologia/MEC/SESU).

### **A new phorophyte record for *Pseudolaelia corcovadensis* (Orchidaceae), an endemic threatened species from the Brazilian Atlantic Forest**

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*Pseudolaelia corcovadensis* Porto & Brade is an epiphyte restricted to inselbergs in the Atlantic Forest. Small populations are recorded in five municipalities in the State of Rio de Janeiro (RJ), and one in the State of Minas Gerais where it can be considered extinct. These populations are severely threatened by granite extraction, city development and forest fires. Bibliographical information mentions *Vellozia* spp. as its only phorophyte. However, we recorded in Petrópolis (RJ) *Pseudobombax petropolitanum* A. Robyns as a new phorophyte. Ecological studies on *Pseudobombax* spp. classify its species as nucleating species. With the aim of correlating dendrometric data with the number of *P. corcovadensis* specimens and epiphytic richness, we visited all specimens of *P. petropolitanum* inside a 200 × 100 m area. Our results indicate that trunk diameter is not determinant for the establishment of epiphytes, and only trees taller than 2 m presented vascular epiphytes. We recorded four species of Orchidaceae: *Anathallis* sp. (one specimen), *Polystachya estrellensis* Rchb. f. (66), *P. corcovadensis* (44), and an unidentified morphospecies and for Bromeliaceae three species: *Tillandsia recurvata* (L.) L. (81), *Tillandsia*

*gardineri* Lindl. (26), and *Vriesea bituminosa* Wawra (two). And finally, two ferns: *Phlebodium aureum* (L.) J. Sm. and *Pleopeltis hirsutissima* (Raddi) de la Sota.

**Key words:** conservation, ecology, epiphytes, inselbergs, *Vellozia*.

**Funded by:** CAPES - CNPQ

### **Rediscovery of *Griffinia colatinensis* (Amaryllidaceae): an endemic, critically endangered and poorly-known species from southeastern Brazil**

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*Griffinia* Ker Gawler is constituted by sixteen species, most of them considered highly threatened. It's an endemic genus for Brazil and the Atlantic Forest is its main center of diversity. Knowledge about morphological variation in different populations is limited. In this context, we highlight *Griffinia colatinensis* Ravenna, a species currently considered nearly "Extinct" because the only known populations were the type-collections collected by Kuhlmann in 1934 and 1943 near the city of Colatina, Espírito Santo state, Brazil. While performing a taxonomic survey of Amaryllidaceae from Espírito Santo, a new population was found in the municipality of Águia Branca, composed of 20-30 individuals, occurring in the understory of a fragment of semideciduous forest, and near a stream. The species presents leaves with elliptic to oblanceolate leaves, a single bract with bifid apex, flowers with a white base and lilac apex, and a hypanthial tube 0.8-1 cm long. The single bract is described for the first time for the species. Although extremely threatened and with a very restricted distribution, the new known population for *Griffinia colatinensis* is a relief for conservation purposes since it can still be found in nature.

**Key words:** Atlantic Forest, conservation, Espírito Santo, Griffinieae, taxonomy.

**Funded by:** CAPES

### **Temporal changes in the most effective pollinator of *Edmundoa lindenii* (Bromeliaceae)**

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The spatio-temporal variation of the most effective pollinator is the most cited factor to explain the emergence and maintenance of generalist pollination systems. In this study we detect whether the effectiveness (i.e., frequency associated with efficacy) of the floral visitors varied among blooms during a period of four years. For this, we studied the *Edmundoa lindenii* (Bromeliaceae) pollination system. We analyzed floral visitors (large bees, small bees and hummingbirds) frequency through focal observations, efficacy (seed set), and pollen limitation index comparing seed set by hand cross and natural pollination experiments. The frequency of floral visitors varied among the years, influencing their effectiveness. Hummingbirds are more efficacious than large bees. Small bees were not efficacious. The pollen limitation varied among the years, occurring in only one. Some studies documented the existence of year-to-year changes of floral visitors but did not consider the reproductive success of the population sampled. Our results allow us to conclude that the reproductive success of the plant may vary over time even in the presence of effective pollinators. Furthermore, this study clarifies that results obtained in different years allow estimation of a more trustworthy analysis of pollination systems, contributing to the still scarce knowledge about variable pollination environments.

**Key words:** Bromeliaceae, effectiveness, generalist, pollination, temporal variation.

**Funded by:** National Council for Scientific and Technological Development - CNPq.

### **Germination rate in four bromeliad species from ironstone outcrops, Central Brazil**

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The bromeliad species that occur in ironstone outcrops (“cangas”), in Corumbá and Ladário, Mato Grosso do Sul, are important because of their high carbon fixation capacity, with CAM metabolism that allows them to survive water shortages and high temperatures. The objective of this study was to evaluate the germination rate of native bromeliads with potential for use in urban areas. Seeds of *Deuterocohnia meziana*, *Dyckia excelsa*, *Dyckia leptostachya* and *Bromelia balansae* were collected. Seeds of all species were disinfected, sown on filter paper in Petri dishes and maintained under controlled conditions for 30 days. Only *B. balansae* seeds were submitted to chemical scarification using 98% sulfuric acid. Cultures were moistened daily with distilled water. Our results showed, after 30 days of observation, 100% seeds of *D. meziana* germinated; in *D. excelsa*, 33% of the seeds germinated; and in *D. leptostachya* only 16.6% of the seeds germinated. No germination occurred in *B. balansae*. *Deuterocohnia meziana* presented the highest germination rate among the species evaluated to date, presenting high potential for its plantation in urban areas.

**Key words:** *Bromelia*, Bromeliaceae, CAM, *Deuterocohnia*, *Dyckia*.

### **Dissociation between phylogenetic and functional structure in communities of Velloziaceae, a family diversified by evolutionary radiations**

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Niche conservatism is a basic premise of studies about phylogenetic structure of communities, predicting that species functions depend on phylogenetic relationships. However, phylogeny and functionality can be unrelated, especially in clades that diversified explosively. We investigate phylogenetic and functional diversity in Velloziaceae, a monocot family dominant in *campos rupestres*. Velloziaceae radiations are associated with functional traits involving water use strategies. We tested predictions expected for rapidly diversifying clades and the effect of water availability on Velloziaceae communities. We sampled species in 30 plots among different soil conditions, measured 13 functional traits, and used a molecular phylogeny to estimate phylogenetic structure. Most plots showed no phylogenetic structure (MPD/MNTD:  $p > 0.05$ ; only 4 plots: MPD < 1). Two functional traits displayed phylogenetic signal (K: 0.281 and 0.288 for LDMC and leaf width;  $p < 0.01$ ). Soil influences species and functional traits in the plots ( $r^2 = 0.2$  and  $0.43$ ;  $p < 0.001$ ), but not phylogenetic structure ( $r^2 = 0.14$ ;  $p = 0.061$ ). Functional diversity is crucial to understand ecosystem functioning, while phylogenetic diversity reflects historical patterns and evolutionary potential. Our findings highlight the importance of directly assessing both aspects, especially in habitats where many species have arisen by evolutionary radiations.

**Key words:** *campos rupestres*, convergence, hotspot, phylogenetic signal, soil heterogeneity.

**Funded by:** FAPESP, CNPq.

## Comparative analysis of Orchidaceae flora of some Brazilian eastern mountains and Central Plateau areas

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We present a comparative analysis of Orchidaceae flora among 32 localities from the Central Plateau (*Planalto Central*) and eastern Brazil. The similarity was calculated by Jaccard's index and the relationship between areas was verified by clustering analysis (UPGMA). The data matrix compiled a total of 145 genera and 725 species. Jaccard's values were relatively low, denoting floristic particularities for each area. Some interesting patterns were found: despite the Espinhaço Range (*Cadeia do Espinhaço*) areas having a predominance of rocky field (*campos rupestres*) formations, they did not group together. The areas of Chapada Diamantina form a well-defined group including also Grand Mogol, while the southern areas of Espinhaço Range are more correlated with those of Brazilian Atlantic Forest located in southeastern of Minas Gerais and São Paulo states. The areas of Central Plateau form a separate group as well as those near the coast in Rio de Janeiro.

**Key words:** clustering, floristic similarity, Jaccard's index, orchids, UPGMA.

**Funded by:** We would like to thank the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) for the Master's Degree Scholarship granted to the first author from 2013 to 2015.

## Spatio-temporal variation in the reproductive phenology of *Euterpe edulis* (Arecaceae) at superior and inferior distribution limits in the Atlantic rain forest

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Phenology of the species *Euterpe edulis* Mart. was studied, from June 2014 to May 2017, observing monthly flowers and fruits, green and ripe, of thirty individuals in three populations of the species situated in an altitudinal gradient of the Atlantic Rain Forest, Rio de Janeiro state: ReBio Poço das Antas (PA, 0 m, mean annual temperature 25.5°C, annual precipitation 1995 mm) and PARNASO (17.8°C, 2821 mm) in trails Primavera (PR, 900 m) and Rancho Frio (RF, 1100 m). The study addressed the questions: (i) is there intrapopulation synchronism? (ii) is reproduction seasonal? (iii) how is the reproduction of the species influenced by temperature and precipitation? Flowering and ripe fruits were seasonal for all populations. Green fruits were seasonal in PA and RF populations, except for the first year, while in PR population, seasonality was verified only in the last year. In PARNASO, flowering is influenced by low temperature (negative and meaning correlation), however exhibits a low synchrony, and in PA, flowering is influenced by high temperature and precipitation (positive and meaning correlation) and the population synchrony is high. The results for RF and PR were different from PA probably due to spatial distance and most conspicuous environmental variables in the lowest altitude area.

**Key words:** altitudinal gradient, phenology, phenophases, seasonal, synchronicity.

## Monocots in society and tools to spread knowledge about monocots

### Wild monocots of ornamental potential from Western Ghats, India

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Western Ghats is one of the eight “hottest hot-spots” of biodiversity in the world and also home of more than 1/4 of flowering plant species reported in India. There are many flowering plant species of ornamental value in Western Ghats. In the monocot group, plants such as orchids, gingers, lilies, palms, sedges, grasses and many more have great ornamental potential and are of great floricultural importance. Monocots have ornamental potential for their foliage and flowers. Wild ornamental monocot genera from Northern Western Ghats include species of *Aerides*, *Alpinia*, *Amorphophallus*, *Aponogeton*, *Ariopsis*, *Arisaema*, *Arundinella*, *Asparagus*, *Bambusa*, *Bulbophyllum*, *Burmannia*, *Calamus*, *Chlorophytum*, *Commelina*, *Corypha*, *Costus*, *Crinum*, *Curcuma*, *Cyanotis*, *Cymbidium*, *Cyperus*, *Dendrobium*, *Dinebra*, *Dioscorea*, *Dipcadi*, *Eleocharis*, *Eragrostis*, *Eria*, *Eriocaulon*, *Eulophia*, *Fimbristylis*, *Gloriosa*, *Habenaria*, *Hedychium*, *Iphigenia*, *Kaempferia*, *Kyllinga*, *Limnophyton*, *Monochoria*, *Murdannia*, *Ottelia*, *Pancratium*, *Pecteilis*, *Pennisetum*, *Peristylus*, *Pholidota*, *Rhynchelytrum*, *Rhynchospora*, *Rhynchostylis*, *Saccharum*, *Setaria*, *Smilax*, *Thunia*, *Typha*, *Vanda*, *Zingiber*, etc. The present study brings out the ornamental potential of some of the monocot species and the need for their domestication and introduction in botanical gardens.

**Key words:** Konkan, monocotyledons, Northern-Western Ghats, ornamental potential, wild.

**Prospects and limitations for using small Unmanned Aerial Vehicle Systems (UAVs) to examine and document high-canopy epiphytic bromeliads (Bromeliaceae)**

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Tropical forest canopies have been characterized as perhaps the last frontier in terrestrial biology, and a primary reason for this is canopy access. Canopy access techniques are typically placed into two categories, high-tech, and low-tech. The high-tech access methods equate to high-cost and include canopy towers, cranes, scaffolding, walkways, hot air balloons, and canopy rafts. Low-tech canopy access methods, which tend to be lower cost, include ground-based methods, such as binoculars and clipper-poles, or tree climbing. We describe results from a May 2018 expedition in Costa Rica to test proof of concept for using small, relatively low-cost commercially available Unmanned Aerial Vehicles (UAVs, or drones) to examine and photograph epiphytic bromeliads. The research confirms that small multi-propeller UAVs can be added to the lower cost ground-based methods for canopy research. The benefits and prospects of UAV use in tropical epiphytic plant research, as well as some of the limitations encountered with comparative use of three different UAV systems, will be presented.

**Key words:** Bromeliaceae, canopy-access, epiphytes, photography, UAVs.

**Funded by:** University of Wyoming A&S Seed Grant

**An educational poster of monocot characters, relationships and floral diversity**

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Many biology students only learn basic plant taxonomy, resulting in many young biologists graduating with little knowledge of botanical diversity and its importance. Identification and taxonomic skills are also rarely taught in university curricula, and this results in perpetuation of “green blindness” even among ecologists and other biologists. Diversity and classification of monocots have been particularly problematic because traditional morphological characters used for identification and to support the classification did not always coincide with the results from molecular data. During the molecular revolution in the field of plant taxonomy over the last 25 years, thousands of phylogenetic trees have been generated using DNA sequences from thousands of plant species, which have unravelled relationships and allowed taxonomists to revisit the classification and circumscriptions of orders, families and genera. Despite monocots being a well-defined group, numerous changes have taken place at every taxonomic level in recent years. Particularly, circumscriptions of families like Liliaceae, Asphodelaceae and Asparagaceae have

changed substantially. Here we present a visually attractive, informative and exciting new educational aid for showcasing relationships, morphology and characters for the eleven orders and 77 families of the monocots families.

**Key words:** diversity, education, green blindness, identification, phylogeny.

**Funded by:** Plant Gateway LTD

### ***Dipcadi concanense* and *Dipcadi goaense* (Asparagaceae) – threatened wild species from Northern–Western Ghats of India with unexplored potential as vegetable and ornamental plants**

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*Dipcadi concanense* and *Dipcadi goaense* are two wild species of restricted distribution in the Northern – Western Ghats. *Dipcadi concanense* is locally abundant but area of distribution is limited. *Dipcadi goaense* is restricted to two nearby areas of about 5 sq. km. Both the species fall under plants of India. The major threat is habitat destruction and habitat modifications. Both the species need conservation measures. The leaves and bulbs of *Dipcadi concanense* are used as a vegetable. Other species are also edible. Similarly, both the species have large glistening white and sweet-scented flowers of ornamental potential. Therefore, introduction of these plants in horticultural trade would help in conservation and utilization of these species. The present work emphasis on the nutritive and potential of these species.

**Key words:** conservation, *Dipcadi*, Northern Western Ghats, ornamental, threatened.

### **Zingiberaceae Phylogeny Poster**

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This educational phylogeny poster provides a graphic overview of the Zingiberaceae (ginger family) with its subfamilies, tribes, and genera along with relevant apomorphic, plesiomorphic as well as diagnostic traits – a compilation of published research – intended as a useful teaching, studying, and research tool on the current relationships among gingers. The poster is currently available in English, Portuguese, Indonesian, Chinese, Japanese, Vietnamese, and Thai. All versions are accessible to the community via ResearchGate and other open online platforms. Regular updates of the poster will be made as new data becomes available. Within the tribe

Alpinieae, the genera *Alpinia*, *Amomum*, and *Etilingera* are largely polyphyletic and currently being revised; recircumscriptions and reassignments to new or other genera are to be expected in the near future.

**Key words:** botany education, botany outreach, ginger, systematics, teaching.

## Genetics, genomics and bioinformatics

### Clues from genetic variability in *Ipheion* (Allioideae) using genotyping-by-sequencing

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The tribe Leucocoryneae is monophyletic and includes 6 genera and about 100 species occurring only in South America. The genus *Ipheion* comprises three species: *I. sessile*, *I. tweedieanum* and *I. uniflorum* distributed in the Pampean region (Argentina, Uruguay, and Brazil). The species are morphologically differentiated and earlier molecular studies indicate a single origin during the Middle Miocene. For the first time, a population-level analysis was carried out for *Ipheion* using genome-wide single-nucleotide polymorphism (SNP) data from genotyping-by-sequencing (GBS), a next-generation sequencing method. This approach allowed us to investigate phylogenetic relationships and genomic variability among natural populations of *Ipheion*. In 93 analyzed individuals a total of 2,424 loci were retained after filtering, and a concatenated matrix of 240,568 bp length was generated. The level of missing data was lower than 90%. Phylogenetic analyses provided robust relationships and showed that *Ipheion* is composed of three genetically, cytogenetic and morphologically differentiated entities that agree with previously defined species. We found that *I. sessile* and *I. tweedieanum* might have diverged from within *I. uniflorum*. Chromosome rearrangements and polyploidization are the major drivers of speciation within *Ipheion* promoting reproductive isolation among sympatric/parapatric populations.

**Key words:** Amaryllidaceae, GBS, phylogenomics, population genomics, SNP.

### Use of ISSR nuclear molecular markers to estimate genetic diversity in *Bromelia hieronymi* (Bromeliaceae) populations, Pantanal region, Brazil

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Bromeliaceae species are considered natural indicators of environmental health, playing an important role in local communities due to their social, economic and medicinal value. The objective of this study is to analyze the polymorphism of ISSRs (Inter Simple Sequence Repeats), and estimate genetic diversity of *Bromelia hieronymi* Mez, a typical species from the humid Chaco, in the Pantanal “sul-mato-grossense”, and to develop the use of ISSR markers in bromeliads. The study species is self-incompatible and mainly pollinated by hawkmoths. For this study, we collected samples in natural populations in Porto Murtinho city, Southwest of Mato Grosso do Sul state, Brazil. We conducted DNA extractions using the CTAB protocol in a sample of 20 individuals from three natural population. For amplification of 10 ISSR primers we performed PCRs in a Veriti thermocycler, and products were visualized in 1% agarose gel. ISSR are DNA fragments from 100 to 3000 bp amplified by PCR using a single primer constructed from a microsatellite sequence, exhibiting a dominant gene expression. From the 10 ISSR primers tested, six amplified satisfactorily, showing polymorphism among the individuals tested.

**Key words:** bromeliads, Bromelioideae, Chaco, Pantanal, polymorphism.

**Funded by:** CNPq, CAPES and FUNDECT

### Comparative plastid genomes of *Anathallis* (Orchidaceae, Pleurothallidinae)

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*Anathallis* is a Neotropical orchid genus with over 150 species usually grouped into three morphological assemblages: *Acuminatae*, *Alatae* and *Panmorphia*. Previous phylogenetic studies indicated that the genus is polyphyletic due to the inclusion of some species of *Acuminatae* into the genus *Stelis*. In this study we characterize and compare the complete plastid genome sequence, structure and content between *A. rubens* (*Acuminatae*) and *A. obovata* (*Alatae*), the type species of the genus. Plastid genomes range from 155,515 (*A. obovata*) to 156,382 bp (*A. rubens*), both structured in the typical quadripartite structure, with slight length variation between regions. *Anathallis obovata* presents *accD*, *ndhB* and *ndhF* genes with premature stop codons, causing a reduction of ~400 aa and ~240 aa in *accD* and *ndhF* genes, respectively. Similarly, the premature stop codon results in a loss of ~100 aa in the first exon of *ndhB* gene. All these cases may be caused by a pseudogenization process and produces a nonfunctional protein product. *Anathallis rubens* has the *accD*, *ndhB* and *ndhF* genes of the same size as *Masdevallia coccinea* (NC\_026541) and *M. picturata* (NC\_026777). These results reinforce the molecular distance between species in the *Acuminatae* group and other species of *Anathallis*.

**Key words:** *accD*, Epidendroideae, NADH, NGS, plastome.

**Funded by:** CAPES (134737/2016-4) and CNPq (Proc.308460/2017-0).

### Genetic diversity of *Deuterocohnia meiziana* (Bromeliaceae) natural populations from ironstone outcrops, Central Brazil

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The Bromeliaceae family is composed of 3475 species, distributed in 51 genera, native mainly to the tropical and subtropical Americas. The genus *Deuterocohnia* Mez comprises 17 species, all adapted to extremely dry environments. They have a terrestrial growth habit, generating dense rosettes with thorny leaves. In Brazil, there is only *D. meiziana* Kuntze ex Mez occurs, which is self-incompatible and polinated mainly by bees. *Deuterocohnia meiziana* is threatened due to its restricted occurrence only on ironstone outcrops, also known as “cangas”, and to habitat loss. In Brazil, *D. meiziana* occurs in lateritic crusts of the Residual Plateau of Maciço do Urucum on the western edge of the Pantanal. This study aims to analyze the genetic diversity of *D. meiziana*, considering natural populations found on ironstone outcrops using ISSR markers. For this study, we will analyze individuals from six populations from Corumbá and Bodoquena cities. We collected fresh leaves from at least 20 individuals from each populations, which were stored in silica gel until DNA extractions. At least 30 ISSR markers will be tested by PCR amplification. As *D. meiziana* is self-incompatible, we expect a high genetic diversity and small genetic distance among populations. **Key words:** bromeliad, “cangas”, ISSR markers, Pitcairnioideae, polymorphism.

**Funded by:** CNPq, CAPES, FUNDECT

### **Repetitive DNA and karyotype diversity in *Philodendron* (Araceae)**

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*Philodendron s.l.* is the second largest genus of Araceae and has been the focus of recent taxonomic and phylogenetic studies. Karyotypic data is limited to chromosome counts and genome size measurements. In this work, karyotypes of 29 *Philodendron* and five *Thaumatococcus* ( $2n = 28$  to 36) species were analyzed by fluorescence *in situ* hybridization with rDNA and telomeric probes, besides analysis of the repetitive DNA fraction. *Philodendron* exhibited two to 16 35S-rDNA sites with heteromorphisms for nine species, whereas *Thaumatococcus* had two or four sites. In turn, the number of 5S-rDNA sites was conserved (two sites) in both genera. In addition, the use of telomeric probe revealed only terminal in *P. giganteum*, while *P. callosum* presented interstitial sites associated to satellite DNA clusters. The repetitive DNA fraction, characterized for six species, ranged from 33.75% (*P. quinquelobum*, 2C/4.60 pg) to 67.71% (*P. melinonii*, 2C/4.20 pg) by retrotransposons Ty3-Gypsy and Ty1-Copia, mostly belonging to the lines Chromovirus and Ogre-Tat. This work provides an overview of the composition and diversity of the genomic repetitive DNA fraction of *Philodendron s.l.* species, with large variations observed, probably due to the rapid evolution and significant diversity of these genomes.

**Key words:** FISH, Fluorescent In Situ Hybridization, RepeatExplorer, ribosomal RNA, telomeric probes, transposable elements.

**Funded by:** CNPq, CAPES, FACEPE.

### **Cytogenetic investigation of *Eriochrysis* (Poaceae: Andropogoneae) species and their putative hybrids**

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*Eriochrysis* comprises ca. 7–12 species distributed mainly in tropical regions of America, Africa, and India. The circumscription of its species is complex and hampered by the occurrence of interspecific hybrids. *Eriochrysis cayennensis*, *E. laxa*, and *E. villosa* are found in sympatry in southern Brazil, together with individuals with intermediate morphology. Previous molecular studies reported the occurrence of natural polyploid hybrids between *E. laxa* and *E. villosa*. Considering the scarcity of cytogenetic studies in *Eriochrysis*, this study aims to characterize those three species and individuals with intermediate morphology, investigating their possible hybrid status. For meiotic and pollen analyses, staining with propionic carmine and Alexander's method were used. All taxa analyzed have a diploid number of  $2n = 2x = 20$ . The three species and the individuals with intermediate morphology showed high meiotic regularity (more than 99% of normal cells) and 100% pollen viability. All taxa have prolate spheroidal pollen grains. Taking into account molecular data, regular meiotic behavior, high pollen viability, and diploid chromosome number were not expected for those putative hybrids. The individuals with intermediate morphology analyzed in this study are probably not interspecific hybrids, but only an expression of the high morphological plasticity of the species of *Eriochrysis*.

**Key words:** chromosome number, *Eriochrysis*, genome size, hybrids, meiosis, hybrids.

**Funded by:** CNPq and PRAE/UFRGS

### **Cross-amplification of nuclear microsatellite markers in *Aechmea distichantha* (Bromeliaceae)**

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*Aechmea distichantha* Lem. is a bromeliad with a terrestrial or epiphytic habit, with tubular rosettes that accumulate water, and simple inflorescences. This species occurs in the Cerrado and Brazilian Atlantic Forest, and also in Bolivia, Paraguay, Argentina and Uruguay. In this study, we tested the cross-amplification of 43 nuclear microsatellites, originally developed for other species of bromeliads in *A. distichantha*. Of the 43 loci tested, 17 were polymorphic, and among these we selected ten to be amplified and genotyped in three populations of the species. The observed and expected heterozygosity per locus in the *A. distichantha* populations ranged from 0.182 to 0.735 and 0.297 to 0.830, respectively. On average, the observed and expected heterozygosity were 0.530 and 0.624, respectively. The loci Acom\_82.8 (*A. comosus*), Op30, Op77A (*O. ofiuroides*), PaD07, and PaZ01 (*P. albiflos*), showed significant departure from HWE for the *A. distichantha* populations analyzed. The loci will be used for studying population the diversity and genetic structure and phylogeography of *A. distichantha* and will also contribute to conservation decisions.

**Key words:** bromeliad, Bromelioideae, genetic diversity, SSR markers, transferability.

**Funded by:** CNPq, CAPES, FUNDECT

### Cytogenetic characterization of *Prosthechea* (Orchidaceae)

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The genus *Prosthechea* Knowles & Westc. has about 100 species with Neotropical distribution, of which 25 are endemic to Brazil. The genus is characterized by fusiform pseudobulbs and a free labellum. The present work aimed to characterize cytogenetically four *Prosthechea* species using the CMA/DAPI fluorochromes. All species analyzed presented  $2n = 40$ , differing only in the pattern of heterochromatic bands. *Prosthechea pygmaea* had chromosome sizes ranging from 1.2 - 2.7  $\mu\text{m}$ , with predominantly submetacentric chromosomes (32SM+8M), two pairs with CMA<sup>+</sup> terminal bands and one pair with CMA<sup>+</sup> pericentromeric bands. *Prosthechea aemula* had chromosomes ranging from 1.5 - 2.1  $\mu\text{m}$ , a karyotype consisting mainly of metacentric chromosomes (27M+13SM), with two terminal and two interstitial CMA<sup>+</sup> bands. The chromosomes of *P. fragrans* ranged from 1.5 - 3.9  $\mu\text{m}$  with the karyotypic formula consisting of 18M+22SM, and a chromosome pair with a conspicuous terminal CMA<sup>+</sup> band. In *P. pamplonense* it was not possible to estimate chromosome size due to the degree of condensation, while fluorochrome banding revealed a single pair with terminal CMA<sup>+</sup> bands. Although the analyzed species are stable in regard to the chromosome number, they presented variation in the patterns of heterochromatic bands.

**Key words:** chromosome banding, Epidendreae, heterochromatin, Orchidaceae, *Prosthechea*.

## Using ISSR nuclear molecular markers to estimate genetic diversity *Dyckia ferruginea* (Bromeliaceae)

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*Dyckia ferruginea* Mez, a species of the Bromeliaceae family native to Brazil, has a unique tone of silver color, and its inflorescence is yellowish. This species is found in various regions in Mato Grosso do Sul and Mato Grosso. There are no records that this species is included in any conservation unit. Bromeliads have an extensive geographical distribution, occurring from the south of the United States to the North of Patagonia in Argentina. The present study aims to analyze the genetic diversity in *D. ferruginea* using ISSR nuclear molecular markers. To do this, we will use genomic DNA samples that were collected in several localities of Mato Grosso do Sul: in the cities of Coxim and Aquidauana, and in the Municipal Park of Piraputangas in Corumbá. The amplifications will be performed in the Laboratory of Genetics of UFMS/CPAN, using ISSR nuclear markers, which will be initially tested in some individuals of the species, and only polymorphic primers will be used in the population analysis. The analysis will estimate genetic variability, similarity and genetic distance. These data will stimulate future research and promote the use of molecular ISSR markers in different species of bromeliads.

**Key words:** Bolivia, genetic variability, ISSR markers, Pitcairnioideae, polymorphism.

**Funded by:** CNPq, CAPES and FUNDECT.

## Complete plastid genome sequence of *Butia eriospatha* (Arecaceae)

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*Butia eriospatha* (Mart. ex Drude) Becc. is an endemic palm species from the Atlantic Rainforest in Brazil. This biome is considered a biodiversity hotspot, but historically its exploitation resulted in severe fragmentation. As a consequence of this exploitation, *B. eriospatha* is currently listed in the IUCN red list as vulnerable. Here we characterized, for the first time, the complete plastid genome (plastome) sequence, structure and content of a species from the *Butia* genus (*B. eriospatha*). The complete plastome sequence is 154,048 bp in length, with the typical quadripartite structure. This plastome length and gene content is consistent with other four species from tribe Cocoseae already sequenced, with 8 ribosomal RNA genes, 38 tRNA genes, and 85 protein-coding genes. However, the Inverted Repeat (IR) borders show some variation within Cocoseae tribe. Most species from Cocoseae tribe have the *rps19* gene completely duplicated in the IR region, but all plastomes sequenced from Attaleinae subtribe, including *B. eriospatha*, have this gene partially duplicated in

the IR borders, with a functional protein only in the LSC-IR<sub>A</sub> junction. These results provide new insights into the evolution of plastomes, especially regarding the IR/SC borders expansion/contraction characteristics.

**Key words:** Attaleinae subtribe, evolution, IR borders, next generation sequencing, plastome.

### **What forces drive genomic abundance of satellite DNAs in plants? Evolution of holocentromeric satellite *Tyba* in the genus *Rhynchospora* (Cyperaceae)**

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Repetitive elements such as satellite DNAs comprise the most abundant fractions of plant genomes. However, it is still unclear what forces regulate genomic satellite abundance. We investigated the evolution of *Tyba*, the first centromere-specific satellite DNA of holocentric plant chromosomes, discovered in *Rhynchospora* species. We reconstructed *Tyba* abundance along an NGS-based phylogeny of 111 species (30% of the genus) and its possible correlations with karyotype and environmental variables. *Tyba* abundance varied from 0.001% (*R. speciosa*) to 5.34% (*R. glaziovii*) and showed a strong phylogenetic signal ( $\lambda=0.92$ ). No correlation with chromosome number or genome size was observed. A *Tyba* increase was observed predominantly in North American species, but no correlation was observed with all 25 environmental variables. A *Tyba*-based phylogeny was able to solve all main clades of the genus, supporting that its evolution is highly dependent on phylogenetic relationships and most likely not correlated to ecological niche. Monomer sequences of satellite DNAs evolve concertedly with mutations being homogenized and fixed in lineages, resulting in sequence divergence in reproductively isolated groups of organisms. These direct the evolution of satellite DNAs motifs present in the functional centromeres of different lineages.

**Key words:** chromosome number, ecological niche, genome size, phylogenetic comparative methods, repetitive DNA.

### **New chromosome counts of the annual species of *Paspalum* L., Plicatula group (Poaceae: Paspaleae) from Brazil.**

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*Paspalum* L. includes 350 species distributed mainly in America. Of the informal taxonomic groups into which the genus *Paspalum* has been subdivided, the Plicatula group is of the greatest

agronomic interest because it encompasses species well accepted by cattle. The base chromosome number of *Paspalum* species is  $x = 10$ . Diploid and polyploid levels normally related to sexual and apomictic reproduction are common. This work provides new counts and confirms others for annual species of the Plicatula group. The chromosome numbers were determined from pollen-stem cells (meiosis) according to the usual techniques. Chromosome numbers reported for the first time: *Paspalum foveolatum* Steud. ( $2n = 20$  and  $40$ ), *P. cordaense* Swallen ( $2n = 32$ ) and *P. macranthecium* Parodi ( $2n = 20$ ). We confirmed counts for *P. boscianum* Flügge ( $2n = 20$ ), *P. convexum* Humb. & Bonpl. ex Flügge ( $2n = 32$ ) and *P. melanospermum* Desv. ex. Poir. ( $2n = 20$ ). Base chromosome numbers other than  $x = 10$  have been considered exceptional in *Paspalum*. The counts  $2n = 32$  for *P. convexum* and *P. cordaense* is a disparate case with relatively few others reported. The finding suggests that more cytogenetic characterization is necessary to assess the whole chromosomal in the genus.

**Key words:** cerrado, cytogenetics, grass, meiosis, mitosis.

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### **Comparative plastid genome of the tribe Cranichideae (Orchidaceae, Orchidoideae) from the Neotropics**

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Cranichideae is a terrestrial orchid tribe with eight subtribes; two of them are especially diverse in the Neotropics: the subtribe Goodyerinae and the subtribe Spiranthinae. Here we characterized, for the first time, the complete plastid genome sequence, structure and content of *Aspidogyne longicornu* (Cogn.) Garay and two species from the subtribe Spiranthinae: *Sauroglossum elatum* Lindl. and *Eurystyles cotyledon* Wawra. Plastomes length ranged from 139,811 to 154,562 pb with the typical quadripartite structure, but the Short Single Copy (SSC) region showed high length variation, from 11,177 bp in *E. cotyledon* to 22,018 bp in *A. longicornu*, caused by the deletion of *rpl32* and five *ndh* complex genes (*ndh-F*, *D*, *G*, *I*, and *A*). The *rpl22* gene is part of the IR border in *E. cotyledon*. All three species show the same 8 rRNA gene and 38 tRNA gene content. These relevant differences were identified inside the subtribe Spiranthinae: whereas the plastome of *S. elatum* has a complete *ndh* complex, *E. cotyledon* has only three *ndh* genes (*ndh-B*, *H*, *E*). Moreover, *A. longicornu* has *accD* and *matK* genes with premature stop codons, which usually result in a nonfunctional protein product.

**Key words:** Goodyerinae, NGS, *ndh* complex, plastome, Spiranthinae.

**Funded by:** CAPES (grant 88881.065009/2014-01) and CNPQ (Proc. 308460/2017-0).

### **Comparative plastid genome of Neotropical *Bulbophyllum* (Orchidaceae, Epidendroideae) lineages**

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*Bulbophyllum* Thouars is the largest Pantropical orchid genus with 2,200 spp. The Paleotropical region is the richest in species, followed by Africa and then the Neotropics that comprises about 60 spp. In the Neotropics, the genus has six lineages, two above the Equator and four mainly in the Brazilian Atlantic Rainforest. Although the phylogeny and taxonomy of the genus is well understood in the Americas, some species-complexes need more studies of their delimitation. Here we characterized the complete plastid genome sequence, structure and content of *B. mentosum* (sect. *Micranthae*), *B. plumosum* (sect. *Xiphizusa*) and *B. regnellii* (sect. *Napelli*). Chloroplast genomes ranged from 151,493 to 146,401 bp with the typical quadripartite structure. The gene order, GC content, and gene compositions are similar between the three species (8 ribosomal RNA genes, 38 tRNA genes and 76 protein-coding genes). We identified *ndhJ* and *ndhH* pseudogenes that were lost in *B. mentosum*; *ndhF*, *ndhE* pseudogenes, and one copy of *ycf1* were lost in *B. regnellii*; and *ndhF*, *ndhI*, *ndhA*, *ndhJ*, and *ndhH* pseudogenes were lost in *B. plumosum*. The *matK* gene presents a nucleotide deletion at the end of the sequence and the *ndhB* gene presents premature stop codon, which usually produces a nonfunctional protein product.

**Key words:** Dendrobiinae, NADH, NGS, plastome, pseudogene.

**Funded by:** This work was supported by CAPES (grant 88881.065009/2014-01) and CNPq (Proc. 311001/2014-9).

### **Past rainforest connections explain the demographic history of *Acrocomia intumescens* (Arecaceae) through Brazilian Caatinga**

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The occurrence of a wetter and cooler climate associated with humid vegetation had been inferred for the Brazilian Caatinga during the late Pleistocene and early Holocene. Modern forest enclaves - "Brejos de Altitude"- are presumed to be remnants of this once widespread rainforest that retracted as the current Caatinga expanded. Here we investigate how past vegetation dynamics have influenced the geographical distribution of genetic variation in *Acrocomia intumescens* Drude. We sampled 62 individuals from 10 localities representing its range and analyzed polymorphisms at five microsatellites and two non-coding nuclear regions, using ecological niche modeling (ENM). We found low genetic diversity, eight haplotypes, and two clusters: one corresponds to "Brejos de Altitude" and the second includes populations from the Atlantic Forest. Differentiation among the

populations and correlation between genetic and geographical distances were detected. Our results suggested a contemporary and historical gene flow restriction between "Brejos de Altitude" and Atlantic Forest populations. The clusters identified represent genetic breaks, which were geographically coincident with past forest invasions reported in the literature and concordant with past connections between forests through Caatinga showed by ENM. Our findings support the hypothesis that past expansions of the Atlantic Forests over the Caatinga shaped the phylogeographical pattern of *A. intumescens*.

**Key words:** ecological niche modeling, microsatellite DNA, non-coding nuclear DNA, phylogeography, population genetics.

**Funded by:** Doctoral Sandwich Program Abroad-CAPES (006795/2015-03), Agricultural Research Service (USDA/ARS), CNPq (447697/2014-5) and Postgraduate Program in Genetics -FMRP-USP

### **The genome of *Alloteropsis semialata* reveals extensive lateral gene transfer among Panicoideae**

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Genetic exchanges among distantly related species, known as lateral gene transfers (LGT), have contributed to the functional diversification of bacteria. In contrast, this phenomenon is usually perceived as marginal in eukaryotic evolution. Thanks to high-throughput sequencing, reports of eukaryotic LGT are accumulating, and genes for two key enzymes of C<sub>4</sub> photosynthesis have been laterally acquired by the grass *Alloteropsis semialata*, from the Paniceae tribe. To evaluate the extent and importance of LGT in grasses, we generated a chromosome-level reference genome for *A. semialata*, and compared it to genome data for 148 other grasses to identify all genes of unambiguous LGT origin. Our phylogenomic approach identifies tens of laterally-acquired genes, which are clustered in large genomic fragments that include non-coding DNA and transposable elements received from other species within the Panicoideae. Transcriptome analyses show that most LGT are expressed and added functional diversity to the genome of *A. semialata*. We conclude that LGT can be a potent force in grass evolution.

**Key words:** genomics, grasses, lateral gene transfer, phylogenetics, transcriptome.

### **Strong genetic structure in *Dyckia excelsa* (Bromeliaceae), an endangered species from ironstone outcrops, Pantanal, Brazil**

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The plant communities associated with ironstone outcrops in the Pantanal biome are still little known relative to genetic aspects. Rock outcrops are often considered as being "terrestrial islands", and gene flow among populations is presumed to be hampered by the isolation effect provided by the surrounding matrix. We studied the diversity and genetic structure of *Dyckia excelsa*, to test the hypothesis that its genetic variability is highly structured due to the disjunction of outcrops. We

used nuclear microsatellites and intergenic plastid DNA spacers aiming to access patterns that led to the diversification of this species. The species exhibited low haplotype and allelic diversity and high levels of divergence among outcrops, indicating a prolonged genetic isolation. The diversification of haplotypes dates from the Pleistocene (1.4 Mya), when the Pantanal experienced major modifications in its landscape. Pollen movement may occur between neighboring populations, resulting in a pattern of isolation by distance. It is highly recommended that *D. excelsa* be preserved in as many locations as possible, since it has a unique identity wherever it is found. The peculiar environment in which this species occurs contributes to the evolution of rare and endemic species, showing the great importance of Pantanal landscapes for conservation purposes.

**Key words:** Bromeliaceae, conservation, genetic structure, ironstone outcrops, Pantanal.

**Funded by:** CNPq

### **Polymorphism in *Ledebouria revoluta* (Asparagaceae): a case study from the Indian Peninsula**

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*Ledebouria* Roth of Asparagaceae (Hyacinthaceae) has about 45 species distributed in tropical and South Africa and 2 species in both Madagascar and India. Presently four species of the genus, viz. *Ledebouria revoluta* (L. f) Jessop, *L. viridis* S.R. Dutta and P. Harvey ex M.R. Almeida, *L. karnatakensis* Puneekar and Lakshmin., and *L. hyderabadensis* M.V. Ramana, Prasanna and Venu, have been recorded from India. Field observations, analysis of morphological characters, molecular data and cytology of various populations have revealed that there is a single species of *Ledebouria*, i.e., *L. revoluta*, in peninsular India and the other three species reported from India represent simply the variants. Four cytotypes viz.  $2n=30$ , 45, 60 and 90 have been observed in *Ledebouria revoluta*. Autopolyploidy has resulted into cytotypes and great diversity in morphotypes adapted to various climatic, geographic and edaphic zones. *Ledebouria revoluta* has adopted a means of vegetative propagation by formation of bulbils at leaf tips. The present investigation deals with geographical distribution, morphological variations, cytotypes and means of reproduction in *L. revoluta* based on more than 50 populations from various parts of peninsular India which support the merger of *L. viridis*, *L. karnatakensis* and *L. hyderabadensis* under a highly polymorphic species, *L. revoluta*.

**Key words:** bulbils, chromosomes, cytotypes, *Ledebouria*, vegetative morphology.

### **The road to evolutionary success in a Neotropical palm: the role of natural selection and genetic drift**

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Evolutionary success, as demonstrated by high abundance and a wide geographical range, is related to genetic variation and historical demography. Here we carried out a population genomics study in the Neotropical palm *Mauritia flexuosa*, sampling 16,262 SNPs by target sequence capture in 260 individuals across 22 populations, covering the entire geographical range across both humid Amazonian forests and arid Cerrado savannas. Only modest evidence for local adaptation was detected by outlier loci and Bayesian correlations of allele frequency and environmental variables, and by analysis of selective sweeps and genetic hitchhiking. The small difference in allele frequencies among widespread populations and the limited evidence for adaptive sweeps suggest a polygenic model of adaptation involving a large number of small, genome-wide effects and phenotypic plasticity as a potential mechanism for species adaptation in different biomes. Moreover, the low differentiation among *M. flexuosa* populations is consistent with environmental tracking leading to a change in population mean, but is mostly indicates that neutral evolution drives differentiation by genetic drift. As a model for tropical forest evolution, results from this widespread palm provide important insight into processes governing species diversity and distributions, and particularly the roles of phenotypic plasticity and neutral evolution.

**Key words:** Arecaceae, *Mauritia flexuosa*, population genomics, natural selection, target sequence capture.

## Using genomic barcoding to study global trade and evolution of the iconic monocot genus *Aloe* (Asphodelaceae)

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The genus *Aloe* L. is receiving increased global interest for its medicinal properties and horticultural value, placing already-threatened species at greater risk of extinction. Inclusion of all *Aloe* species (except *Aloe vera*) in CITES provides a form of legal protection. However, the identification of intercepted samples is often problematic, even using available molecular markers, and the international trade remains poorly understood. Expanded DNA barcodes using the whole genome hold promise for the identification and authentication of *Aloe* products in trade. Using target capture high-throughput sequencing (HybSeq), a genomic barcoding tool for *Aloe* will be designed, tested and optimised for use by stakeholders in the conservation and trade of herbal products. Barcodes will be produced for a representative sample of 200 species, providing 1) a reference database for sample identification and 2) genomic sequence information to build a high-resolution phylogenomic tree. Barcoding a variety of aloe-derived samples will lead to a checklist of traded *Aloe* species,



revealing areas of high conservation priority. Our phylogenomic tree will, in turn, provide a framework for studying the evolutionary history and use-related traits of the genus.

**Key words:** *Aloe*, barcoding, conservation, evolution, phylogenomics.

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## Physiology and biochemistry

### **Aquaporins facilitate the transport of nitrogen through membranes of *Vriesea gigantea*, an epiphytic bromeliad**

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Organic molecules can be the main input of nitrogen for plants in nitrogen-limited environments, such as the epiphytic habitat. *Vriesea gigantea*, an epiphytic tank-forming bromeliad, has a high capacity to absorb urea through their leaves, making it an excellent model to study urea metabolism. Three foliar aquaporin genes, *VgPIP1;1*, *VgPIP1;2* and *VgTIP2;1*, were cloned from urea-treated plants and functionally tested for urea, NH<sub>4</sub><sup>+</sup>/NH<sub>3</sub> and water transport by uptake studies in *Xenopus laevis* oocytes and complementation assays in *Saccharomyces cerevisiae*. In addition, aquaporin expression analyses were performed through qPCR in juvenile and adult plants of *V. gigantea*. The results showed that the three cloned aquaporins act as water channels. However, *VgTIP2;1* was the only aquaporin tested able to facilitate the diffusion of urea through the membrane, while *VgPIP1;2* seems to be capable of transporting NH<sub>4</sub><sup>+</sup>/NH<sub>3</sub>. Relative transcript abundance analyses showed that *VgPIP1;1* and *VgPIP1;2* were up-regulated in response to urea or ammonium only in juvenile plants, while *VgTIP2;1* were up-regulated in response to urea only in the adult plants. These results indicate that the ontogenetic shift from juvenile to adult-tank-forming-plants occur with metabolic changes regarding nitrogen metabolism regulation.

**Key words:** ammonium, aquaporins, epiphytic bromeliads, nitrogen metabolism, urea.

**Funded by:** FAPESP

### **Physiological responses to long-term water restriction in *Guzmania monostachia* (Bromeliaceae)**

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Water restriction is one of the crucial environmental factors in modulating foliar metabolism and morphogenesis in plants. *Guzmania monostachia* is a heteroblastic epiphytic tank bromeliad with photosynthetic plasticity capable of expressing the CAM under water stress. This study investigated physiological responses along the ontogenetic leaf development of *G. monostachia* under long -

term water restriction. For this, young plants were submitted to a water restriction for 120 days (in the control water was offered daily). Measurements of relative water content (RWC), chlorophyll fluorescence parameters, stomatal conductance and key components of CAM machinery were studied in three leaf groups (younger, intermediate, and mature). The water restriction reduced leaf expansion; the leaves became thinner, delaying the formation of the tank. There was a reduction in the chlorophyll fluorescence and RWC in all leaves. Comparatively, it was observed a higher RWC in the younger leaves. According to the nocturnal organic acid accumulation and stomatal conductance data, there was a gradual progression from classical CAM to the *idling* type in all leaves.

**Key words:** bromeliad, CAM, epiphyte, ontogeny, water restriction.

**Funded by:** CAPES

### **Effects of reduced fumarase mitochondrial activity on CO<sub>2</sub> assimilation rates and growth in *Setaria viridis***

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*Setaria viridis* (Panicoideae subfamily) is one of the most important agronomic groups of plants and has been recently proposed as a model system to investigate C<sub>4</sub> metabolism and bioenergy traits. In order to understand the impact of cell respiratory metabolism on a C<sub>4</sub> plant's growth, we used the RNAi silencing approach in which the mitochondrial fumarase gene was downregulated in *S. viridis* plants. Transgenics were obtained by *Agrobacterium tumefaciens* transformation and independent events resistant to hygromycin were identified. The progeny of T1 plants were grown and gas exchange and growth parameters were performed. The transgenics showed no significant changes in their photosynthesis rates and carbon assimilation before and after flowering compared to wild type plants. However, some transgenic plants showed delayed growth, presenting short plants compared to non-transformed plants. On the other hand, after flowering some events demonstrated no difference in growth parameters. These results indicate that reduced fumarase activity in *S. viridis* may influence their growth. However, it seems to be not a limiting factor for C<sub>4</sub> photosynthesis at ambient CO<sub>2</sub> partial pressure, making these plants interesting targets for exploring the role of fumarase in C<sub>4</sub> plants targeting changes in the use and storage of carbon.

**Key words:** biomass, C<sub>4</sub> metabolism, carbon allocation, fumarate hydratase, malate.

**Funded by:** Fundação de Amparo à Pesquisa do Estado de São Paulo- FAPESP

### **Responses to water deficiency related to foliar ontogeny in *Guzmania monostachia* (Bromeliaceae): potential water variations and different patterns of CAM**

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*Guzmania monostachia* is an attractive bromeliad to study distinct patterns of the Crassulacean acid metabolism (CAM) expression in leaves of different developmental stages. To investigate the CAM patterns and the water status of distinct leaf portions in different ontogenetic phases, we analyzed the variations in the potential water, relative water content, soluble sugar concentration and PEPC activity when the bromeliads were submitted to 8 days of water suspension. Our results indicate that apical and middle leaf portions of different foliar groups were induced to CAM when water was suspended at least for four days. A gradual reduction of the water potential of the apices during the 8 days of drought was observed, as well as the partition of the soluble sugars. The highest PEPC activity was found the apical part of young leaves. The relative water content was higher in the younger leaves compared to the other foliar stages. Thus, these results suggest that CAM photosynthesis is expressed gradually, starting with a classical CAM and reaching an idling form at 8<sup>th</sup> day. Soluble sugars can play a role in the osmoregulation, directing, in part, water transport both to the leaf apex and to the younger leaves.

**Key words:** Bromeliaceae, *Guzmania*, photosynthesis, physiological plasticity

**Funded by:** FAPESP, CAPES.

### **Intensification of CAM in young plants of the epiphytic bromeliad *Acanthostachys strobilacea* subjected to drought**

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CAM photosynthesis can be intensified under drought conditions, avoiding water deficit in plant tissues. CAM metabolism was identified in adult plants of the epiphytic bromeliad *Acanthostachys strobilacea* (Schultz f.) Klotzsch. Considering that establishment of populations depends on the ability of young epiphytes to withstand drought, the objective of this study was to evaluate the effect of drought on CAM activity by assessing phosphoenolpyruvate carboxylase (PEPC) activities and titratable acidity in young *A. strobilacea* plants exposed to irrigation withholding. Ninety-day-old juvenile plants cultivated on *Pinus* bark substrate were submitted to irrigation withholding for 8 days to induce drought stress. An increase in acid content during the day and an increase in the nocturnal activity of the PEPC enzyme was observed. The content of chlorophylls and carotenoids also increased in water deficit, which was accompanied by a reduction in the relative water content from 84.42% to 69.33%. This may have occurred due to the intensification of CAM activity, which is an acclimation to drought conditions.

**Key words:** Bromeliaceae, climate change, Crassulacean acid metabolism, photosynthesis, water shortage.

**Funded by:** Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES)

### **Rupicolous *Alcantarea imperialis* (Bromeliaceae) presents an increase in chlorophyll *a* content under irrigation withholding**

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Rupicolous bromeliads, especially in the juvenile phase, show unusual mechanisms of response to environmental stresses. It has been reported that chlorophyll *a* content of *Alcantarea imperialis* (Carrière) Harms increases at low temperatures, possibly as a cross response to multiple stresses since cooling can cause dehydration. The objective of this study was to evaluate if drought exposure leads to an increase in pigments content in young *Alcantarea imperialis* plants. For the drought treatment, 5-month-old plants did not receive water for 30 days. Plants submitted to drought were rewatered daily for seven more days. Control plants were watered daily throughout the experiment. Each hydric condition consisted of 120 plants. Leaves from plants of each hydric condition were sampled for chlorophyll and carotenoid content analyses. There was a significant increase in chlorophyll *a* content in drought-treated plants when compared to the control. After rehydration, there was a decrease in chlorophyll *a* content, which was similar to the control. Chlorophyll *b* and carotenoids have not undergone changes. These results show that *A. imperialis* juvenile plants show increases in chlorophyll *a* content under drought suggesting a possible remobilization of nitrogen to molecules of pigments in a situation of stress.

**Key words:** *Alcantarea imperialis*, bromeliad, drought, pigments, water availability.

**Funded by:** CNPq

### **Phytochemical characterization and spectroscopy by FTIR-ATR of extracts of leaves and epicuticular wax of *Copernicia prunifera* (Arecaceae)**

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*Copernicia prunifera* (Mill.) H.E. Moore is a native palm tree of Brazil, which is of economic importance. The objective of this study was to characterize the phytochemistry of alcoholic and aqueous extracts obtained from leaf and epicuticular wax from young and mature leaves of *C. prunifera*, using qualitative phytochemistry and analysis of Fourier Transform Infrared Spectroscopy–FTIR. The samples were collected in Caxingó, Piauí, Brazil. For the phytochemical characterization, qualitative tests were used (phenols, tannins, saponins, reducing sugars, organic acids, polysaccharides, alkaloids and flavonoids). The Fourier Transform Infrared Spectroscopy (FTIR) analysis was performed to identify the main chemical groups in the extracts. Condensed tannins, saponins and reducing sugars, alkaloids and flavonols were detected in leaf extracts and epicuticular wax. FTIR analysis revealed the presence of bands associated with these metabolites. The phytochemical characterization through qualitative tests and FTIR-ATR analysis showed classes of biologically active metabolites, which have pharmacological and biological properties. Thus, this technique can be used as a tool to find bioactive compounds for therapeutic purposes.

**Key words:** biological prospecting, Carnaúba, FTIR, northeast Brazil, phytochemistry.

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## Temporal variations in physiological responses to rehydration between desiccation-tolerant species are related to contrasting antioxidant mechanisms

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Desiccation tolerance is an extremely controlled process in which the cells return from vitreous to functionality state. Early studies performed with *Barbacenia purpurea* and *B. graminifolia* (Brazilian Velloziaceae species endemic to tropical inselbergs and *campos rupestres*, respectively) indicated that both species have a recovery of photosynthetic assimilation during rehydration. However, there was a temporal asynchrony of this response between both, leading us to hypothesize the existence of distinct antioxidant mechanisms involved in the rehydration. To test it, both species were submitted to a water deficit and further slow rehydration experiment. *Barbacenia purpurea* had a rapid response mechanism to avoid and quench reactive oxygen species production by means of phenolic compounds. *Barbacenia graminifolia* showed a delay in photosynthetic recovery, with ascorbate being the outstanding antioxidant involved in this response. There is also a distinct pattern of cell responses between species, in which chloroplasts are the pivotal organelle involved in the recovery of *B. purpurea* due to their role in flavonoid biosynthesis, while mitochondria are linked to the production of ascorbic acid by *B. graminifolia*. These findings indicate that species evolved distinct cellular strategies to cope with desiccation–rehydration, probably due to the unique environmental pressures from their respective habitats.

**Key words:** drought, microclimate, redox biology, resurrection plants, rock outcrops.

## Juvenile plants of *Alcantarea imperialis* (Bromeliaceae) cultivated under thermoperiod and drought can be able to perform CAM

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Native species of rupicolous environments are adapted to intense thermal amplitude and water deficit, such as *Alcantarea imperialis* (Carrière) Harms (Bromeliaceae). As daily thermal amplitude in the natural environment occurs at short intervals, plants must be able to change their metabolism rapidly to survive this condition. This study aimed to investigate whether *A. imperialis* has the potential to develop CAM in response to water deficit associated or not with thermal amplitude and if these factors may be linked to anatomical alterations. Six-month-old plants, obtained by *in vitro* germination, were transferred to a medium with polyethylene glycol (PEG 6000) to simulate drought (-8.5MPa) (T1) or without PEG (T2) under thermoperiod (30°C day/15°C night). Another group of plants was grown at constant temperature (25°C) with and without PEG (T3 and T4, respectively). All treatments were maintained under 12-h photoperiod in germination chambers. After 30 days, plants were evaluated in terms of phosphoenolpyruvate carboxylase activity, organic

acid content and anatomy. *Alcantarea imperialis* is able to perform CAM photosynthesis under thermoperiod associated with water deficit using PEG6000. CAM plants leaves did not show higher thickness of aquiferous parenchyma than leaves of C<sub>3</sub>. Thermoperiod associated with drought can induce CAM in this C<sub>3</sub> bromeliad.

**Key words:** inselbergs, leaf anatomy, rupicolous plants, temperature, water deficit.

**Funded by:** CNPq

### **Morphology and ultrastructure analysis of *in vitro* culms of *Guadua chacoensis* (Poaceae) under light and darkness conditions**

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*Guadua chacoensis* Londoño & P.M. Peterson is a native bamboo from the Atlantic forest. The conventional propagation of this species presents some bottlenecks; micropropagation techniques are a suitable alternative. An essential factor that influences micropropagation is light. So, this study aimed to evaluate the influence of light and darkness conditions during *in vitro* culture of *Guadua chacoensis* culms through morphological, histological and ultrastructure analysis. For that, samples were collected at 0, 10, 20 and 30 days and fixed for light microscopy and electron microscopy. Additionally, culm length and number of neoformed shoots were evaluated. Light microscopy analysis showed that culm morphology was not affected by light condition. In addition, morphological analysis demonstrated that culm length did not differ between both conditions and, in contrast, the number of neoformed shoots was higher in light than in darkness condition. Results of electron microscopy analysis revealed that light is an important factor in chloroplast biogenesis, as in darkness the presence of disorganized chloroplasts could be observed. This study demonstrated that cell ultrastructure, but not culm morphology, was affected by light conditions, *in vitro* culture being a suitable model to investigate light effects on plant morphogenesis.

**Key words:** bamboos, micropropagation, morphoanatomy, organogenesis, photomorphogenesis.

**Funded by:** CAPES and CNPq.

### **Endogenous levels of polyamines and hormones in culms of *Guadua chacoensis* Londoño & P.M. Peterson subjected to light and darkness conditions**

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Endogenous levels of polyamines (PAs) and hormones are important biochemical markers that help to elucidate plant physiology response to different light conditions. In this sense, this study aimed to investigate the endogenous contents of PAs, abscisic acid (ABA), gibberellin (GA<sub>4</sub>), jasmonic acid (JA) and zeatin (Z) in culms of *G. chacoensis* subjected to light and darkness conditions. At 0, 10, 20 and 30 days of cultivation, three samples were collected from each treatment for biochemical analysis. PA quantification was carried out by HPLC with UV detector and endogenous hormone levels were assessed by LC-MS/MS. PAs quantification demonstrated that the content of putrescine, spermidine and spermine were similar in both conditions. Hormone quantification showed that the content of ABA, GA<sub>4</sub> and JA decreased in darkness, when compared to light conditions. Otherwise, the content of Z quantification did not differ between treatments. These results showed that in contrast to PAs, the endogenous contents of hormones were significantly affected by light conditions, indicating that light is an essential factor triggering hormone biosynthesis. Possible reasons for that will be discussed during the oral presentation.

**Key words:** bamboos, micropropagation, organogenesis, photomorphogenesis response, biochemical analysis.

**Funded by:** CAPES and CNPq.

### **Effect of NaCl and PEG 8000 in *Setaria viridis* physiological traits**

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*Setaria viridis* (Panicoideae, Poaceae) was proposed as a genetic model system for studies involving C<sub>4</sub> photosynthetic metabolism. Among the interesting characteristics of this species, is its large capability to simulate water deficit. The aim of this study was to evaluate how *S. viridis* physiological traits respond to two well-known methods to simulate water limitation in hydroponic systems: NaCl and polyethylene glycol (PEG) 8000. Plants of *Setaria viridis* accession A10.1 grown in 25% Hoagland solution for 25 days after imbibition (DAI) were submitted to treatments with 150 mM NaCl or to 6, 12 or 24% (m/v) PEG 8000 and evaluated 8 and 13 hours after treatment (HAT). The main traits analyzed were relative water content (RWC), foliar and radicular relative electrolyte leakage (REL<sub>f</sub> and REL<sub>r</sub>, respectively), stomatal conductance (gs) and photochemical efficiency of photosystem II (Fv/Fm). REL<sub>f</sub> and REL<sub>r</sub> were significantly different from control, especially under salt treatments. Cell membrane injury associated with NaCl application was evidenced by electrolyte leakage, mainly in roots. However, treatments did not differ in terms of RWC, gs and Fv/Fm at 8 and 13 HAT.

**Key words:** abiotic stress, chlorophyll fluorescence, grass, leakage, osmoregulation.

## **Allelopathic potential of *Tillandsia tricholepis* (Bromeliaceae) leaf extracts on lettuce and foxtail millet**

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*Tillandsia tricholepis* Baker (Bromeliaceae) is an epiphytic plant, whose genus has a wide distribution in the Americas. Some epiphytic species could damage the host plants, increasing susceptibility to pathogen attack or leaf abscission due to their allelopathic activity. This work evaluated the effects of *T. tricholepis* fresh leaf extracts on seed germination and early growth of *Lactuca sativa* L. (lettuce) and *Setaria italica* (foxtail millet) seedlings. Seeds of lettuce and foxtail millet were germinated in different concentrations (1%, 5% w/v) of *T. tricholepis* extracts and distilled water as the control. The results showed that the extracts did not influence germination percentage, germination speed index, and dry mass compared to the control. Conversely, leaf extracts stimulated the growth of lettuce seedlings, especially root growth. The same effect was not observed in foxtail millet, which was similar to the control. Meanwhile, a significant inhibitory effect was observed in root branching development of lettuce when germinated at both concentrations. The results indicate that, despite concentration, *T. tricholepis* extracts have positive influence on germination and growth of tested species, however negative effect on lateral root development of lettuce at low concentration. The results suggest information for further studies exploring host-epiphyte interactions by allelochemicals.

**Key words:** allelochemicals, Bromeliaceae, epiphytic species, germination, phytotoxicity.

## **Light modulates auxin, abscisic acid, and 1-aminocyclopropane-1-carboxylic acid contents and induces cell wall modifications in aerial roots of the epiphytic orchid *Catasetum fimbriatum***

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*Catasetum fimbriatum* is an epiphytic orchid with a complex and flexible root system. Its roots might grow downward penetrating through organic substratum remaining in the dark, while the thin upward-growing lateral roots are fully exposed to light. Phytohormones are key endogenous signals modulating both root architecture and several photomorphogenic responses. The main goal of this study was to investigate the potential involvement of phytohormones on the phenotypic plasticity of *C. fimbriatum* roots in response to the light condition. For this, growing roots were either protected from or exposed to light. We analyzed the growth rate, morpho-histological modifications, endogenous levels of hormones, and transcripts analyses of genes related to cell wall lignification. Dark-grown roots showed greater growth rate, and lower dry weight than the exposed roots.



Surprisingly, only illuminated roots presented lignin cell wall thickening deposition in cortical cells. Additionally, the levels of IAA and ABA were higher in illuminated roots, whereas ACC content was lower. Finally, light increases the transcript level of genes related to lignification. Altogether, we conclude that light is an important signal mediating root phenotypic plasticity in *C. fimbriatum*, triggering secondary cell wall remodeling and differentially affecting the content of plant hormones and the development.

**Key words:** cell wall, hormones, Orchidaceae, photomorphogenesis, root.

**Funded by:** CAPES/FAPESP/CNPq

### **Epiphytic bromeliad *Acanthostachys strobilacea* rapidly adjusts osmotic potential and leaf temperature after exposure to water deficit**

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*Acanthostachys strobilacea* is an epiphytic bromeliad found at the Cerrado and Atlantic Forest domains in Brazil, hence it is adapted to a wide range of environmental conditions, which makes this species suitable for studies about stress tolerance in epiphytes. We aimed to evaluate if exposure to low water availability induces rapid physiological adjustments in *A. strobilacea*. In a growth room under 25±2°C and a 12-hour photoperiod, three-month-old *A. strobilacea* plants were transferred to dry substrate to induce water deficit. Control plants were maintained in well-watered substrate. Sampling occurred at 0, 2, 5, 10, 24, 48 and 72 hours after transfer. Relative water content was gradually reduced during the first 10 hours by 20% in drought-treated plants. Significant reduction in leaf osmotic potential was first detected at 10 hours of stress exposure when compared to the control (18% reduction). Leaf temperature increased by ca. 1.5°C in drought-treated plants throughout the experiment in comparison to the control. Despite such changes, the photosynthetic apparatus was not affected by water deficit. These results suggest that *A. strobilacea* rapidly employs strategies such as osmotic adjustment and transpiration reduction as a defense against drought.

**Key words:** Bromeliaceae, drought, epiphyte, osmotic adjustment, water deficit.

**Funded by:** CNPq

### Systematics, evolution, biogeography

### **Palynotaxonomy of *Hohenbergia* (Bromeliaceae) in an Atlantic forest fragment in Alagoinhas, Bahia, Brazil**

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*Hohenbergia* Schult. & Schult. f. is one of the most important genera of the Bromeliaceae family in South America. Its center of diversity is situated in Brazil, with 48 of 54 species, and the large majority of these are endemic, especially in the Atlantic Forest. Description of the pollen grains has always been relevant for Bromeliaceae taxonomy, mostly types of aperture and exine. Thus, this study aimed to describe the pollen morphology of *Hohenbergia* species that occur in an Atlantic Forest fragment in Alagoinhas, Bahia, Brazil. First, a survey was carried out to determine the species present in the Atlantic Forest fragment. After that, the floral buds were collected, and then, the material was processed using acetolysis method. Five microscopy slides were assembled, of these one used stained gelatin with safranin. The processed material was analyzed on a light microscope at 1000x magnification. *Hohenbergia catingae* Ule: monad, medium-sized, 2-porate, heteropolar, oblate shape, micro reticulated. *Hohenbergia stellata* Schult. & Schult. f.: monad, medium-sized, 1-sulcate, heteropolar, suboblate shape, reticulated. This work describes, for the first time pollen morphology of these two species and classifies the genus as euripalynous. *Hohenbergia* morphology is divergent, and pollen grains can provide additional characteristics to support the taxonomy.

**Key words:** Atlantic Forest, Bromeliaceae, palynology, pollen, taxonomy.

## Phylogenetic analysis of *Palisota* (Commelinaceae) using chloroplast and nuclear regions

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*Palisota* (Commelinaceae) consists of approximately 26 species. The genus, which differs from other Commelinaceae genera in androecial and pollen characters, a fleshy berry-type fruit, anatomical characters, and a basic chromosome number  $x=20$ , has been divided into two sections based on uniseriate vs. biseriate seed arrangement. Molecular phylogenetic analyses in Commelinaceae have placed *Palisota* near the base within the family, although its precise position is unclear. Chloroplast (*matK*, *rbcL*, *rps16*, and *trnL-trnF* and *psbA-trnH* intergenic spacers) and nuclear (AT103) regions in 17 accessions representing 15 species of *Palisota* and 15 outgroup species were sequenced and analyzed using parsimony, maximum likelihood, and Bayesian methods, with the goal of resolving *Palisota*'s placement within Commelinaceae and relationships among species. Preliminary results place *Palisota* sister to tribe Tradescantieae (maximum likelihood), or sister to both Tradescantieae and Commelineae (parsimony and Bayesian analysis). Within *Palisota*, all analyses yielded similar results. *Palisota schweinfurthii*, *P. alopercus*, and *P. preussiana*, all caulescent plants with red berries, are united, although relationships among them are unresolved. *Palisota ambigua*, *P. brachythyrsa*, and *P. tholloni*, all shrub-like or climbing plants, are also united. Results will be discussed in the context of sectional classifications, fruit color, and seed arrangement.

**Key words:** Commelinaceae, molecular, *Palisota*, phylogenetics, systematics.

## Population genetic structure of *H. catingae* (Bromeliaceae), a polymorphic species from Brazilian SDTFs.

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The Neotropical bromeliads from the genus *Hohenbergia* comprise species from the Atlantic Rainforest, as well from the Caatinga. The most conspicuous species from this group is *H. catingae*. Four varieties are recognized among them. The populations in the field show no geographic pattern where each one belongs to a single variety. In order to evaluate the genetic link between populations this work aimed to apply four nuclear and two chloroplast microsatellites to eight populations of *H. catingae*. The statistical analysis was conducted with commonly used programs. Genetic diversity resulted in low levels of observed and expected heterozygosity (0.0583 between populations). In addition, nuclear genetic analysis resulted in high genetic differentiation among populations and a highly structured population genetic structure. Pairwise  $F_{st}$  showed high differentiation between populations. Chloroplast analysis resulted in four haplotypes, and only a single haplotype was private from the GUA population. HAP1 showed the higher frequency, present in all populations. Our results suggest that gene flow between populations may be low, and the harsh Caatinga environment may prevent populations to breed triggering genetic and morphological differentiation. Furthermore, this species should be considered threatened due to the high levels of genetic differentiation coupled with a low level of genetic diversity.

**Key words:** bromeliad, evolution, microsatellite, phylogeography, SSR.

### **Taxonomy, phylogeny and biogeography of *Leiothrix* (Eriocaulaceae)**

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*Leiothrix* was described by Ruhland in 1903 with 28 species in five subgenera. Following the Flora do Brasil 2020, the genus contains 47 species all occurring in Brazil, especially in the Espinhaço Range in Minas Gerais and Bahia States. *Leiothrix* is a well supported monophyletic group, both in morphological and molecular analyses. It is characterized by having pistillate flowers with free petals as in *Paepalanthus*, with the styles fused into a column whose branches become free at different levels, flattened filaments, basifixed anthers and striate seed coat. During the last 20 years, species of *Leiothrix* have been the subject of diverse studies, including anatomy, taxonomy and phylogeny. Based on intense fieldwork and analysis of specimens in 51 herbaria, 39 species and eight infraspecific taxa are recognized. Molecular analyses were realized using 31 species in the ingroup, using plastid (*trnL-trnF*, *psbA-trnH*) and nuclear (ITS and ETS), employing parsimony and Bayesian inference. Two well-supported clades appear, one in Bahia and the other in MG and Itatiaia. A date for *Leiothrix* suggests that the ancestor of the genus may have arisen c. 23 Mya, with two isolated lineages originating after a vicariance event. The lineage at MG initiated the diversification process first.

**Key words:** Brazil, Espinhaço Range, molecular phylogeny, monocots, taxonomy.

**Funded by:** CNPq, FAPESB

## New insights into the evolutionary history of *Vriesea* species (Bromeliaceae) from RAD-seq data

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*Vriesea* is a hyperdiverse genus practically exclusive to the Atlantic Forest and the Brazilian dry diagonal. Because of its recent origin and diversification, it is difficult to develop variable molecular markers to infer infrageneric relationships, which prevents its detailed study. The attempts carried out to date to establish natural groups inside the genus have failed. The use of RAD-seq (Restriction Site Associated DNA Sequencing) data has been showing high resolution in studies at this taxonomic level and is quite promising. We seek to access a great part of *Vriesea* species genome to (1) obtain infrageneric phylogenetic resolution, inferring divergence times and relationship among the lineages; (2) trace key morphological characters; and (3) test the two traditional sections (*Vriesea* and *Xiphion*) linking their diversification with the specialized polinization by hummingbirds and bats, respectively. A total of 95 terminals were sampled. The preparation of genomic libraries and the Illumina sequencing were carried out by a sequencing facility. Total DNA was double-digested with the *sbfl* and *PstI* enzymes. We got 85GB of data containing 358.425.268 reads of 100bp each. The data was demultiplexed, the reads were quality-filtered and assembled using the software pipeline pyRAD v.3.0.4 Analyses are in progress.

**Key words:** Brazilian Atlantic Forest, Bromeliaceae, phylogenomics, Tillandsioideae, *Xiphion*.

**Funded by:** We thank the Coordenação de Aperfeiçoamento Pessoal (Capes), the Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ) and the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for fellowships and financial help for field and lab works.

## Chromosome number and heterochromatin band patterns in species of the genus *Scaphyglottis* (Orchidaceae)

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*Scaphyglottis* Lindl. has approximately 63 species distributed from Mexico to Brazil and Bolivia, with the probable basic chromosome number  $x=20$ . Cytogenetic analyses may reveal strategies of chromosome evolution and useful cytotaxonomic markers in chromosomally stable groups. The objective of this paper was to identify the chromosome numbers as well as the heterochromatin band patterns in *Scaphyglottis* species without previous chromosome records. For that, the technique of double staining with the fluorochromes CMA/DAPI was used. All species presented  $2n=40$ . In *S. amethystina* four CMA<sup>+</sup>/DAPI<sup>-</sup> bands were observed, two terminal and two pericentromeric. *S. bidentata* presented four CMA<sup>+</sup>/DAPI<sup>-</sup> bands, two terminal and two pericentromeric, in addition to 8-12 inconspicuous pericentromeric DAPI<sup>+</sup>/CMA<sup>0</sup> bands. In *S. sickii* two CMA<sup>+</sup>/DAPI<sup>-</sup> terminal bands were observed, 14 DAPI<sup>+</sup>/CMA<sup>0</sup> terminal bands of which two covered the entire long arm. In *S. stellata*, three CMA<sup>+</sup>/DAPI<sup>-</sup> terminal bands and ten DAPI<sup>+</sup>/CMA<sup>-</sup> pericentromeric were observed. The band diversity in *Scaphyglottis* analyzed here indicates that the group possesses different families of repetitive sequences. Despite the stability of chromosome numbers, heterochromatin distribution is a promising cytotaxonomic marker for species delimitation of the genus.

**Key words:** cytotaxonomy, evolution, fluorochromes, heterochromatin, Laeliinae.

**Funded by:** Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Instituto Nacional do Semiárido (INSA), Universidade Federal da Paraíba (UFPB).

### Phylogenetic biogeography of the family Cymodoceaceae (Alismatales)

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Cymodoceaceae is a family of sea grasses (5 genera, 17-18 species) common in tropical and subtropical littorals. With the aim to reconstruct the processes of historical distribution of Cymodoceaceae, a complex morphological and molecular genetic analysis was carried out (36 morphological features and the 5.8S ribosomal RNA gene) for 9 species of all genera of the family. *Scheuchzeria palustris* F. Muell was chosen as an outgroup. Using the WinClada program and bootstrap analysis, “molecular”, “morphological”, and “complex” cladograms were built for Cymodoceaceae. The complex cladogram was obtained as a result of combining molecular-genetic and morphological data matrix; it became the basis for reconstruction of the history of current distribution of Cymodoceaceae in combination with paleobotanical data. The oldest representatives of Cymodoceaceae (genera *Amphibolis*, *Halodule*, *Thalassodendron*) supposedly arose in the tropical waters of East Gondwana (Australia-Antarctica) in Cretaceous and later settled along the World Ocean by various tracks, the most important of which passed along the southern coast of Eurasian continental masses (through the “residual pool” of Tethys) and reached the waters of

modern Atlantic. More advanced genera *Cymodocea* and *Syringodium* originated in these areas and moved “back” to the waters of the Indian Ocean and the southwestern Pacific.

**Key words:** cladistics analysis, Cymodoceaceae, dispersal history, molecular data, sea grasses.

## **New and reassessed endangered species of *Griffinia* (Griffinieae, Amaryllidaceae) from the Brazilian Atlantic Forest**

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The Atlantic Forest is the center of diversity for the tribe Griffinieae, the only American tribe endemic to Brazil. In the context of our ongoing revision of Griffinieae, we describe a new species of *Griffinia* known only from one forest fragment located in the northeast of the state of Minas Gerais. The region is one of the most deforested of Brazil and leads the ranking of Atlantic Forest deforestation in the last decades. In addition, we present a taxonomic reassessment of *G. intermedia*, a species published in the 1820s from material in cultivation in Europe and historically of very uncertain identity. Although included in all taxonomic treatments published later for the genus *Griffinia*, the species remained poorly understood, as it was known only from the description, with unknown origin and lost cultivated material. We provide a detailed description and comments on its geographic distribution, including previously unpublished records, plus photographs of the species in its natural habitat. We also compare foliar anatomy and surface micromorphology of the two species with very similar flowers, evaluate their conservation status, both being considered threatened with extinction. An identification key for the species with large leaves and flowers of the genus is presented.

**Key words:** endemism, leaf anatomy, scanning electron microscopy, taxonomy, threatened species.

## **Diversity and conservation of Amaryllidaceae from the Serra da Mantiqueira and Serra do Mar**

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The Atlantic Forest, recognized for its high biodiversity and endemism levels, is reduced to about 5% of its original area. Close to a quarter of the remnant vegetation is located in the Serra do Mar and Serra da Mantiqueira ranges, in southeastern Brazil, the two major mountain chains of the Atlantic Forest. This study aims to improve our knowledge of the Brazilian Amaryllidaceae, focusing on the endemic and rare species of the mountain chains of the Atlantic Forest. A floristic treatment was prepared, including an identification key for the taxa found in four previously selected sites, besides photographs of all the species in their natural habitat. Floristic similarities with other regions in the Atlantic Forest, as well as conservation status and spatial distribution of these species were analyzed. Our preliminary results reinforce the unique importance of the

remaining fragments in mountainous areas of the Atlantic Forest for the conservation of Brazilian Amaryllidaceae. The studied localities are suffering disturbances such as the presence of invasive species and disorganized visitation, accompanied by illegal collection, which aggravate the threatened status of the species, requiring immediate measures to help guarantee conservation.

**Key words:** Amaryllidoideae, endangered species, endemism, *Griffinia*, *Hippeastrum*.

### **Phylogeography of *Dyckia* species (Bromeliaceae) and relationship with the biomes where they occur**

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The species of *Dyckia* (Pitcairnioideae) have characteristics that allow them to inhabit environments with drought conditions, such as the dry diagonal (DG) in the center of South America. Mato Grosso do Sul state (Midwest Brazil) is located in the DG and harbours four biomes (Cerrado, Pantanal, Chaco, Atlantic Forest), being one interesting area for phylogeographic studies. The aim of this study was to estimate the genetic structure and diversity within and among five *Dyckia* species endemic to the Cerrado and Pantanal/Chaco, and also one more widespread species, *D. leptostachya*. For this, we used two plastid markers (*rpl32-trnL*; *rps16-trnK*) and one nuclear (*phyC*) to carry out analyses with a phylogeographic approach. The cpDNA haplotype tree and network revealed three haplogroups, one formed by *D. leptostachya*, another by the species from Cerrado and one by the Pantanal/Chaco species. The genetic diversity was low within populations, but some indices were high when considering the total value of each species. We found a higher genetic diversity and a slightly structured network and tree with the *phyC*, being weakly correlated with biomes. Our results suggest that the biogeographic history of these biomes may have influenced the speciation process of this *Dyckia* species.

**Key words:** biomes, cpDNA, dry diagonal, *Dyckia*, phylogeography.

### **Diversity study of Mediterranean annual *Bromus* species (Bromeae, Pooideae, Poaceae) by using new polymorphic microsatellite markers**

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*Bromus* is one of the most diverse grass genera in the Mediterranean region, including some 40 species that are plastic and affected by reticulate evolution, making taxonomy difficult. Therefore, the study of diversity is essential to discover boundaries within and among species. New microsatellite markers were developed in two selected species belong to the subgenus *Bromus*: *B. lanceolatus* and *B. scoparius* with the aim to investigate their transferability as suitable markers. A DNA library was constructed and sequenced in Illumina MiSeq®platform-PE300 (Solexa, Cambridge, UK). From the five hundreds primer pairs generated, forty aleatory selected were tested and fifteen cross-amplified used to detect polymorphism in 217 individuals belonging to six Mediterranean species. Allele sizes were assigned with Geneious 9.1.7 (Biology Matters, Auckland, New Zealand). Descriptive statistics, PCoA, UPGMA cluster, AMOVA, and population structure were performed. A total of 79 alleles were recovered, ranged from 2 to 11, and the polymorphic information from 0.572 to 0.309 per loci. Cluster analysis revealed close relationship between *B. scoparius* and *B. chrysopogon*. The *B. lanceolatus* group was structured into four subgroups, segregating Canarian populations. New markers are promising tools to study populations and to discover boundaries and population structure within closely related species influenced by reticulate evolution.

**Key words:** *Bromus*, microsatellites, Next Generation Sequencing (NGS), Poaceae, taxonomy.

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### **A black sheep in the sedges: insights on the systematics and biogeography of *Carex* sect. *Phacocystis***

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Section *Phacocystis* (~120 spp.) is one of the largest, most widespread and taxonomically complex sections in genus *Carex*. It is mainly distributed in temperate and cold regions of the Northern Hemisphere, with disjunct species in the Southern Hemisphere. Species circumscription has remained obscure due to weak morphological boundaries and hybridization phenomena. We reconstructed a phylogeny based on a comprehensive sampling including about 80% of the species and using four markers (nuclear: ETS, ITS; ptDNA: *rpl32-trnL*<sup>UAG</sup>, *ycf6-psbMR*). Our preliminary results show sect. *Phacocystis* as a paraphyletic group in which the allied sections *Forficulae* and *Scitae* p.p. are nested. The morphologically similar section *Praelongae* was however excluded from it. A basal grade with species distributed in the North Pacific may point to this area's role in the origin of the section. In *Phacocystis* s.s., three large lineages with geographical congruence were recovered: (1) a mainly Western Palearctic clade; (2) a mainly East and Central Asian clade and (3)



a North American lineage which was the source for most colonizations of South America and Oceania. Several widely distributed and highly morphologically variable species displayed a rampant polyphyly, suggesting either their use as taxonomic hotchpotches or a major role of hybridization.

**Key words:** disjunction, Holarctic, hybridization, phylogeny, taxonomy.

### **Plastome phylogenomics of sugarcane and relatives confirms the segregation of the genus *Tripidium* (Poaceae–Andropogoneae)**

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Sugarcane (*Saccharum officinarum*) is one of the most important crops in the world and a major source of sugar for human consumption. Despite this immense value, the circumscription of the genus *Saccharum* is complex, contentious, and largely unresolved. *Saccharum* is either accepted in a broad sense by some authors or split into various genera such as *Erianthus* and *Tripidium* by others. A plastome phylogenomic analysis of sugarcane and relatives was performed in order to investigate generic delimitation, with emphasis on *Tripidium* (= *Erianthus* sect. *Ripidium*). Our plastome analysis demonstrates that *Saccharum s.l.* is polyphyletic and *Tripidium* (distributed in the Old World) belongs to a distinct lineage from *Saccharum s.s.* (Old World) and *Erianthus s.s.* (= *Erianthus* sect. *Erianthus*, New World). Therefore, this study confirms the recognition of *Tripidium* as a genus distinct from *Saccharum* and *Erianthus*, which is also supported by morphology and nuclear markers. The circumscription of *Erianthus s.s.* remains unclear since our results are consistent with either considering it as a distinct genus or including it in *Saccharum*. Better understanding of the evolutionary relationships of sugarcane and relatives may be useful for the selection of potential taxa for interspecific and intergeneric crosses in the genetic improvement of sugarcane.

**Key words:** *Erianthus*, high throughput sequencing, Old World, plastid genome, *Saccharum*.

### **Heterochromatin variation in Orchidoideae (Orchidaceae)**

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In the subfamily Orchidoideae, chromosome numbers vary from  $n=7$  to  $n=84$ , but little is known about the distribution of heterochromatin. We analyzed the patterns of CMA/DAPI bands in five species aiming to analyze the composition and distribution of heterochromatin in different genera. *Habenaria* presented  $2n=42$  and  $2n=100$  in *H. bicornis*, *H. petalodes* and *H. obtusa*, respectively. In *H. bicornis* heterochromatin CMA<sup>+</sup>/DAPI<sup>-</sup> was restricted to NORs on a single chromosome pair, in *H. petalodes*, the karyotype showed proximal regions DAPI<sup>+</sup>/CMA<sup>0</sup>, terminal regions CMA<sup>+</sup>/DAPI<sup>0</sup>, beside two pairs with a band DAPI<sup>+</sup>/CMA<sup>-</sup> and three eucromatic pairs. *Habenaria obtusa* presented a tetraploid cytotype with  $2n=100$  and chromosomes with proximal regions weakly stained with DAPI and CMA, terminal regions of the short arms CMA<sup>+</sup>/DAPI<sup>-</sup> and interstitial regions of the short arms DAPI<sup>+</sup>/CMA<sup>-</sup>. In *S. lanceolata* ( $2n=46$ ) heterochromatin was restricted to two bands CMA<sup>+</sup>/DAPI<sup>-</sup> terminals, while in *S. grandiflora* ( $2n=46$ ), the chromosomal terminals were CMA<sup>+</sup>/DAPI<sup>-</sup>. There was a variable distribution in quantity, composition and location of the heterochromatic fraction, even in species of the same genus.

**Key words:** chromosome evolution, CMA/DAPI, *Habenaria*, karyotype evolution, polyploidy.

**Funded by:** thanks to CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) and CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) for financial support.

### **Assessing the molecular diversity of *Hilidae* (Poaceae): reaching a compromise between the splitter and the lumpers**

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*Hilidae* is a tropical genus of grasses recently segregated from *Ichnanthus* sect. *Foveolatus*, mainly characterized by its paniculate inflorescences and the upper antherium with scars on the base of the lemma. It is mostly composed of species with obscure boundaries. This group was revised by two previous authors with very distinct opinions. One of them recognized more than 30 taxa whereas the other reduced this number to six, but with a long list of associated synonyms. We re-evaluated current and previous classifications through a comprehensively sampled phylogeny using plastid and nuclear data. Our data demonstrate that the diversity within *Hilidae* is a middle ground between the splitter and the lumpers, indicating that part of the names currently treated as synonyms should be reinstated at the species level. The Old World *Hilidae* were found to belong to two clades, one including plants from Asia and Oceania, and other with plants from the Neotropics and Western Africa. Topological incongruences suggest that hybridization may be a possible cause for the morphological variation observed. Additional studies on reproductive biology, cytology, and population genetics should be performed in order to better understand the biological processes involved in the evolution of this grass lineage.

**Key words:** Panicoideae, Paspaleae, phylogeny, species delimitation, tropical grasses.

**Funded by:** CAPES. CNPq. FAPESB.

## Relationship of the West Indian species *Arthrostyidium farctum* to other Neotropical woody bamboos

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*Arthrostyidium*, a genus of 31 mostly clambering Neotropical woody bamboos, has long been a catchall for ambiguous species thought to belong in subtribe Arthrostyldiinae. The genus is diagnosed by having racemose synflorescences and branches arising from a promontory. Owing to its equivocal taxonomic history, several species of *Arthrostyidium* have extraordinary morphologies. One particular case of this is observed in the West Indian species *Arthrostyidium farctum*, which has stiff, thread-like leaves emanating from the nodes giving the plant the appearance of pom-poms arranged along the culm. We investigated the relationship between *A. farctum* and other native West Indian woody bamboos using detailed morphological, anatomical and molecular analyses. We generated a phylogenetic tree based on four chloroplast DNA markers (ndhF, trnC-rpoB, trnD-trnT, rps16-trnQ) and recovered *A. farctum* as forming a well-supported clade with sister species *A. angustifolium* and *A. pinifolium*. Parametric bootstrap tests uphold this topology as significant. Detailed analysis revealed that these three species also share morphological and anatomical characteristics, which supports the phylogenetic result. Our findings help to improve the disordered taxonomy of *Arthrostyidium* and reinforce the taxonomic value of leaf anatomical characters for classification among the Neotropical woody bamboos.

**Key words:** Arthrostyldiinae, bamboo classification, Guaduinae, New World woody bamboos, West Indies.

## Niche conservatism explains differences in palm (Arecaceae) species richness amongst tropical habitats

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Seasonality may increase in the tropics due to the global climatic changes causing an expansion of seasonally dry habitats, shifts in species distribution and a decrease in species richness. Here we use a macroevolutionary framework to understand the differences in palm richness and lineage composition between seasonally dry and moist tropical habitats. We use trait-dependent

diversification models to test whether speciation rates in palms are different in seasonally dry and moist habitats. We also analyse phylogenetic dissimilarity across biogeographical regions and test whether niche conservatism affected differences in lineage composition within biogeographical regions. Our findings showed that palms colonized seasonally dry habitats *c.* 60 Ma, yet clades only diversified after *c.* 30 Ma. The high phylogenetic dissimilarity between habitats at both global and continental scales was driven by high lineage turnover, at least for Africa and Neotropics. We also found no evidences that differential speciation rates are responsible for the seasonally dry and moist palm-richness discrepancy. However, over the evolutionary history of palms, most lineages fail to colonize seasonally dry habitats due to a tendency to retain ancestral habitat. Indeed, seasonally dry palm assemblage was the result of the diversification of particular lineages.

**Key words:** Arecaceae, macroevolution, phylogenetic beta diversity, trait-dependent diversification, tropical dry forest.

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### **Heterochromatin banding patterns in species of the genus *Dichaea* (Orchidaceae: Zygopetalinae)**

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The Neotropical genus *Dichaea* Lindl., with about 100 species, presents controversial taxonomy and an infrageneric classification that is considered artificial. The genus is cytologically little known, and the available chromosome data refer to chromosome counts with conventional staining. We aimed to test the efficiency of the fluorochromes CMA (which binds preferentially to GC-rich DNA sequences) and DAPI (which binds preferentially to AT-rich DNA sequences) to detect heterochromatin variation in four species of the genus. *Dichaea anchoraelabia* and *D. cogniauxiana* presented  $2n=56$ , while *D. panamensis* and *D. pendula* presented  $2n=50$ . The use of CMA/DAPI made it possible to identify different band patterns in *Dichaea*, with CMA<sup>+</sup>/DAPI<sup>-</sup> bands on terminal and pericentromeric regions, and variable DAPI<sup>+</sup>/CMA<sup>-</sup> band patterns on interstitial regions, sometimes covering the entire chromosome. *Dichaea anchoraelabia* stood out by having four chromosomes totally DAPI<sup>+</sup>/CMA<sup>-</sup>. The other species, with the exception of *D. pendula*, had two to five chromosomes totally DAPI<sup>+</sup>/CMA<sup>0</sup>. The band pattern diversity observed here is an important indication that the genus presents different types of heterochromatin, which makes the use of CMA/DAPI a promising cytotaxonomic tool for the infrageneric delimitation of *Dichaea*.

**Key words:** chromosome banding, CMA/DAPI, cytotaxonomy, fluorochromes, heterochromatin.

**Funded by:** Universidade Federal da Paraíba / Instituto Nacional do Semiárido - INSA

### **Phylogeny of the genus *Dryadella* (Orchidaceae, Pleurothallidinae)**

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*Dryadella* Luer comprises ca. 54 species distributed in two main centers of diversity: the Atlantic forest of eastern Brazil and the Andean region. No phylogenetic study of *Dryadella* is currently available, but in previous molecular analyses of subtribe Pleurothallidinae, two species were included in a strongly supported clade containing Andean genera. The aim of this study was to provide a phylogenetic framework of the genus based upon molecular and morphological data, and a better understanding of the evolution of morphological characters and biogeography of the genus. This preliminary phylogenetic analysis of *Dryadella* was based on nrITS, and includes Brazilian and Andean species, representatives of phylogenetically-related genera (*Andinia*, *Muscarella*, *Phloeophilla*, *Platystele*, *Scaphosepalum*, *Specklinia*) as an ingroup and *Octomeria* as an outgroup. The data was analyzed using maximum parsimony. The results obtained in this study support the monophyly of the genus. Except for *D. guatemalensis*, the Brazilian species formed a separate clade from the Andean species. We provide the first evidence of monophyly and interspecific relationships of the genus. Additional studies are needed to determine morphological synapomorphies of the infrageneric groups.

**Key words:** Andes, Atlantic forest, evolution, nrITS, systematics.

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### **Speciation process in *Pontederia* (Pontederiaceae, Commelinales): an ecological niche models insight**

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Ecological niche modeling and its subsequent quantitative analyses have become useful tools to assess the ecological and evolutionary forces that act on geographical distributions and species diversification. *Pontederia* is a monophyletic genus with a probable early rapid radiation, but it has never been tested. Here, we provide the first statistically testable results related to niche divergence in a Neotropical group of aquatic plant, seeking to understand the diversification events in *Pontederia*. We reconstructed ecological niche models for three taxa arranged in four lineages of the well supported whitish-flowered clade using climatic and geographic data. These models were used in niche identity tests and multivariate analyses to explore the possible differences among their climatic niche envelopes. We found low overlap on potential distributions among species, influenced by differences in precipitation levels, especially rainfall distribution throughout the year, acting as an important factor in potential areas of occurrence delimitation and differentiation. The niche breadth, species distribution, and overlap patterns found in this study suggests a peripheral budding speciation action in the diversification of the group analyzed. Besides that, the use of niche characteristics of whitish-flowered *Pontederia* species was shown to be a promising criterion to help in solving taxonomic conflicts in the group.

**Key words:** aquatic monocots, budding speciation, environmental niche modelling, peripheral isolation, species diversification.

**Funded by:** CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) and NGC (Neotropical Grasslands Conservancy), through the Sue Wijdenes Memorial Award.

## **Eriocaulaceae in the Itacolomi State Park (Ouro Preto, MG)**

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Eriocaulaceae are monocots with capituliform inflorescences mainly diversified in tropical quartzitic mountains, especially in the *campos rupestres* of Southeastern Brazil. The Itacolomi State Park is visited and its flora collected by naturalists since the XVIII century, providing type specimens for several species of Eriocaulaceae. The Park is located in the Atlantic Forest Domain, in Ouro Preto, MG. We studied the Eriocaulaceae in Itacolomi State Park, based on field collections and from specimens of the herbaria OUPR and BHCN, as well as the virtual herbaria VIC, NYBG and CESJ. Results recorded 22 species for the area, being *Paepalanthus* the richest genus (13 species), followed by *Actinocephalus* (3), *Leiothrix* (3), *Syngonanthus* (2) and *Eriocaulon* (1). However, only 12 species were actually found in the field and five species remain known from type material only. These absences suggest that these species may have become rare or extinct, as major antropogenic disturbances have affected the Park in the last centuries. Descriptions for all species were provided, as well as photos, identification key, maps, and general comments on the plants biology.

**Key words:** *campos rupestres*, flora, Poales, *quadrilátero ferrífero*, threatened species.

**Funded by:** CNPq

## **Micromorphological and anatomical features of the spikelet and the upper anthecium of *Axonopus* and *Digitaria* (Poaceae: Panicoideae)**

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A micromorphological and anatomical study of the spikelet and the upper anthecium of 40 species of *Axonopus* and 36 of *Digitaria* is presented. This study is based on scanning electron microscopy (SEM). For the spikelet and upper anthecium, the shape, colour, texture, ornamentation, callus, germination lid, type of papillae, bicellular microhairs, unicellular macrohairs, silica bodies, prickles, stomata, and epidermal cells were studied (lower glume, lower lemma, upper lemma, and upper palea were analyzed). Five types of macrohairs for the spikelet were identified; while for the upper anthecium, two types of bicellular microhairs, one type of macrohair, six types of silica bodies, two types of prickles, two types of stomata, six types of epidermal long cells, and five types of papillae were observed. As partial results, the phenomenon of 3-flowered spikelets is recorded

for the first time for the genera *Axonopus* and *Digitaria* and it was verified in five species: *Axonopus anceps*, *A. schultesii*, *A. scoparius*, *Digitaria dioica*, and *D. nuda*. A new type of 3-flowered spikelets is recorded for the first time in the Paniceae. The analysis suggests that micromorphological and anatomical characters have the potential to serve as phylogenetic markers at the specific level.

**Key words:** anatomy of grasses, micromorphology of grasses, phylogenetic markers, SEM studies, tropical grasses.

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### Species delimitation in the *Cryptanthus zonatus* complex (Bromeliaceae)

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*Cryptanthus burle-marxii* and *Cryptanthus zonatus* (Vis.) Vis. are two species restricted to the north of the Brazilian Atlantic Forest and have difficult taxonomic delimitation due the overlapping of morphological characters. Both taxa belong to the *Cryptanthus zonatus* complex of species and are present in lists of threatened species. We used morphology and population genetics data, where ten loci nuclear microsatellites were genotyped in 147 individuals of eight populations of the *C. zonatus* complex in order to get the best delimitation in this complex. Samples were collected in Northeastern Brazil (states of Pernambuco, Paraíba and Rio Grande do Norte). The genetic structure analysis was performed with  $K=2$  and the clusters were formed based on geographic distribution instead of morphology. One cluster arises with the northern populations and the other with the southern ones. Thus, we refute the hypothesis of *C. burle-marxii* and *C. zonatus* as two distinct species, mainly because within each cluster there are populations with morphological characteristics of both taxa, showing us that there are not enough genetic differences to consider the two as different species. Thus, we synonymize *C. burle-marxii* under *C. zonatus* and we reevaluate their conservation status that was classified as Endangered.

**Key words:** Atlantic Rainforest, Brazil, genetic structure, microsatellites, population genetics.

### Biogeography of Bromeliaceae in the northernmost distribution of the Brazilian Atlantic forest and Caatinga

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This study aims to understand distribution patterns of Bromeliaceae along the extreme Northeast of Brazil, thus contributing to conservation planning of the group. Using herbarium specimens compiled from online databases, we analyzed species richness, collection effort and how environmental variables may affect the distribution and endemism of genera in a water resource region. Our sampling recorded 22 genera and 138 species of Bromeliaceae in the study area. The analyses show that altitude, mean temperature and rainfall are relevant factors affecting richness and genera distribution. Species richness is concentrated in the Atlantic forest and the Borborema plateau. Together, both these areas harbor highest richness, sampling effort and endemism. Four main patterns of genera distribution were recognized, each reflecting well-documented ecophysiological features of different lineages across the family. A region of extremely low precipitation might act as a biogeographic barrier blocking a northward dispersion of mesophytic genera, although differences in seed dispersion strategies must also be considered and further investigated. We conclude that, despite the family's adaptations to xeric environments, Bromeliaceae richness in the study area is concentrated in regions of higher altitudes or fragments of humid forests, which should be considered priority areas for conservation of the group.

**Key words:** bromeliads, mapping, monocotyledons, phytogeography, WorldClim.

**Funded by:** UFRN Sistemática e Evolução and Ciências Florestais Graduate Programs. CNPq, Proap/Capes. \*AC and FAC contributed equally to this work.

### **Pollen morphology of the *Xyris teres* complex (Xyridaceae)**

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Palynological studies in Xyridaceae are still scarce and pollen morphology is only known for a few species. This study aims to describe the pollen morphology of eight species of *Xyris*, which belong to the *Xyris teres* L.A. Nilsson complex, to improve the morphological characterization of the group. Pollen grains were collected from herbarium sheets and then acetolysed, measured, described and photographed under a light and a scanning electron-microscope. The data were statistically treated. The results showed that the main pollen pattern of the *Xyris teres* complex is stenopalynous due to the similarity in the pollen morphology, characterized as monads, ellipsoidal in polar view, medium to large sized, flattened/convex in equatorial view and monosulcate. The sulci are pontoperculate and covered with an insulae type membrane. The pollen wall ornamentation presents a microreticulum with perforations (with slight differences in the features and thickness of the muri). The sexine is thicker than the nexine. The contribution of pollen morphology to the taxonomy of this species offers few parameters, pointing to an assemblage of species of the *Xyris teres* complex palynological-related. Although previous studies showed that some groups of *Xyris* could be separated by pollen wall ornamentation, this was not observed here.



**Key words:** monocots, palynotaxonomy, Poales, pollen morphology, Xyrid clade.

## **A preliminary survey of Araceae diversity in Serra do Brigadeiro State Park, Minas Gerais, Brazil**

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The Araceae are a family composed of species with diverse life forms, ranging from aquatic to epiphytic. Currently, the family has 144 recognized genera and about 3,650 species. Araceae is characterized by the presence of an unbranched inflorescence containing flowers, the spadix, which is surrounded by a modified bract, the spathe. A preliminary survey of Araceae diversity in Serra do Brigadeiro State Park was realized using virtual herbaria (*SpeciesLink* and *Jabot*) and through bibliographic survey. Thirteen species were found, representing three genera, *Anthurium* and *Philodendron*, with six species each, and *Asterostigma* with only one species. The species found were: *Asterostigma luschnathianum* Schott., *Anthurium bocainense* Cath. & Nadruz, *Anthurium fontellanum* Nadruz & Leoni, *Anthurium gaudichaudianum* Kunth, *Anthurium longifolium* (Hoffm.) G. Don, *Anthurium lucioi* Nadruz, *Anthurium scandens* (Aubl.) Engl., *Philodendron acutatum* Schott, *Philodendron glaziovii* Hook. f., *Philodendron appendiculatum* Nadruz & Mayo, *Philodendron cordatum* Kunth ex Schott., *Philodendron minarum* Engl. and *Philodendron propinquum* Schott. Three of these species, *A. bocainense*, *A. gaudichaudianum* and *P. glaziovii*, were registered for the first time in Minas Gerais State. Field trips will help with an accurate taxonomic identification and possibly amplify the species' geographical distributions.

**Key words:** *Anthurium*, *Asterostigma*, Atlantic Rain Forest, *Philodendron*, Serra da Mantiqueira.

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## **Karyotype evolution and distribution of heterochromatin in some *Epidendrum* (Orchidaceae: Epidendroideae)**

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The genus *Epidendrum* L. includes groups of species with high variation in the number of chromosomes and other groups that are numerically stable. In this work we aimed to analyze numerically stable species of *Epidendrum*, seeking to obtain cytotaxonomic markers that allow distinguishing species or groups of species considering their karyotypes. Double CMA/DAPI staining was used in species with  $2n = 40$  belonging to different lineages. All species had a chromosome pair with CMA<sup>+</sup>/DAPI terminal bands, corresponding to RONS. However, *E. ciliare*, *E. ramosum* and *E. rigidum* presented terminal DAPI<sup>+</sup> regions in almost all chromosomes, in

addition to pericentromeric CMA<sup>+</sup>/DAPI<sup>-</sup> bands. On the other hand, *E. avicola* presented, by monoploid complement, five CMA<sup>+</sup>/DAPI<sup>-</sup> pericentromeric bands, while *E. paniculatum*, *E. proligerum* and *E. tridactylum* presented pericentromeric DAPI<sup>+</sup>/CMA<sup>-</sup> bands in three, two and one chromosomal pair(s), respectively. Morphologically distinct species such as *E. rigidum* and *E. ciliare*, *E. proligerum* and *E. tridactylum*, or *E. avicola* and *E. paniculatum* formed groups with similar banding patterns. This analysis suggests that distantly related species of *Epidendrum* may present correlated patterns of CMA/DAPI bands.

**Key words:** Brazilian orchids, chromosome number, CMA/DAPI, different patterns of heterochromatin, karyotype stability.

## Characterization of the molecular diversity in Tigridaeae (Iridaceae) species

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Iridaceae is an amazing family of Asparagales, characterized by extensive morphological and cytogenetic diversity. In contrast, previous studies reported that the molecular variation is not extensive within Iridaceae (e.g., in Sisyrinchieae). As Sisyrinchieae, Tigridaeae is placed in Iridoideae, but their species occur exclusively in the Neotropics. The understanding of the phylogenetic relationships within Tigridaeae has increased, but there is no information about molecular diversity for phylogeography and DNA barcoding studies. The aim of this study was to characterize molecular dataset to understand how the currently employed markers provide information within Tigridaeae. Four plastid and one nuclear datasets from representatives of Tigridaeae (18 species, 68 populations) were employed. Considering all sampled populations, plastid *matK-trnK* was the most polymorphic in number of haplotypes ( $h=35$ ) and haplotype diversity ( $Hd=0.975 \pm 0.006$ ). In 59% of sampled populations, a single haplotype was found in 4/5 or 5/5 of the screening markers. Estimated K2P distances were extensive between lineages ( $0.514 \pm 0.052$ ), within clades A ( $0.029 \pm 0.052$ ) and B ( $0.079 \pm 0.008$ ). The extensive proportion of monomorphic species demonstrates the need to expand the search for other potentially polymorphic markers able to characterize the complex genetic diversity in Tigridaeae species.

**Key words:** DNA Barcoding, phylogeography, plant evolution, population genetics, taxonomy.

**Funded by:** Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES); Universal MCTI/CNPq N° 14/2013

## Barcoding of Bromeliaceae

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The angiosperm family Bromeliaceae comprises 3906 species, almost all of them restricted to the Neotropics. The family is characterized by exceptionally high morphological and ecological plasticity, but very low genetic variability. In several genera, plants are vegetatively very similar which makes determination of bromeliads difficult. Especially in botanical collections this is a problem, when plants are cultivated several to many years without flowering. Barcoding is a very promising approach to provide fast and cheap determination of bromeliads, however, the observed low genetic variability causes specific problems. In the scope of a project funded by the German Federal Ministry of Education and Research (BMBF) to improve access and scientific use of living collections in Botanical Gardens, a number of markers was tested for their suitability for barcoding (nuclear: *Agt1*, ETS, *PHYC*; plastid: *matK*, *ycf1*). The low copy nuclear marker *Agt1* was identified as a potential genetic barcode suitable for identification and applied to taxonomically comprehensive and reliably determined sampling provided by several botanical gardens as well as from the private collection of one of the authors.

**Key words:** bromeliads, DNA barcoding, integrative taxonomy, low copy nuclear marker, species identification.

**Funded by:** German Federal Ministry of Education and Research (BMBF)

### **Phylogenetic analyses of subtribe Parianinae with emphasis on *Eremitis* (Poaceae: Olyreae) based on DNA sequences and morphology**

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*Pariana* and *Eremitis* are herbaceous bamboos included in the subtribe Parianinae, characterized by fimbriae at the apex of the leaf sheaths and exclusively spiciform inflorescences. The proximity between them has been historically discussed and often they were considered as a single genus (*Pariana* sensu lato). We analyzed the relationships within Parianinae based on combined data from nuclear (ITS), plastid DNA spacers and morphological aspects, including ectexine patterns of pollen grains, using Maximum Parsimony and Bayesian Inference methods. Parianinae includes three lineages represented by 1) *Parianella*, exclusively from Southern Bahia, Brazil; 2) core *Pariana*, distributed in Central America and northern South America, mainly in the Amazon Basin; and 3) *Eremitis*, endemic to the Brazilian Atlantic Forest, from Pernambuco to Rio de Janeiro. Dimorphic and multiple inflorescences on the leafy culm apex and rarely on decumbent culms, as well as a pubescence on the style, may represent morphological synapomorphies of *Eremitis*. Core *Pariana* and *Eremitis* display pollen with areolate-microechinate ectexine, in contrast to the microechinate ones of *Parianella*. Based on such characters and a high support in all molecular analyses, *Pariana multiflora*, endemic to the Espírito Santo State, Brazil, is transferred to *Eremitis*.

**Key words:** Bambusoideae, molecular phylogenetics, Neotropical grasses, *Pariana*, *Parianella*.

**Funded by:** CNPq, CAPES, FAPESB

### **High gene flow maintains range-wide species cohesion in a Neotropical riparian epiphyte (*Tillandsia aeranthos* – Bromeliaceae)**

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Phylogeographic frameworks allow testing hypotheses about organisms' evolutionary responses from patterns of genetic diversity and structure, phylogenetic relatedness and ecological traits across species' geographic ranges; data which is scarce for South American clades. Here we present a study of genetic diversity, structure and reproductive biology in a subtropical bromeliad endemic to the Plata River basin, *Tillandsia aeranthos* (Lois.) L.B. Smith, based on seven nuclear microsatellite markers and 13 chloroplast regions from 13 populations from Brazil and Argentina and controlled pollination experiments. Considering that *T. aeranthos* is widespread across various topographies, we expected some degree of population differentiation. Alternative hypotheses were: H1: Populations are not genetically structured, with gene flow maintaining species cohesion; and H2: Genetically structured populations with moderate to elevated levels of differentiation due to limited gene flow. Microsatellite results show high gene flow between populations, low genetic structure ( $F_{ST}=0.031$ ,  $p<0.001$ ) and high genetic diversity ( $H_O=0.745$ ,  $F_{IS}=0.162$ ). There was no evidence for isolation-by-distance ( $p=0.258$ ,  $r=0.035$ ). Chloroplast regions were monomorphic across all populations. Controlled pollination experiments showed complete self-incompatibility. Results indicate panmixia, possibly allowed by the reproductive system and connectivity of the riparian environments *T. aeranthos* inhabits.

**Key words:** microsatellites, pampas, panmixia, phylogeography, reproductive biology.

**Funded by:** FAPESP, CAPES

### **Dioscoreaceae of the Brazilian Flora 2020**

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Brazil has 141 cataloged species of Dioscoreaceae, being the country with the largest number of endemic species (105). Field data were collected mainly by our studies of regional floras (Espírito Santo and Rio de Janeiro states) and taxonomic revisions, but additional data were also compiled from herbaria aggregated to the Re flora Program and from the literature. The family is represented by two genera: *Tacca*, with only 1 species (*T. parkeri* Seem.) and *Dioscorea*, the largest one, which occurs in almost all Brazilian domains (except Pantanal). Eighty one species of *Dioscorea* occur in Atlantic Forest, 69 occur in Cerrado, 25 occur in Amazonian Forest, 23 occur in Caatinga and 3 species occur in Pampa. The two physiognomies with the highest number of *Dioscorea* species are

Rainforests, with 66 species, 29 of them exclusive to this vegetation and Cerrado (sensu lato) with 55 species, 17 of them exclusive. Southeast is the region with the largest number of species, 98. In the past years, three new species were described for *Dioscorea*. We are currently describing at least more 5 species. Thus, Brazilian richness tends to grow.

**Key words:** *carás*, *Dioscorea*, Neotropics, taxonomy, yams.

## **Phytoliths of African and Amazonian palms and their applications in taxonomy, paleoecology and archaeology**

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Phytoliths are silica bodies found in the cells of vascular plants. They are especially abundant in palms, occurring in all organs, with the highest concentrations in leaves. Phytolith morphologies are often diagnostic for specific plant groups. Since these structures can remain in the soil for millions of years, they provide physical evidence for the presence of specific taxa in time and space. Applications include reconstructing ancient floras and landscapes, and documenting plant use by ancient peoples. We are currently producing regional catalogues for identification of palm phytoliths in two palm-rich regions of the world: continental Africa and the Amazon. We surveyed contemporary phytoliths, extracted primarily from herbarium specimens (COL, G) in 113 species of Amazonian palms (31 genera), and 45 species of African palms (16 genera); all five subfamilies were sampled. We characterized 14 morphotypes, of which two are unique to American palms and four to the Paleotropical species sampled. These morphotypes permit diagnosis of subtribes and genera, and in some cases species. Phytoliths are proving to be a powerful line of evidence linking palms to ancient environments and human activity in both study regions. They are also a promising source of data for taxonomic studies in palms.

**Key words:** Africa, Amazonia, Arecaceae, palm phytoliths, phytolith morphotypes.

## **The Devil is in the details: Novel insights about the genus *Bromus* (Bromeae, Pooideae, Poaceae) in Italy**

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After 35 years of standstill a fundamental text for Italian botany, Flora d'Italia (Pignatti 2017 and forthcoming), is undergoing an extensive review and amplification. However, in the new edition some genera show only minor revision. In the last decade major collaborative efforts were directed to update the checklists and map the biogeographical regions of the country; as a result, a deeper

systematic screening was put temporarily on hold. Among the genera needing a closer look there is *Bromus* L., represented at least by 32 species in Italy. This study proposes an herbaria-based analysis of Italian specimens of 12 species belonging to subgenus *Bromus*, and 6 to *Festucaria*. Through the analysis of the morphological features integrated with ecological, space-time, phylogenetic and even archaeobotanical evidence we aim to (1) to delineate most discriminating traits among species, (2) understand the extent of phenotypic plasticity and (3) shed light on the evolutionary pathways of these taxa. The preliminary results support the partitioning in two subgenera (by somatic characters), highlighting a rather complex situation for the subgenus *Bromus*, which shows low resolution – especially for the ranks below species. The archaeobotanical evidence suggests the possibility of indirect artificial selection, partially explaining this complexity. **Key words:** *Bromus*, *Festucaria*, herbaria-based studies, Italian flora, morphological characters.

### Self-pollen deposition inside floral buds of *Wittmackia patentissima* (Bromeliaceae)

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Self-pollen deposition is one type of self-pollination, where the pollen doesn't need a pollinator to reach the stigma. Producing autogamic seeds may be a strategy to maintain the species cohesion. Here we investigated potential cleistogamy in floral buds of *Wittmackia patentissima* (Mart. ex Schult. f.) Mez, living in sympatry with different Bromelioideae species. We dissected and examined floral buds, which were grouped into size classes: B1 up to 3 mm long; B2: 4-7 mm; B3: 8-11 mm with visible petals; B4: 12 mm or in pre-anthesis. Pollination tests with bagged flowers showed that the species is auto-compatible and can produce fruits with self-pollen deposition (57%-fruit set). Floral buds have stamens higher than the stigma, and the anthers were found to be dehiscent in pre-anthesis, favoring the self-pollen deposition. No stigma B1, 10%-B2, 50%-B3 and 60%-B4 presented self-pollen. Fluorescence tests showed that no pistils B1, one B2, thirteen B3 and twenty B4 possessed pollen tubes. The self-pollination and pollen germination inside buds suggest that self-deposition is one possible isolation mechanism in the reproduction of this Bromelioideae, and it may maximize its reproductive success, particularly avoiding hybridization with chasmogamous sympatric taxa.

**Key words:** autogamous flowers, Bromelioideae, floral morphology, germination, self-pollination.

**Funded by:** Capes, Nap/Esalq/USP

### Structural variation of root vessel elements of *Canna* species

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Cannaceae belongs to the Zingiberales order, and comprises 50 Neotropical species which generally have economic importance. This study aims to characterize structural types of vessel elements and to describe their pit membrane remnants. Root fragments were fixed, and the cellular elements were macerated using 40 % formaldehyde and glacial acetic acid (1:1). For Scanning Electron Microscopy, samples were dehydrated and critical point dried, coated with 20 nm gold, and observed in a Zeiss EVO 40 electron microscope. Types of vessel secondary wall thickenings and perforation plates have diagnostic value among *Canna* species. Scalariform secondary wall thickening was observed in all studied species, although partially pitted thickening was restricted to *C. generalis*. Perforation plates were scalariform or reticulate in *C. generalis*, whereas the simple type was also found in *C. patens* and *C. paniculata*. Between these two species, perforation plates in *C. patens* exhibiting more than 40 bars and in *C. paniculata* no more than 20 bars were detected. In all studied species vessel pit membranes were found to be intact, and few areas appeared to be split.  
**Key words:** Cannaceae, perforation plates, pit membranes, root, xylem.

**Funded by:** CNPq

### **Taxonomic revision of the *Dyckia tuberosa* complex (Bromeliaceae, Pitcairnioideae) in the southern cone**

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*Dyckia* is a broad genus of Neotropical Bromeliaceae that occurs essentially in the South American dry diagonal. Several recent taxonomic works in this genus are proposing informal complexes, just like ours. The *Dyckia tuberosa* complex is basically characterized by presenting the following set of morphological characters: simple or composite inflorescence, pedicellate flowers, unguiculate petals, stamens included, filaments with cones at least at the base, and discoid seeds with a subfalciform appendix. Within our circumscription for this complex, most of the species of the genus belong to it. However, accurate analysis of the species occurring in the southern cone, southern Brazil, Uruguay, Argentina and Paraguay, revealed that of the approximately 170 species accepted for the genus cited for this territory, 14 belong to this complex. However, most of the names we consider as synonyms, consisting of a nomenclaturally inflated group, with little pragmatic taxonomy. Starting from our analysis we accept as species: *D. minarum*, *D. tuberosa* (with the synonyms: *D. pseudococcinea*, *D. coccinea*, *D. deltoidea*, *D. croceae*, *D. lutziana*, *D. linearifolia*, *D. cabrerae*, *D. subinermis*, *D. remotiflora* var. *angustior*), *D. dusenii* (with the synonym: *D. ibicuiensis*), and *D. vicentensis* (with the synonym: *D. waechteri*). We also localized two new species.

**Key words:** Argentina, Paraguay, south of Brazil, taxonomy, Uruguay.

### **Advances in understanding the evolutionary history of the herbaceous bamboo genera *Parodiolyra* and *Raddiella* (Poaceae, Bambusoideae, Olyreae)**

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Molecular studies involving the herbaceous bamboos of subtribe Olyrinae are in progress to better understand the inner relationships of this group, which comprises four main lineages. We investigated the phylogenetic relationships within one of them, composed of *Parodiolyra* Soderstr. & Zuloaga and *Raddiella* Swallen, which share several features and were indicated as paraphyletic in previous studies. We increased the sampling of both genera based on material obtained in recent field work, and used five nuclear (ITS1-5.8S-ITS2) and cpDNA (*rpl32-trnL*, *trnD-trnT*, *trnS-trnG* and *ndhF*) regions, analyzed using Maximum Parsimony, Bayesian Inference, and Maximum Likelihood methods. Our results reinforced the paraphyly of *Parodiolyra*, which includes two main lineages, one represented by *P. micrantha*, which is sister to the clade including *Raddiella* and the remaining *Parodiolyra*. Combining this data with morphological evidence, we propose the transfer of *P. micrantha* and *P. colombiensis* to a new genus, rendering *Parodiolyra* monophyletic with *P. aratytiopensis*, *P. lateralis*, *P. luetzelburgii*, and *P. ramosissima* (the type species). Our results were inconsistent for *Raddiella* due to the incongruent placement of *R. malmeana* among data partitions. Thus, any taxonomic decision on this genus depends on the inclusion of other phreatophyte species in the analyses.

**Key words:** molecular phylogeny, Neotropical grasses, Olyrinae, taxonomic novelties, topological incongruence.

### **Biogeographical insights into the Brazilian lineage of Tillandsioideae (Vrieseinae, Bromeliaceae)**

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Vrieseinae is included in Tillandsioideae (the largest Bromeliaceae subfamily) and encompasses about 20% of species in four genera, *Alcantarea*, *Stigmatodon*, *Vriesea* s.s. and *Waltillia*, a practically Brazilian lineage. Here, we aim to infer the biogeographic history of this subtribe, under the hypothesis that its origin occurred in the Brazilian Shield. All of Tillandsioideae genera (except *Waltillia*) were sampled for DNA extraction and PCRs, totaling 130 terminals. Two cpDNA (*rps16-trnK* and *matK*) and one nDNA (*PHYC*) markers were amplified. Cladistic and biogeographic analyses were performed. A vicariant event between the Andes and Brazilian Shield occurred about 10 Ma, when two clades diverged: Cipunopsidinae + Tillandsieae and Vrieseinae.



After an extensive search in the literature we suggest that a set of events influenced divergence of the main clades. While Miocene events pointed to the diversification of the major clades within Tillandsioideae, Pleistocene events have probably had an important role on the diversification of the most recent clades, mainly the Vrieseinae genera. This work sheds light on the biogeographic history of a key group of Neotropical plants, providing a great hypothesis for the evolution of bromeliads.

**Key words:** Andes, Atlantic Rainforest, Brazilian Shield, Miocene, Pleistocene.

**Funded by:** CAPES, CNPq and Museu Nacional

### **Monocotyledons of the Delta do Parnaíba Herbarium, Piauí, Brasil**

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The monocotyledons are represented by 60,100 species distributed in 60 families. In Brazil, there are around 9,067 species (5,355 endemic) and 773 genera, with 2,848 species in the Northeast. Despite their economic importance and wide distribution, there are still gaps in knowledge of their diversity and distribution in the northeastern region of Brazil. The HDELTA collection has approximately 5,000 specimens distributed in 143 families, including plants, fungi and algae, 569 genera and 987 species. The great relevance of the Herbarium is due to its contribution to the advancement of research on the floristic composition of the Parnaíba River Delta, in addition to holding specimens from other parts of neighboring states such as Maranhão and Ceará. A survey of the monocotyledon species in the collection was carried out. There were 27 families, 103 genera and 201 species; Cyperaceae (51 species), Poaceae (50) and Araceae (19) had the highest number of taxa. The most abundant genera were *Cyperus*, *Eleocharis* and *Paspalum* (16, 10 and 8 spp., respectively).

**Key words:** diversity, floristics, forage plants, monocotyledons, northeast of Brazil.

**Funded by:** Edital Mac-Doubles Fernandes do Nascimento de apoio a Ciência, Tecnologia e Inovação, Prefeitura de Parnaíba, PI

### **Heterochromatin in species of the subtribe Pleurothallidinae (Orchidaceae)**

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Pleurothallidinae is little studied karyologically with regard to its patterns of heterochromatic bands. In this work we determined chromosome number, composition and distribution of heterochromatin in six subtribe species through CMA/DAPI staining. In *Acianthera*, chromosome numbers varied from  $2n=40$  in *A. panduripetala* and *A. saundersiana*, to  $2n=34$  in *A. macuconensis*, with five CMA<sup>+</sup>/DAPI pericentromeric bands in the first, 10 terminal or proximal bands in the second and at least three proximal bands and three terminals per monoploid complement in the third. In *Anathallis rubens* and *Stelis aprica*,  $2n=30$ , without observable heterochromatic bands, while *Specklinia picta*, with  $2n=20$ , revealed, by monoploid complement, a pattern of bands formed by nine pericentromeric bands DAPI<sup>+</sup>/CMA<sup>-</sup>, in addition to CMA<sup>+</sup>/DAPI<sup>-</sup> in both arms of seven chromosome pairs. With the exception of *Stelis aprica*, all counts were unpublished and constitute new records of chromosome numbers for Pleurothallidinae. In this subtribe, preliminary information suggested composition and differentiated distribution of heterochromatin among different genera, with a tendency to a greater amount in *Acianthera* and *Specklinia* species and small amounts in the genomes of *Stelis* and *Anathallis*. In this work, seems to confirm this tendency.

**Key words:** chromosome number, cytogenetics, Epidendroideae, fluorochromes, karyotype.

**Funded by:** Universidade Federal da Paraíba (UFPB), Instituto Nacional do Semiárido (INSA).

### **Population genetic analysis of a bromeliad species complex in Northeastern Brazil reveals a geographic structured gradient genetic differentiation**

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Bromeliaceae are known for their morphological diversity and adaptative radiation. Among the bromeliads, *Hohenbergia ridleyi* (Baker) Mez is treated as a species complex and occurs along the Atlantic rainforest on northeastern Brazil. This complex is polymorphic and includes species that can be easily misidentified. To evaluate the species integrity, this work applied five nuclear and three chloroplast microsatellites in 181 individuals belonging 16 populations (BA, PE, RN and SE states). Statistical analysis was performed in commonly used programs. Nuclear and plastid primers showed an average of 17 and 15 alleles per locus, respectively. Population analysis showed an expected heterozygosity (0.40-0.77) higher than observed (0.13-0.58) for all populations. An AMOVA indicate high genetic differentiation within populations (83.88%). Analysis comparing regions and species resulted in a relatively low  $F_{ST}$  (0.16). However, a hierarchical AMOVA comparing each geographic region, excluding the type region (PE), resulted in considerable differentiation between them (0.20-0.24). In other words, populations from BA, SE and RN are similar to PE's populations but different between them. Such evidences reinforce the idea that PE holds the higher genetic differences within the *H. ridleyi* complex, acting as a bridge region between northern and southern populations.

**Key words:** biogeography, bromeliads, evolution, *Hohenbergia*, SSR.

**Funded by:** FACEPE

## Updates on phylogenetic studies of *Chusquea* from Brazil, focusing on the complex *Euchusquea* clade

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*Chusquea* represents the most diverse genus of Neotropical woody bamboos (tribe Bambuseae). It comprises 180 described species, occurring from Mexico and the West Indies to Argentina, Chile and Brazil, from sea level to over 4,000 m.a.s.l. *Chusquea* is currently the sole genus included in the subtribe Chusqueinae and comprises the subgenera *Magnifoliae*, *Platonina*, *Rettbergia*, *Chusquea*, and *Swallenochloa*. The last two form the strongly supported *Euchusquea* clade, encompassing ca. 75% of the species diversity of the genus, but neither is supported as monophyletic based on plastid or plastid plus nuclear sequence data. Previous phylogenetic studies focusing on the *Euchusquea* clade recognized five main lineages, one of them including most Brazilian species of the genus. We have increased the number of Brazilian *Chusquea* in our phylogenies based on nuclear internal transcribed spacer (ITS) and plastid DNA sequence data (*ndhF* and *trnD-trnT*), confirming the monophyly of this lineage and also recognizing a sixth one within the *Euchusquea* clade, which we named clade VI–*Chusquea meyeriana*. Internal relations within this lineage are obscure, but includes a main clade formed by the species of the informal group *C. meyeriana*, which must be investigated by taxonomic and molecular criteria.

**Key words:** Chusqueinae, nuclear and plastid DNA, phylogeny, systematics, woody bamboos.

**Funded by:** CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico)

## Chromosomal characterization in three species of Orchidoideae (Orchidaceae)

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The subfamily Orchidoideae comprises terrestrial plants of cosmopolitan distribution (except Antarctica), with chromosome numbers varying from  $n=7$  to  $n=84$ . The present study aimed to examine the chromosomal variation of two species of Spiranthinae and another of Orchidinae, using the CMA/DAPI banding technique. In Spiranthinae, *Sarcoglottis curvisepala* and *Sauroglossum elatum* presented asymmetrical karyotypes with  $2n=46$ , highlighting a larger chromosome pair entirely CMA<sup>+</sup> in *S. elatum*, while in *S. curvisepala* this pair was CMA<sup>+</sup> in the long arm and DAPI<sup>+</sup> in the short arm. Both species were DAPI<sup>+</sup> in the proximal regions, while the terminal regions were CMA<sup>+</sup> in *S. elatum* and neutral in *S. curvisepala*. The third analyzed species, *Habenaria josephensis*, presented proximal regions weakly stained for both fluorochromes and neutral terminal regions, except for bands CMA<sup>+</sup>/DAPI<sup>-</sup> bands in three chromosome pairs. The genus *Habenaria* is variable in chromosome numbers and heterochromatic banding patterns. However, the genera *Sarcoglottis* and *Sauroglossum* are numerically stable and, although they varied in terms of

CMA/DAPI bands, in the sample, both species were characterized by presenting DAPI<sup>+</sup> bands in the proximal regions of all chromosomes, except in the larger chromosome pair.

**Key words:** chromosome number, CMA/DAPI, heterochromatin, karyotype evolution, terrestrial orchids.

### **Australasian orchid diversification in space and time: phylogenomic insights from the beard orchids (*Calochilus*, Diurideae)**

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The Australasian orchid genus *Calochilus* R.Br. comprises ca. 30 geophytic species. So far, phylogenetic relationships and the spatio-temporal evolution of the genus are poorly understood. Here we present a phylogenomic study based on a supermatrix of 81 loci, representing ca. 70% of the species diversity within the genus. Divergence time estimations were conducted based on an uncorrelated relaxed molecular clock model and secondary calibrations in Diurideae and ML ancestral range reconstructions were carried out based on the dispersal-extinction-cladogenesis model including jump dispersal. Divergence time estimations inferred that the genus originated in Australia ca. 12.0 Mya in the mid Miocene. Divergence of *Calochilus* into two major lineages, a tropical and a temperate clade, was inferred to have occurred ca. 7.6 Ma in the late Miocene. Northern Australia was reconstructed as the ancestral area of the tropical clade and southeast Australia for the temperate clade, with both lineages having predominantly diversified within their ancestral areas. Range expansions to other Australasian regions, such as New Zealand and New Guinea, were inferred to have occurred in recent geological times, commencing in the Pliocene. Most of the contemporary species diversity of *Calochilus* was inferred to have arisen during the Quaternary.

**Key words:** divergence dating, genomics, historical biogeography, Orchidaceae, Thelymitrinae.

### **Bambusoideae (Poaceae) of Maranhão, Brazil: preliminary data**

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Brazil is one of the largest centers of Bambusoideae (Poaceae) diversity in the Americas. However, in some Brazilian states the bamboos are poorly studied, such as Maranhão state. Within the scope of the project Flora of Maranhão, the aim of this work is the study of the flora of Bambusoideae in the state, with elaboration of morphological keys and taxonomic treatment for genera and species. Maranhão is an ecotone area with influence of the Amazon biome, Cerrado biome and the coastal zone. Field collections for bamboos are being made in Maranhão, as well as analysis of herbarium specimens and literature survey. To date, 21 native bamboo species (four woody and 17 herbaceous) in 10 genera have been recorded for Maranhão. *Rehia nervata* Fijten was found in Maranhão after over 80 years since the type collection was recorded in the state. Due to the high degree of vegetation devastation in the state, with Maranhão's Amazon reduced to only 25% of its original coverage and the extensive fragmentation of Cerrado, it's estimated that many species of Bambusoideae are endangered or critically endangered. At the end of this work, the data will help conservation initiatives in Maranhão.

**Key words:** bamboo, Bambuseae, Gramineae, Olyreae, taxonomy.

### **Idiosyncratic grasses of lateritic plateaus of Western Ghats, India**

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Lateritic plateaus in Western Ghats of India are known for their unique ephemeral monsoon vegetation composed of perennial herbs, grasses and monsoon perennials. The plateaus are known for high endemism. Based on altitude, the plateaus can be further recognized as high elevation plateaus (above the 800 meters from MSL) in main crest of Northern Western Ghats and Low elevation plateaus (0 – 300 meters from MSL) of West Coast of India. Grasses are the most dominating component of plant community of plateaus. Most of the grasses are uniquely adapted to these plateaus and many of them are endemic. An account of grasses of lateritic plateaus of both high and low elevation, their cytology, adaptations, spikelet structure and seed germination are discussed. Polyploidy has played major role in speciation of the grasses.

**Key words:** adaptations, endemic grasses, India, lateritic plateaus, West coast and Western Ghats.

### **Scientific collections and catalogues of grass phytoliths: fundamental tools for paleoecological and archaeological studies in the tropics**

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Phytoliths, also known as plant opal, are found in many taxonomic groups, but are most abundant in commelinid monocots, especially in grasses. These structures have diagnostic morphologies that can be useful in taxonomic studies; they can also persist in sediments during thousands or millions of years, making them a valuable line of evidence in paleoecological and archaeological studies. We have been developing phytolith reference collections for the Amazon region, based on which we are producing a series of catalogues for the identification of phytoliths in ancient contexts. We present the *Illustrated Catalogue of Contemporary Phytoliths for Archaeology and Paleoecology I. Amazonian Grasses of Colombia*. For this study phytoliths were extracted from herbarium specimens following confirmation of their taxonomic identification. We prepared samples from 164 specimens, corresponding to 143 Amazonian grass species in 55 genera found in the National Colombian Herbarium (COL). In this sample, taxonomic resolution of the phytoliths was possible at the genus level and in some cases at species level. We highlight the importance of herbaria as a source of material for phytolith extraction and for broadening the taxonomic and geographic coverage of reference collections.

**Key words:** Amazonian grasses, archaeology-paleoecology, Illustrated Catalogue of Phytoliths, scientific collections, silica bodies.

### **Hybridization among bromeliads (*Aechmea* spp.) from the southern Atlantic Forest, Brazil**

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Species boundaries can be difficult to define in pairs of species that diverged recently or that continue to exchange genes. In this study, we investigated the occurrence of hybridization and the taxonomic boundaries between *A. comata* (Gaudich.) Baker, *A. caudata* Lindm. and *A. kertesziae* Reitz. To do so, we used 10 nuclear (nuSSR) and six plastid (cpSSR) microsatellite markers, morphological and ecological data, and controlled pollination experiments. We sampled 244 individuals belonging to the three species from four allopatric and four sympatric populations. Bayesian analyses indicated that each species presents a distinct genetic profile, although hybrids have been identified by molecular analyses, three individuals between *A. comata* and *A. caudata* and 17 individuals between *A. comata* and *A. kertesziae*. The hybrids identified by Bayesian analyses did not show intermediate morphology, making their identification in nature difficult. Artificial crosses confirmed the reproductive compatibility between *A. comata* and *A. caudata* and with *A. kertesziae*, since viable seeds were obtained from interspecific pollination experiments. The morphological and ecological data supported molecular results, indicating that *A. comata*, *A. caudata*, and *A. kertesziae* are distinct taxonomic entities. However, *A. kertesziae* presents overlapping morphological features with *A. comata* and *A. caudata*, due to great intraspecific variability.

**Key words:** ecology, hybridization, microsatellites, morphology, pollination experiments.

## New insights into infrageneric classification and systematics of *Chascolytrum* (Poaceae, Poaeae Chloroplast Group 1) based on a molecular phylogeny

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*Chascolytrum* Desv. comprises at least 22 species with major distribution in South America. The taxonomic history of the genus is controversial and several circumscriptions were proposed in the past. Phylogenetic studies supported *Chascolytrum* as monophyletic when including a pool of small and morphologically diverse genera. However, interspecific relationships remain poorly understood, hindering the acceptance of infrageneric categories and the understanding of its evolutionary history. We attempt to reconstruct the interspecific relationships to better understand the systematics, taxonomy and evolution of *Chascolytrum*. We used three molecular markers (nuclear GBSSI, plastid spacer *trnL-trnF* and plastid intron *rps16*) for 33 accessions, representing 18 taxa of *Chascolytrum*, plus *Briza* and *Bromidium* as outgroups. Since no incongruences were detected between different markers, we used a total evidence approach. All the analysis recovered *Chascolytrum* as monophyletic, split into two major clades and other small clades. Due to unresolved polytomies between the clades, the acceptance of distinct genera within this group is impracticable. However, based in morphology, it is possible to recognize at least seven infrageneric categories, corroborating the proposition of past authors. Thus, we suggest the recognition of seven sections to accommodate the species of *Chascolytrum*: sect. *Chascolytrum*, *Poidium*, *Microbriza*, *Rhombolytrum*, *Lombardochloa*, *Calotheca* and *Erianthecium*.

**Key words:** GBSSI, grass, section, South America, taxonomy.

## *Paepalanthus thomasianus*, a new species of *Paepalanthus* subg. *Platycaulon* (Eriocaulaceae) from São Paulo, Brazil

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*Paepalanthus* subg. *Platycaulon* forms a group of plants easily recognized by its fused scapes that gathers many capitula in its apex, which can be sessile or not. After 20 years, two new taxa were discovered in this group, one endemic to Minas Gerais and the other one to São Paulo, named *Paepalanthus thomasianus*. So, here we describe and illustrate this species and compare it with other similar species. *Paepalanthus thomasianus* is characterized mainly by its leaf indumentum, composed of long multicellular trichomes. Undulations formed by the vascular bundles in the leaf surface and the wide ratio between the leaf base and the middle region of the leaf are other features easily recognized in this species. There are just four species in this group that are similar to *Paepalanthus thomasianus*, but they can be differentiated mainly by the indumentum, habit and membrane in the leaves. *Paepalanthus thomasianus* is known just from three small populations in São Paulo State, and it is possible that the species could become extinct within a very short time, because of the effects of human activities where it occurs.

**Key words:** endemism, everlasting plants, IUCN Red List, Poales, taxonomic novelty.

**Funded by:** UNIJUÍ, PET/MEC/SESU and CNPq.

## **Divide to conquer: Karyotypic consequences of the geographic isolation of the three Allioideae (Amaryllidaceae) tribes**

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Allioideae is divided into the tribes Allieae, Gilliesieae and Tulbaghieae, all of them with large genomes and high variability in chromosome number and rDNA distribution. We investigated phylogenetic relationships (based on ITS, *matK*, *ndhF* and *rbcL*) of Allioideae to reconstruct biogeographic and karyotypic trends. Additionally, we present original cytomolecular data for Chilean genera *Gethyum*, *Miersia*, *Solaria* and *Speea*. The monophyletic Allieae and Gilliesieae + Tulbaghieae clades diverged 37 and 24.9 Mya respectively, with Gondwanic origin and posterior vicariance. Gilliesieae varied in regard to genome size ( $1C_x = 4.52$  to  $28.41$  pg) and chromosome number ( $2n = 8$  to  $32$ , with  $x = 6$ ), with high number of 35S rDNA sites on the short arm of acrocentrics. Tulbaghieae also had  $x = 6$ , but with numerical stability. The historical geomorphological stability of the African continent in contrast to the instability of South America may be related to these patterns. The conservation of  $x = 8$  in *Allium* can be related to a recent colonization of the Northern hemisphere from the Himalayan region. Our data suggest that the history of each Allioideae lineage impacts in very particular tribe-specific tendencies of karyotypic evolution.

**Key words:** biogeography, ChromEvol, Gilliesieae, karyotype, phylogenetic comparative methods.

**Funded by:** Financial support: FACEPE, CAPES and CNPq, Brazil.

## **Diversity of *Cyperus* subg. *Pycreus* (Cyperaceae) in Brazil**

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*Cyperus* L. is a large sedge genus comprising 950 species. *Cyperus* subg. *Pycreus* (P. Beauv.) A. Gray includes ca. 120 Pantropical species that occur mainly in humid and flooded environments. The subgenus *Pycreus* is characterized by the combination of laterally compressed dimerous pistils and deciduous glumes. We studied the diversity of subgenus *Pycreus* in Brazil. We carried out a review of herbarium collections (ASE, BHCN, CGMS, EAC, FLOR, FUEL, FURB, HRCB, HUCCS, HUEFS, HUFU, HVSF, ICN, IPA, MBM, MG, MOSS, PACA, RB, SP, SPF, VIES, UB, UFMT, UFP and UPCB), field collections, and morphological analysis including Scanning Electron Microscopy of the fruit. We recognized the occurrence of 17 species in *C.* subg. *Pycreus* from Brazil: *Cyperus barrosianus* Herter, *C. capillifolius* A. Rich, *C. flavescens* L., *C. fugax* Liebm., *C.*



*jaegii* Boeckeler, *C. lanceolatus* Poir., *C. longiculmis* Pereira-Silva, Hefler & R. Trevisan, *C. lorentzianus* Boeckeler, *C. macrostachyos* Lam., *C. megapotamicus* (A. Dietr.) Kunth, *C. mundii* (Nees) Kunth, *C. niederleinianus* Boeckeler, *C. polystachyos* Rottb., *C. pumilus* L., *C. tuckerianus* Pereira-Silva, Hefler & R. Trevisan, *C. unioloides* R. Br., and *C. valiae* Pereira-Silva, Hefler & R. Trevisan. *Cyperus longiculmis* and *C. valiae* are new species described for Brazil.

**Key words:** biodiversity, Cyperaceae, Neotropics, Poales, taxonomy.

## A preliminary overview of Orchidaceae from Uruguay

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Orchidaceae is one of the most diverse plant families. Although Neotropical species are well known, few studies in Uruguay are available and a list of species has not been published yet. It holds diverse ecosystems ranging from grassland to subtropical and humid forests. The aim of this study is to present the first checklist of Orchidaceae from Uruguay, with comments on geographical distribution and habitat where the species are found. A preliminary list of vouchers and species was based on herbaria collections (MBJB, MVFA, MVFC, MVN, SI, SP) and published data including 23 genera and 70 species which belong to Epidendroideae (19 spp.) and Orchidoideae (46 spp.). The most representative genera are *Habenaria* (16 spp.), and *Brachystele*, *Cyclopogon* and *Skeptrostachys* (6 spp. each). The departments of Montevideo and Maldonado showed the highest species richness (15 spp., most of them terrestrial), probably because of sampling bias. In Cerro-Largo department, 13 species were reported (eight of them epiphytes) probably associated to higher levels of rainfall and temperature allowing a high environmental humidity. This study presents an updated overview for the distribution and species composition of Orchidaceae in Uruguay, and it will allow future studies in taxonomy, biogeography and conservation programs.

**Key words:** Cono Sur, diversity, Neotropics, Orchidoideae, species list.

## Orchidaceae in the Atlantic Forest of Paraíba State – Brazil

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Orchidaceae is one of the richest families with a high degree of endemism in the Atlantic Forest. In the state of Paraíba, this domain reaches 6,743 km<sup>2</sup> and includes forest, *restinga* and mangrove

ecosystems. However, forest vegetation is only residual, accounting for no more than 0.4% of forest fragments in the Atlantic Forest. The objective of this work was to survey the Orchidaceae species in the Atlantic Forest of Paraíba state. Field work to collect botanical material and consultation of herbarium specimens were carried out for the survey. A total of 21 genera and 32 species were recorded, *Habenaria* being the richest in species. Among the species, 31 are native and only 12 are endemic to Brazil. *Oeceoclades maculata* was recorded as a naturalized species. Four species are new occurrences for the state, including one for the Northeast region. Regarding habit, 34% are epiphytes, 56% terricolous and 10% hemiepiphytes. Considering threat level categories, 6% are classified as vulnerable.

**Key words:** conservation, endemism, flora, orchids, Paraíba.

### **Genetic insights into the hybrid origin of *Dyckia julianae* (Bromeliaceae) an endemic species from Southern Brazil**

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When related species with incomplete reproductive isolation are distributed in sympatric populations, hybridization may occur. One or more of these species may have arisen through the historical hybridization between taxa and today occur in hybrid zones. *Dyckia hebdingii*, *D. choristaminea* and *D. julianae* are related and endemic species of a small portion of the south of Brazil and can occur in sympatry. We aim to elucidate the occurrence of hybridization among these species and investigate the origin of *D. julianae*. We used seven nuclear and six plastid microsatellite loci to assess genetic diversity, population structure and hybridization patterns. Furthermore, we performed manual pollination experiments to test compatibility and fertility. The main results showed that *D. julianae* has an intermediate molecular profile. Also, we showed low gene flow between *D. hebdingii* and *D. choristaminea* and higher gene flow between *D. julianae* and the other two species. The cpSSR identified 12 haplotypes broadly shared among species. Manual pollination between *D. julianae* and the other two species produced viable seeds, but none of the crosses between *D. hebdingii* and *D. choristaminea* generated fruits or seeds. The information obtained in this study will also help to delineate strategies for the conservation of the three species.

**Key words:** hybridization, interspecific gene flow, microsatellites, pollination experiments, sympatry.

### **Gingers of India: an overview**

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The gingers include the members of the family Zingiberaceae, which consist of about 53 genera and more than 1200 species, with the centre of distribution in the Indo Malayan region. In India, the family includes 21 genera and about 200 species, mostly confined to the Andaman and Nicobar Islands, NE India and South India, of which, one genus and 35% of the taxa are endemic to India. The gingers were used traditionally in India since time immemorial and were documented in many Sanskrit and Ayurvedic writings. The comprehensive work on the Revision of the Zingiberaceae in the whole of India started in 2000 in Calicut University. Since Hooker (1892), 42 new taxa were described from India. The Calicut University Botanical Garden holds the largest collection of gingers in India with about 22 genera and over 200 species with more than 2000 accessions. The major distributional areas of the family, NE India and Western Ghats, come under Biodiversity Hotspots, which led to the moving of some taxa under IUCN red list categories. In the present paper, all taxa from India with colour photos, uses if any, classification, IUCN status etc. are discussed.

**Key words:** conservation, endemism, gingers, India, Zingiberaceae.

## Monocotyledons in a fragment of submontane forest in Northeast Brazil

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Featuring nine acres and ca. 700 m of elevation, the Private Reserve (RPPN) Serra do Contente is an "island" of rainforest placed in the semi-arid region of the Borborema Plateau, Pernambuco State. A floristic survey was carried out in the area from 2015 to 2016, with samples treated following usual techniques and incorporated into UFP and RB herbaria. The study area presents 57 species of monocotyledons recognized to date, 15% of the angiosperm flora, belonging to 47 genera and 18 families. 23 species (40%) are endemic to the country, restricted mainly to the Atlantic Forest. The richest families are Orchidaceae (14 species), Bromeliaceae (9), Cyperaceae (7) and Poaceae (6), corresponding ca. of 63% of the area's monocotyledons. Nine species are threatened of extinction, seven of which are restricted to the Northeastern Brazil. Bromeliaceae (4 spp.) and Orchidaceae (3 spp.) are the most threatened families, e.g. *Cryptanthus zonatus* (Vis.) Vis. and *Notylia inversa* Barb. Rodr. Twenty species are epiphytes (5 of them Bromeliaceae) and three are vines (two Dioscoreaceae). Areaceae is represented by three species, two of which are endemic. Poaceae is sampled by one member of Pharoideae, *Pharus parvifolius* Nash, and five Panicoideae.

**Key words:** Atlantic Forest, Bromeliaceae, Northeast, Orchidaceae, taxonomy.

## Revisiting the taxonomy of the Neotropical Haemodoraceae (Commelinales)

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Haemodoraceae is a small monocot family of ca. 14 genera and 110 species, currently placed in Commelinales as sister to Pontederiaceae, with both sister to Philydraceae. These families share unifacial leaf-blades with alternate xylem and phloem; the presence of styloid crystals; connate and petaloid perianth whorls with tannin cells; pollen released with raphides, the presence of placental sclereid idioblasts; seeds longer than wide; and abundant helobial endosperm. Furthermore, the relationship between Haemodoraceae and Pontederiaceae is morphologically supported by the presence of endothecium with a basal thickening, non-columellate-tectate exine, and septal nectaries. Haemodoraceae is clearly monophyletic being characterized by its peculiar inferior ovary. The family possesses an unusual disjunctive distribution, having Australia and New Guinea as its primary diversity center, and the Americas and South Africa as its secondary diversity center. The Neotropical region was the focus of a comprehensive floristic study on the Haemodoraceae. However, recent field, herbaria, and phylogenetic studies have evidenced the need for taxonomic changes, especially in *Schiekia* and *Xiphidium*. As an attempt to clarify the taxonomy and systematics of Neotropical Haemodoraceae, we propose the description of a new genus, two new species, and a new combination, and provide an updated identification key.

**Key words:** Commelinales, Haemodoroideae, Neotropical flora, *Schiekia*, *Xiphidium*.

**Funded by:** CAPES, Fundação Flora de Apoio à Botânica, and the Smithsonian Institution.

### Origins and geographic diversification of African rice (*Oryza glaberrima*)

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Rice is a staple food for the majority of our world's growing population. Whereas Asian rice (*Oryza sativa* L.) has been extensively studied, the exact origins of African rice (*Oryza glaberrima* Steud.) are still contested. Previous studies have supported either a centric or a non-centric origin of African rice domestication. Here we review the evidence for both scenarios through a critical reassessment of 206 whole genome sequences of domesticated and wild African rice. While genetic diversity analyses support a severe bottleneck caused by domestication, signatures of recent and strong positive selection do not unequivocally point to candidate domestication genes, suggesting that domestication proceeded differently than in Asian rice – either by selection on different alleles, or different modes of selection. Population structure analysis revealed five genetic clusters localising to different geographic regions. Isolation by distance was identified in the coastal populations, which could account for parallel adaptation in geographically separated demes. Although genome-wide phylogenetic relationships support an origin in the eastern cultivation range followed by diversification along the Atlantic coast, further analysis of domestication genes shows distinct haplotypes in the southwest - suggesting that at least one of several key domestication traits might have originated there. These findings shed new light on an old controversy concerning plant domestication in Africa by highlighting the divergent roots of African rice cultivation, including a separate centre of domestication activity in the Guinea Highlands. We thus suggest that the commonly accepted centric origin of African rice must be reconsidered in favour of a non-centric or polycentric view.

**Key words:** Biogeography, plant domestication, Poaceae, rice.

## **A dated phylogeny of the genus *Goepertia* (Marantaceae) with focus on the Atlantic Forest species biogeography**

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*Goepertia* Nees is the most diverse genus of Marantaceae, a relevant component of Neotropical forest understories and with horticulturally important species. A dated phylogeny of *Goepertia*, including ca. 30% of the genus diversity and about 50% of the Atlantic Forest (AF) species, indicates that the genus diversified during Late Eocene (42.73–38.65 Ma). All species of AF belongs to the Breviscapus Clade, despite morphological features. Ancestral area reconstruction analysis places the Amazon as the ancestral area of the genus with 75% likelihood; a vicariant event led to the colonization of the AF (14.85–9.89 Ma). The estimated dates suggest that the assembly of Cerrado (Brazilian Savanna) may have played a crucial role in the diversification *Goepertia* in the AF and Cerrado; diversification rates in these two areas were higher than in the Amazon. Distribution of extant species follows the proposed areas of the Atlantic Forest Pleistocene refugia, indicating that Late Quaternary climate fluctuations influenced the distribution of these species, rather than its diversification. These new insights about the evolutionary history of *Goepertia* not only shed light on the diversification of plants in the AF but also should guide a new infrageneric classification of the genus.

**Key words:** Atlantic Forest, biogeography, diversification, *Goepertia*, molecular dating.

**Funded by:** FAPESP 2012/22713-6; CNPq 148985/2012-2 and 232561/2014-1

## **Individual variation in form and near infrared spectroscopy in *Ischnosiphon arouma* (Marantaceae), a understory terrestrial herb from the Amazon and the Antilles**

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Morphometric variation among individuals can be used to correlate morphology to environmental and biogeographic variables and to promote solutions to taxonomic challenges. Additionally, spectral patterns of Near Infrared (FT-NIR) irradiated to plant tissues can also be used to characterize variability of individuals. NIR is a practical tool which can capture spectral responses of herbarium leaf tissue, revealing chemical composition and anatomical structure. *Ischnosiphon arouma* (Marantaceae) is a terrestrial rhizomatous herb of forest understories of tropical America, which displays great morphological variation along its geographical range. We sampled morphometric variables and spectral readings of upper and lower surfaces of 68 individuals of *I. arouma* from NY and INPA herbaria material. NIR analyses were performed using the average absorbance at all wavelengths over the entire FT-NIR spectrum (1000 to 2500 nm); the most

informative wavelengths were selected by discriminant functions (stepwise). Here, we compare individual variation along a species geographic range on morphometry and near infrared spectroscopy since individual traits are essential to characterize specific variation in nature.

**Key words:** Amazonia, *arumã*, biogeography, FT-NIR spectroscopy, morphometrics.

## **Systematic and biogeographic studies of bromeliads in the Brazilian Amazon: Preliminary checklist**

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Bromeliaceae is an essentially Neotropical family. Distributed throughout the Americas, from the southern United States to the Argentine South, the three main diversity centers are the Guyanan plateaus, the Andean mountains and the east of Brazil. According to data from the Brazilian flora species list, the Amazon can be considered a region of low diversity for the Bromeliaceae family, with 143 species and 22 genera, when compared to other Brazilian regions such as Atlantic Forest (925/31) and even Cerrado (257/20). In order to better understand how the historical-evolutionary relations may have influenced the current distribution of Bromeliaceae in the American continent, since 2014, we have been carrying out expeditions and revision of herbarium collections to obtain a verified list of species in the Brazilian Legal Amazon (BLA). Our checklist increases to 252 species (+ 75%) and 26 genera (+ 20%) the Brazilian flora species list. This result demonstrates that even with collection efforts, a more accurate and up-to-date approach to botanical collections is needed. The next step of this work is to focus on an SDM approach and to analyze high-density spots to determine the most relevant areas of Bromeliaceae in the BLA.

**Key words:** Amazonia, Bromeliaceae, diversity, mountains, tepuys.

**Funded by:** CNPq

## **Checklist of Orchidaceae from an Amazonian area in the state of Maranhão, Brazil**

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The state of Maranhão is rich in biomes and plant physiognomies, with high floristic diversity. In spite of that, there are few and fragmented studies on the botanic families of the state, including Orchidaceae. Thus, the aim of this study was the survey of species of Orchidaceae through field collection expeditions in Candido Mendes, northern Maranhão, in the Amazon region of the state. Forty-one orchid species (31 genera) were collected and 13 of them (31.7% of the total) are new records for the Flora of Maranhão. In addition, eight genera (62%) were collected for the first time in the state: *Coryanthes* Hook, *Dichaea* Lindl., *Laelia* Lindl., *Macradenia* R. Br., *Oncidium* Sw., *Stelis* Sw., *Trichosalpinx* Luer. and *Palmorchis* Barb., three of them (38%) collected for the first time in the Northeastern Brazil: *Macradenia* (*M. lutescens* R. Br.), *Oncidium* (*O. baueri* Lindl.) and *Palmorchis* (*Palmorchis* sp.). The most representative genera are *Epidendrum* L. (4 spp.),

*Scaphyglottis* Poepp. & Endl. (3 spp.), *Vanilla* Plum. (2 spp.), *Campylocentrum* Benth. (2 spp.) and *Polystachya* Hook. (2 spp.). The other genera were represented by only one species each (2.4%), totalling more than half of the species found in the region (64.8%).

**Key words:** Amazon, Maranhão, new record, Orchidaceae, taxonomy.

### **Listing species of *Anthurium* sect. *Pachyneurium* ser. *Pachyneurium* (Araceae) from Brazil**

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*Anthurium* (19 sections) which is the most species-rich genus of the family and is represented by only four of these sections in Brazil, amongst them, *Anthurium* sect. *Pachyneurium*. It was later divided into two series. *A.* sect. *Pachyneurium* ser. *Pachyneurium* (Schott) Croat can be recognized by the presence of dark-brown leaf blades in dry material, primary lateral veins generally with more than three centimeters spacing between of them. This project propose a listing species of *Anthurium* sect. *Pachyneurium* ser. *Pachyneurium* from Brazil. An analysis was realized of the specimens deposited in the RB herbarium, in addition to samples available at *speciesLink*, Jabot and Re flora-Herbário Virtual. This series has 18 species and distributed mainly in the Amazon region with 11 species: *A. atropurpureum* var. *atropurpureum*, *A.* var. *arenicola*, *A. bonplandii*, *A. cataniapoense*, *A. ernestii* var. *ernestii*, *A. galactospadix*, *A. krukovii*, *A. jenmanii*, *A. oxycarpum*, *A. pranceanum* and *A. uleanum*. *A. leonii*, *A. santaritensis* have distributions restricted to the Atlantic Forest. And species-wide distribution: *A. affine*, *A. solitarium* in the northeastern, central-western, southeastern of Brazil and species occurring in more than one biome: *A. lindmanianum*, *A. plowmanii* in Amazon region and Cerrado, *A. paraguayense* in Cerrado and Pantanal.

**Key words:** Amazon region, Atlantic forest, cerrado, monocots, Pothoideae.

**Funded by:** CAPES-JBRJ.

### **Biogeography patterns of *Barbosella* (Orchidaceae, Pleurothallidinae)**

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*Barbosella* is a small orchid genus in the subtribe Pleurothallidinae with 20 species. Distributed from Central America through the Andes to southeastern of Brazil with a disjunct distribution between coastal forests, it was recovered as monophyletic with high support in a previous phylogenetic study. We generated a time-calibrated phylogeny based on a matrix of 13 species of

*Barbosella* plus related genera and outgroup taxa using molecular nrITS, five plastid regions and 43 morphological characters, inferred with BEAST; then we conducted a biogeographical analysis using BioGeoBEARS. The results obtained showed the most recent common ancestor (MRCA) of *Barbosella* originated through the Middle-Late Miocene at 11 (9.3–18.06) Mya, where the Atlantic Forest and Andean Forest are nowadays distributed and separated by a dry area. The early diversification within the genus occurred in the Late Miocene around 8 (5.12–10.8) Mya involving a vicariance event and subsequently multiple dispersals in both areas, where the groups of species are restricted today. The findings from this study contribute to a better understanding of the evolution of *Barbosella* and the evidence of historical connection of the coastal forests of South America.

**Key words:** ancestral area estimation, Andean, Atlantic Forest, molecular dating, Neotropical flora.

**Funded by:** This work was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for grant Doutorado - GD (Proc. 140172/2014-9) and for grant Bolsa de Produtividade em Pesquisa do CNPq - Nível 2 (Proc. 311001/2014-9).

### **Niche modelling of *Pitcairnia* (Bromeliaceae) species in Hispaniola**

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Five species of *Pitcairnia* occur in Hispaniola, all endemic to the island: *P. domingensis*, *P. elizabethae*, *P. fuertesii*, *P. jimenezii*, and *P. samuelssonii*. *Pitcairnia ariza-juliae*, which remains undescribed, is known from three herbarium specimens and was excluded from the analysis. Using environmental data from BioClim, and QGIS and MaxEnt softwares, I modeled the distribution of these species to determine the climatic variables that best explain them and identify other suitable locations where no collections exist. The analysis shows that precipitation is one of the most important variables in determining their distribution, although differences exist between taxa. For every species, except *P. jimenezii*, collections exist from areas outside of what the model determined to be their suitable habitat. Possible new populations of *P. domingensis* and *P. jimenezii* are reported.

**Key words:** biogeography, Dominican Republic, Haiti, Pitcairnioideae, West Indies.

### **Taxonomic overview of *Maranta* (Marantaceae) from the Northeastern Brazil: a center of diversity for the genus**

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*Maranta* L. is the second largest genus in Marantaceae, it is endemic to the Neotropical region and it has about 40 species. In Brazil about 30 spp. are recorded, 21 cited for the Northeast region, growing in Caatinga, Cerrado and mainly in the Atlantic Forest, where most of them occur. The aim of this study was a taxonomic treatment of the species from this region. Fieldwork in five states of the Northeast region was conducted, and specimens from the main Brazilian herbaria were



analyzed. Among the taxa recorded for the area (21 species), five are endemic to the Atlantic Forest and one to Cerrado. Two species are considered threatened, *M. gigantea* (CR), and *Maranta rugosa* (VU), the last one is also a new record to the area. Four new species are found in the states of Ceará, Maranhão and Bahia. Four names are also synonymized to *M. divaricata* and *M. noctiflora* as well as the necessary typifications are provided. Northeastern Brazil was confirmed as highly relevant to the diversity of the genus, where half of the species occur, mainly ones from *M.* subgen. *Maranta*, since 70% of its species grow in the region.

**Key words:** Atlantic forest, cerrado, Neotropics, taxonomy, Zingiberales.

**Funded by:** CNPq

### **Volatile organic profiles from floral fragrances of *Brasiliorchis* orchids**

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Floral volatile organic compounds (VOCs) represent key-characters to understand pollinator attraction. In morphologically similar, sympatric species we expect floral VOCs to vary among species to maintain reproductive isolation barriers. To test this hypothesis, we described the VOCs chemical composition of five species of *Brasiliorchis*. These are morphologically similar orchids typical of the eastern Brazilian Atlantic Forest. VOCs were collected through the floral *headspace* and the composition determined by GC-MS. Results showed distinct major compounds among species: *B. barbozae* emitted 1-hexanol, 2-ethyl- (19.85%) and *trans*-geranylacetone (0.61%); *B. gracilis* emitted heptadecane (3.17%) and tetradecane, 2,6,10-trimethyl- (0.15%); *B. phoenicanthera* emitted pentadecane (53.16%) and tetradecanal (12.51%); *B. porphyrostele* emitted tetradecane (31.97%) and pentadecane (24.16%); *B. marginata* emitted hexadecane (27.44%) and nonanal (23.03%). Our results provide new data regarding the chemical variation of VOCs for Maxillariinae orchids and contributes to the understanding of the reproductive isolation within this group.

**Key words:** animal-plant interaction, chemical ecology, Orchidaceae, pollination, speciation.

**Funded by:** Coordenação de Aperfeiçoamento de Pessoal de Nível Superior

### **Climatic niche models and species distribution of the genus *Vellozia***

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The "campos rupestres" are a species rich vegetation from Brazil, occurring in mountain areas that are particularly sensitive to the climatic changes. The genus *Vellozia* (Velloziaceae, Pandanales) is one of the most representative groups in this habitat. Thus, to quantify the environmental factors that determine the occurrence of *Vellozia* species is important to understand its distribution as well

as to evaluate the conservation of "campos rupestres". The aim was characterizing distributions, climatic preferences and niche overlap of 12 species of *Vellozia*. We used climatic niche models, performed in MaxEnt, to evaluate the distribution of 12 *Vellozia* species widespread across the areas of "campos rupestres". Based on herbarium and field records, we compiled 813 points of occurrence. Our analyses indicated that precipitation of warmest season, isothermality, temperature seasonality, and precipitation of wettest month are the main variables affecting species distribution. Species occupied similar niche amplitudes, although they differed by their climatic preferences. The highest probability of occurrence of each species varied mainly due to isothermality and precipitation seasonality. Niche overlap was mostly low, indicating niche separation among species regarding climatic conditions.

**Key words:** bioclimatic envelope, Neotropics, niche differentiation, species occurrence.

**Funded by:** Coordenação de Aperfeiçoamento de Pessoal de Nível Superior

### ***Carex* (Cyperaceae) in South America: diversity, phylogenetics and biogeography of a Boreotemperate element in the Neotropics.**

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With about 2000 spp, *Carex* (Cyperaceae) is the largest monocot genus and one of the three largest angiosperm genera. Originated in eastern Asia during the Oligocene, its current distribution is cosmopolitan. Remarkably, *Carex* has remained as a temperate-cold element during its entire radiation, being absent from true tropical forests and deserts. Here we analyze the diversity patterns and phylogenetic and biogeographic history of *Carex* in South America. The subcontinent harbors 215 species (11% of the total), belonging to the four major clades of the genus and 40 sections (>25%). Phylogenies point to multiple colonization events, mostly from North America, but also involving long-distance dispersal from the Western Palearctic and Australasia. Typical patterns of distribution include North-South Andean and East-West temperate South Cone disjunctions within the continent, and bipolar and trans-Caribbean disjunctions between North and South America. Most groups seem to have undergone in situ diversification. Preliminary diversification analyses show that the innovation of a hooked fructification in the species of the former genus *Uncinia*, may have contributed to triggering the diversification of the group in South America, although additional factors may need to be invoked to explain such findings.

**Key words:** Boreotemperate, graminoid, sedges, South America, taxonomy.

### **Taxonomic revision of *Rhynchospora* section *Glaucæ* in South America**

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*Rhynchospora* Vahl (Cyperaceae) is a worldwide genus with about 300 species and has its distribution center in the Americas, especially in the tropics. The genus is characterized by spikelets with spiral scale arrangement, the upper scales fertile, with bisexual flowers or one lower bisexual flower and the upper ones staminate; and they have persistent stylobase on the fruit and achene usually with perigonal bristles. This study is a taxonomic revision of *Rhynchospora* sect. *Glaucæ* CB Clarke in South America. Analysis of relevant literature, review of materials from national and international herbaria and field collections of specimens were performed. This section presents 14 species in South America, of which 13 occur in Brazil, four are endemic of Brazil and one endemic of the Guayana Shield. A new species *Rhynchospora caduciglumis* was described. It's similar to *R. polyantha* Steud., but it has shorter spikelets (2.8–4.0 vs. 4.0–5.0 mm), fascicles with fewer spikelets, deciduous scales when the achenes are mature and narrower leaves (1.6–2.0 (–3) vs. 2.0–4.0 (–4.5) mm). Six lectotypifications were made. Descriptions, data on geographic distribution, habitat, phenology, IUCN categorization, distribution maps, photographic record and a dichotomic key to identify the species are presented.

**Key words:** IUCN, Marisculae, Poales, Stenophyllae, taxonomy.

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### **Total evidence phylogeny of Pontederiaceae (Commelinales) sheds light on the recircumscription of *Pontederia***

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Pontederiaceae is a small aquatic monocot family, placed in Commelinales. It can be distinguished from the remaining families of Commelinales by its dimorphic ligulate leaves, ptyxis enclosing the petiole of the preceding leaf; bisulcate pollen grains; and the presence of an anthocarp. The family is currently arranged into four genera (i.e., *Eichhornia*, *Heteranthera*, *Monochoria*, and *Pontederia*). Despite being unquestionably monophyletic, generic boundaries in Pontederiaceae are still in need of revision. A total of 30 genera have been traditionally assigned to Pontederiaceae, and some authors have accepted up to nine genera. All phylogenetic studies invariably recover most genera as non-monophyletic, with *Eichhornia* and *Heteranthera* being the most problematic. Recently, *Heteranthera* was recircumscribed and rendered monophyletic with the inclusion of *Hydrothrix* and *Scholleropsis*. Nonetheless, the *Pontederia* clade remains neglected, with the hopelessly polyphyletic *Eichhornia* being recovered as three distinct lineages. Two opposing approaches are available to solve this issue: sinking *Eichhornia* and *Monochoria* into a broader, but morphologically cohesive *Pontederia*; or splitting *Eichhornia* into three poorly-defined genera. The first option is more taxonomically stable and would greatly facilitate the identification of Pontederiaceae specimens. Thus, based on a total evidence phylogeny for Pontederiaceae, we recircumscribe *Pontederia* to include *Eichhornia* and *Monochoria*.

**Key words:** aquatic flora, *Eichhornia*, *Monochoria*, pickerelweed, water-hyacinth.

**Funded by:** CAPES, Fundação Flora de Apoio à Botânica, and the Smithsonian Institution.

### **Systematics studies on *Aechmea* subgenus *Ortgiesia* (Regel) Mez**

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*Aechmea* is the largest and most polymorphic genus of the subfamily Bromelioideae (Bromeliaceae). It occurs from Mexico to Argentina, in several types of environments, and is represented by more than 240 species. It is currently subdivided into eight subgenera. The subgenus *Ortgiesia* presents species exclusive to the Atlantic Forest and is distributed in Brazil, between the states of Espírito Santo and Rio Grande do Sul, Paraguai, Argentina and Uruguai, occurring in forests, restingas or mangroves, as epiphytes, or rupicolous or terrestrial plants. *Ortgiesia* species are characterized by their sessile flowers, with sepals connate up to ½ of the length, with long mucros; petals with the apex cucullate and provided with well developed appendages. This study aims to monograph the taxonomic treatment of the subgenus, and improve the morphological characterization of the foliar anatomy of the taxa. The studies were carried out on the basis of herbarium specimens from about 1400 collections, as well as analyses of live plants obtained during several expeditions. The results treat 17 species, further two new to science and in the process of description, and four species treated as dubious. Full descriptions, updated nomenclature, illustrations, geographic and conservation data are presented and bring a better distinction to the taxa.

**Key words:** Atlantic forest, Bromelioideae, leaf anatomy, Poales, taxonomy.

**Funded by:** Capes, CNPq (scholarships)

### **Towards a monograph of *Araeococcus* (Bromeliaceae, Bromelioideae)**

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*Araeococcus* Brong. is a small and rare genus of Bromeliaceae, placed in the Bromelioideae subfamily. It has morphological affinities with *Lymania* Read, being differentiated mainly by its globose and smooth-walled ovary and non-winged fruits. It is endemic to the Neotropical region, occurring in Central America, Amazon, and in the Atlantic humid and semi-deciduous forests, as an epiphyte. The objective of this work is to fully revise the genus, including morphology and molecular data to better delimit its species and their relationships. Field trips in Brazil, to the Amazonian forest and along the Atlantic Forest were undertaken. A total of 300 specimens were studied, 7 herbaria were visited. As results, four new species and one variety for the group are proposed, totaling 14 taxa, besides new modifications and changes to the descriptions (amendments

to poorly known species). Anatomical studies (light microscopy and scanning electron microscopy) were performed, revealing that seed testa and trichomes density on the leaf surface are useful characteristics to separate some species. The systematic revision includes distribution maps, illustrations, photographic plates, morphological descriptions and conservation status for each taxon, plus floral anatomy and phylogenetics works based on the low-copy nuclear gene *PHYC*.

**Key words:** Amazonian Forest, Atlantic Forest, epiphytes, Neotropical region, systematics.

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### **Cytogenetics and genome size analysis of *Hohenbergia* highlight a recent diversification of the bromeliad group**

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Bromeliaceae have been raising the interest of evolutionists in recent years, with phylogenetic, phylogeographic, population genetics and cytogenetic studies. This family has high representativeness on the Neotropics, but only 4% of the Bromeliads are studied for cytogenetic characters, and much less is known about their genome sizes. The absence of cytogenetic studies with modern techniques generates a gap in the knowledge about the evolution of the group. The present work aimed to apply molecular cytogenetic techniques (n=10) and size the genome (n=16) of native *Hohenbergia* species and correlate with their evolutionary history. The cytogenetic and genome size results indicate no variation in chromosome number (x=25) and genome size 2C~2. Fluorochrome banding identified that GC rich bands in *Hohenbergia* is associated with NORs. The distribution pattern of the 5S and 35S rDNA sites presented a terminal localization predominance. The number of 5S rDNA sites (2) is conserved in the genus, with a pair of bands for all studied species. Variations presented in two of the species, and indicate possible positioning of the species outside *Hohenbergia*. Adversely to the number, size heteromorphism on banding between the species is a possible sign of recent hybridization.

**Key words:** Bromeliaceae, CMA/DAPI, evolution, FISH, flow cytometry.

**Funded by:** FACEPE, CNPq.

### **Karyotype variability and genome size in an interploidy hybridization zone between *Epidendrum xanthinum* and *E. secundum* (Orchidaceae: Epidendroideae)**

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Hybrid zones involving species of *Epidendrum* with different ploidy levels have been reported; e.g., between *E. xanthinum* ( $2n=28$ ) and *E. secundum* ( $2n=56$ ) at Nova Friburgo (Rio de Janeiro). We aimed to analyze the karyotype variation in that hybridization zone by chromosome characterization by CMA/DAPI fluorochrome staining and also flow cytometry to infer genome size. *Epidendrum xanthinum* presented  $2n=28$ ,  $2C=2.31$  pg, 4 CMA<sup>+</sup>/DAPI<sup>-</sup>, 5 DAPI<sup>+</sup>/CMA<sup>-</sup> and 5 CMA<sup>0</sup>/DAPI<sup>-</sup> bands, while the *E. secundum* presented  $2n=56$ ,  $2C=3.9$  pg, 8 CMA<sup>+</sup>/DAPI<sup>-</sup>, 12 CMA<sup>0</sup>/DAPI<sup>-</sup> bands. The putative hybrid presented  $2n=42$ ,  $2C=3.19$  pg, 4 CMA<sup>+</sup>/DAPI<sup>-</sup>, 2 DAPI<sup>+</sup>/CMA<sup>-</sup> and 12 CMA<sup>0</sup>/DAPI<sup>-</sup> bands. Our results suggest that hybridization plays an important role in *Epidendrum* evolution, what is reflected by the karyotype diversification. The CMA/DAPI banding patterns were unexpected, indicating a rapid evolution of the repetitive sequences. The intermediate nature of the chromosome number and genome size observed in the hybrids show the absence of polyploidization, suggesting the formation of a new homoploid hybrid.

**Key words:** fluorochrome banding, genome size, hybrid, karyotype, orchids.

### Comparative analysis of root vessel elements in Heliconiaceae

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Heliconiaceae belong to the Zingiberales order and comprise about 250 Neotropical species, many of them with ornamental uses. This study aimed to characterize structural types of vessel elements and to describe their pit membrane remnants. Root fragments from six Heliconiaceae species were fixed and macerated using 40 % formaldehyde and glacial acetic acid (1:1). For Scanning Electron Microscopy, samples were dehydrated and critical point dried, coated with 20 nm gold, and observed in a Zeiss EVO 40 electron microscope. Heliconiaceae species showed some plesiomorphic characters, such as long scalariform perforation plates in oblique end walls. Secondary wall thickenings were either scalariform or partially pitted in all studied species. In *Heliconia bihai* and *H. angusta* root vessel elements exhibited slightly oblique and subterminal perforation plates, as well as long scalariform (> 60 bars) perforation plates. In all studied species a few vessel pit membranes were found to be intact. These observations help to point Heliconiaceae as a sister clade of the ginger group.

**Key words:** Heliconiaceae, perforation plates, pit membranes, root, xylem.

### Genetic profile and phenotypic variation in the *Zygopetalum* (Orchidaceae) complex from high altitude rocky complexes of southeastern Brazil

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The high-elevation rocky complexes (HARC) are among the most diverse areas in the Neotropical region with a distinct floristic composition. *Zygopetalum* (Orchidaceae) occurs in granitic and quartzite HARCs and due to floral morphology variation represents one interesting model to investigate the association between genetic profile and phenotypic variation. At Ibitipoca State Park (PEI), located in the southeastern of Minas Gerais State, Brazil, the floral morphology of *Zygopetalum* varies over a gradient stream and no data about the genetic variation of these individuals were reported. The chromosome number, DNA content, molecular profile as well as floral morphometric analysis were accessed for up to 89 individuals representing the morphological variation. All specimens showed  $2n=96$  chromosomes and  $2C=14.08 (\pm 0.298)$ , the highest values so far reported for the genus. Floral morphometric analysis showed an overlapping pattern and do not show any particular cluster. The genetic diversity based on ISSR markers was estimated using Jaccard and Dice coefficients suggesting *Zygopetalum* specimens share the same genetic background. The floral morphology variation apparently not associated to a specific genetic profile reinforces the complex scenario behind the phenotypic variation, contributing to understand the origin and the evolution of tropical species.

**Key words:** chromosome number, DNA content, floral morphometry, molecular markers, Orchidaceae.

**Funded by:** FAPEMIG, CAPES.

### Cytogenetical studies in the genus *Pancratium* (Amaryllidaceae) in India

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The genus *Pancratium* L. with about 21 species is distributed mainly in the Old World. It is represented by about 9 taxonomically well recognized species viz. *Pancratium biflorum*, *P. donaldii*, *P. longiflorum*, *P. nairii*, *P. parvum*, *P. sanctae-mariae*, *P. triflorum*, *P. verecundum*, *P. zeylanicum* occurring in India of which 6 are endemic to the country. Delimitation of *Pancratium* species is a hard task as there are no good taxonomic characters and the protologues do not provide proper description for the discrimination of species, usually poorly preserved type specimens. All the species screened for cytology showed  $2n=22$  except for some populations of *P. triflorum* with  $2n=33$ . Experiments on hybridization have shown that all the taxonomically well recognized *Pancratium* species in India cross freely (with artificial pollination) with each other producing viable seeds. Further 95-98% pollen fertility is observed in hybrids and there is normal seed setting. Thus, even though *Pancratium* species in India are well recognized taxonomically but they are not reproductively isolated. The present paper presents field observations on various *Pancratium* species, their cytology, hybridity, hybrid morphology and reports on complexity in delimitation of species in bulbous monocots in general and *Pancratium* in particular.

**Key words:** bulbous, cytogenetics, hybridization, hybrids, monocots.

## **Evolution of adaptations to the temperate climate in the grass subfamily Pooideae**

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The grass subfamily Pooideae dominate the grass flora in Northern temperate regions. It has commonly been assumed that the Eocene-Oligocene (E-O) climate cooling 34 MYA had a strong impact on evolution of adaptations in Pooideae. However, lack of a large-scale, dated phylogeny has hampered studies of evolution of adaptations in Pooideae in a paleoclimatic context. To resolve this, we generated a comprehensive, dated phylogeny and used this for analyses of diversification in response to paleoclimate, ancestral state reconstruction of climatic niche and gene expression responses to cold. Our results so far suggest an old origin (66 MYA) of Pooideae and divergence of all major tribes before the E-O transition. This suggest that adaptations to colder and more seasonal climate emerged independently in separate lineages. This is supported both by molecular genetic data on cold acclimation genes and ancestral niche reconstruction. Nevertheless, molecular data on genetic responses to short-term cold and ancestral niche reconstructions suggests that Pooideae had adaptations to temperate climate long before the emergence of temperate biomes after the E-O transition. We identified climate cooling throughout the Cenozoic as a driving force in Pooideae evolution, and the E-O transition as a trigger of diversification.

**Key words:** cold adaptation, gene expression, grasses, Pooideae, temperate climate.

**Funded by:** Research Council of Norway

## **Taxonomic revision and anatomy of a *Vriesea* species complex from the mountains of southeastern Brazil**

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*Vriesea* is the largest genus of Bromeliaceae family in Brazil. It is conspicuous within the Atlantic Forest and can occur in heterogeneous habitats ranging from the sea level up to 2,000 meters of elevation. *Vriesea* presents a wide morphological variation with some characters overlapping between species. Here we present the taxonomic revision of “*Vriesea itatiaiae* species complex” composed of three species (*Vriesea itatiaiae*, *Vriesea crassa* and *Vriesea hoehneana*) with distribution restricted to mountains above 1,200 meters of elevation in Southeastern Brazil, characterized by yellow flowers, brownish bracts, resulting in inflorescences not very showy and mostly pollinated by bats. Species in this complex have approximately two meters tall when blooming, compound inflorescence with campanulate and secund flowers. An identification key for the species was created allowing species segregation based on flower characters. We also present



distribution maps, photographic plates, morphological and leaf anatomy descriptions, which indicated that a set of anatomical characters may be useful to separate species. Conservation status for each taxon, key and illustrations are provided. Our work is important to clarify the identification of plants occurring in mountains, providing reliable data for conservation initiatives in these fragile habitats.

**Key words:** conservation, Neotropics, rupicolous, species limits, Tillandsioideae.

**Funded by:** Capes, CNPq-SWE (205660/2014-2), CNPq (142354/2016-3), CNPq (304778/2013-3)

### **Species delimitation in *Cypella* (Tigridieae: Iridaceae) based on morphological and molecular data**

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*Cypella* Herb. is one of the largest genera of Tigridieae (Iridaceae) with 36 species accepted by the World List of Iridaceae (WCI). Historically, this genus encompasses taxonomic problems and identification of species requires detailed morphological characterization. The recent description of *C. gloriana*, an endemic species closely related to *C. pusilla*, exposed the need for a detailed taxonomical study of this and related taxa, in order to understand morphological variation and the limits between species. This study focus on five species of *Cypella* assembling morphological and molecular traits to establish species delimitation. Phylogenetic analyses were based in eight plastid and two nuclear markers and multivariate statistical analyses were performed with 38 qualitative and quantitative morphological characters. Phylogenetic and multivariate analyses allowed discrimination of *Cypella aquatilis*, *C. discolor* and *C. ravenniana*, while *C. pusilla* encompassed the remaining samples, including the specimens of *C. gloriana*. Therefore, an expanded description of *C. pusilla* was elaborated, a lectotype was assigned and *C. gloriana* was synonymized. The results demonstrated that increased sampling and iterative approaches as applied in this study should be generalized among species of *Cypella*, to provide robust taxonomic proposals.

**Key words:** discriminant analysis, multiple correspondence analysis, multivariate analyses, phylogenetic delimitation, species recognition.

**Funded by:** Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq); Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES); Bibliothèque du Vivant (Centre National de Séquençage) - France

### **Genome size distribution in *Sisyrinchium* (Iridaceae)**

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Iridaceae is interesting with reference to genome size and ploidy levels and their consequences in diversification. *Sisyrinchium* is largely widespread in the Americas and represents one of the most species-rich genera within Iridaceae. A new infrageneric classification and a phylogeny were provided with ten sections in the genus. Here, we aim to provide a better understanding about the genome size variation and the extension of this in the diversification of *Sisyrinchium*. Representatives of all sections were analyzed encompassing a total of 64 species. Genome size varied from 1.08 pg in *S. micranthum* (diploid) to 8.4 pg in *S. alatum* (tetraploid), thus a variation corresponding to 7.7-fold. Notably, polyploidy is important in the diversification within *Sisyrinchium* but the higher genome size does not correspond to the higher ploidy levels. Monoploid genome size varied from 0.29 to 2.65 pg among sections. In some sections we observed that almost all species present the distribution of genome size in the same class as *Cephalanthum* where 11 of the 18 species presented the genome size from 1.5 to 2.5 pg. In contrast, in *Viperella* it six different classes of genome size distribution were observed. Genome downsizing seems to be evolutionarily important for these species.

**Key words:** DNA content, genome organization, genome size variation, plant evolution, polyploidy.

**Funded by:** CNPq; CAPES/COFECUB

### **Evolutionary history of *Barbacenia paranaensis* (Velloziaceae) in the campos rupestres of the Devonian Scarp in southern Brazil**

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The Southern limit of Velloziaceae's distribution in South America is currently determined by the distribution of *Barbacenia paranaensis*, a species that occurs vertically on rock outcrop walls along fragments of cerrado in Paraná. With an annual and synchronous flowering, it is pollinated by traplining hummingbirds. The pollinator returns to patches according to intervals of nectar replenishment and covers extensive areas in its route, dispersing pollen between close walls. It is a self-compatible species and presents inbreeding depression. We inferred how ecological processes and geographical fragmentation shaped the evolutionary history of the species, by combining information about floral dynamics, pollination and reproduction systems, modeling of geographical distribution, and contemporary (ISSR markers) and historical genetic variability (cpDNA sequences). The ISSR presented high variability and weak genetic structure within geographic regions, a pattern that may be related to the species biological characteristics. The cpDNA presented a strong historical divergence between regional lineages, mirroring the geographical isolation between rocky outcrops along the Devonian Scarp. Clonal reproduction and genetic drift due to the geographical isolation may contribute to population differentiation and cpDNA haplotype endemism. The modeling of the geographical distribution suggests that it remains open which geographical barriers act on limiting the distribution of the species.

**Key words:** breeding systems, geographical isolation, phylogeography, pollination, population genetics.

**Funded by:** MCTI/CNPq 459989/2014-6

### **Morphometrics of the *Paepalanthus aequalis* (Eriocaulaceae) complex**

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*Paepalanthus* Mart. is the second most diverse genus of Eriocaulaceae and the richer in number of species in Brazil. Due to its richness and morphological variation, *Paepalanthus* shows some taxonomic problems. *Paepalanthus aequalis* (Vell.) J.F. Macbr. complex is an example. The lack of a detailed description in its original description contributed to misunderstandings in the circumscription of about 5 most similar species. In order to clarify the morphological limits of these taxa, 10 individuals of 7 populations were analyzed. We measured the plant, leaves, inflorescences, and involucral bracts dimensions. Herbarium sheets from *P. aequalis*, *P. blepharocnemis* Mart. ex Körn., *P. loefgrenianus* Ruhland, *P. cachambuensis* Silveira, and *P. henriquei* Silveira & Ruhland are also analyzed. We performed the linear discriminate analysis. The results showed that the population of Serra da Bocaina's and São Paulo's (city) populations are isolated from the other populations and Baependi's population stayed partially together with the other populations. The leaf size, leaf shape, scape size, and inner involucral bracts width were the most important characters responsible for the segregation of groups in the analysis. As a conclusion, the names *P. blepharocnemis* and *P. loefgrenianus* were accepted and *P. aequalis* and *P. cachambuensis* had their occurrence area expanded.

**Key words:** Eriocaulaceae, morphometrics, *Paepalanthus*, populations, species complex.

**Funded by:** CAPES, CNPq, FAPERJ, and Alexander von Humboldt foundation.

### **Heterochromatin in the subtribe Oncidiinae (Orchidaceae)**

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The subtribe Oncidiinae includes species with a basic number  $x=28$ , with processes of chromosome evolution: polyploidy and dysploidy, although little is known about the distribution and composition of the heterochromatic fraction. In this work we analyzed chromosome number and heterochromatin composition through the CMA/DAPI banding of three species of the subtribe (one species of *Aspasia* and two of *Gomesa*), aiming to characterize the karyotypes of these species. The karyotype of all species analyzed was  $2n=56$ . In *G. laxiflora* we observed six large chromosome pairs carrying heterochromatin DAPI<sup>+</sup>/CMA<sup>-</sup> blocks in more than 50% of the chromosome extension, in addition to two CMA<sup>+</sup>/DAPI<sup>-</sup> subterminal bands corresponding to heterochromatic

RONs. On the other hand, in *G. flexuosa*, no heterochromatic chromosome region was observed except RON located in a small chromosomal pair. Similarly, *Aspasia lunata* also showed only heterochromatin CMA<sup>+</sup> corresponding to heterochromatic RON. The genus *Gomesa* was expanded by the inclusion of most Brazilian species and *Oncidium*, based on data from DNA sequences. Curiously, the only species of *Gomesa* s.s. analyzed was different from the other subtribe species because it presented larger chromosomes with a large amount of proximal heterochromatin rich in AT.

**Key words:** dysploidy, Epidendroideae, evolution, heterochromatic bands, polyploidy.

**Funded by:** Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Instituto Nacional do Semiárido (INSA), Universidade Federal da Paraíba.

### **Comparative analysis of complete chloroplast genomes improves phylogenetic resolution in Smilacaceae**

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Smilacaceae, composed of only one genus, *Smilax*, is a monocot family of lianas, widely distributed across the world. The family comprises more than 200 species characterized by climbing habit, reticulate leaf venation, paired petiolar tendrils, unisexual flowers, and berries. Previous molecular phylogenetic studies have showed the primary framework of systematics in Smilacaceae. However, the deep-level relationships remain poorly resolved, thus impeding our understanding of the evolutionary history and biogeography of the family. Plastome sequencing is now an efficient option for increasing phylogenetic resolution in plant phylogenetic and population genetic analyses. Here, the complete chloroplast (cp) genomes of 59 species covering its phylogenetic diversity and almost all geographic distribution of this family were sequenced to better understand the evolutionary relationships among these plants. Phylogenetic analyses based on whole cp genome data revealed four major lineages within Smilacaceae. Our analyses provided robust support for the deep relationships, verifying *Smilax aspera* to be a basic group of Eurasia clade and the West China–Himalaya clade is sister to the Old World prickly clade. The findings highlight the potential of the whole cp genomes for improving resolution in phylogeny as well as species identification in phylogenetically and taxonomically difficult plant groups.

**Key words:** chloroplast genome, biogeography, phylogenetic, phylogenomics, Smilacaceae.

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### **Both climatic change and geographic isolation have driven the diversification of *Amana* in East Asia**

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East Asia is species-rich and has high levels of endemism, yet the evolutionary processes responsible for this endemism remains largely unexplored. Herein we used the endemic genus *Amana* to gain new insights into the origin and diversification of this important biome. We reconstructed its biogeographic pattern based on plastid and nuclear markers, and combined climatic, geographic and morphologic data with molecular phylogeny. *Amana* is divided into two clades (broad-leafed vs. narrow-leafed), with two and four subclades, respectively. The origination and diversification of *Amana* coincided with the initial and subsequent intensification of the Asian monsoon, respectively. Analyses of ecological disparity (DTT) indicated a departure from a neutral model of evolution, and spatial evolutionary and ecological vicariance analyses (SEEVA) showed that different subclades tend to evolve under different potential climatic niches, especially in terms of Temperature Seasonality, Annual Precipitation, Precipitation of Driest Month, Precipitation of Driest Quarter and Precipitation of Coldest Quarter. In either clade, leaves gradually narrowed as the latitude increased. Geographical ranges showed little overlap between related subclades. Our results suggest that two independent intensifications of the East Asian monsoon and subsequent geographic isolation may have jointly facilitated the origin and diversification of *Amana* in East Asia.

**Key words:** biogeography, East Asia monsoon, ecological divergence, endemism, phylogeny, .

### **Phylogeographic study of the Tethys relic *Smilax aspera* (Smilacaceae) based on a population genomics method**

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*Smilax aspera* L. is a basal taxon placed in the Old World Smilacaceae and is the most widely distributed species in the family. It shows a disjunct distribution pattern in Mediterranean, Eastern African, and Southern Asia. Thirty-five microsatellite loci developed from transcriptome data revealed that *S. aspera* diverged deeply into two lineages, that is, the Mediterranean lineage and the East African-South Asian lineage. Population genetic analysis also indicated that *S. aspera* was isolated by distance among sampling locations. Furthermore, 49,138 single nucleotide polymorphism loci were identified using double digest restriction associated DNA (dd-RAD) sequencing approach among 40 populations. Sequentially, a precise phylogenetic analysis based on maximum likelihood method using the SNPs data revealed that two clades were recovered in Mediterranean and East African-Southern Asian region, of which diverged in middle Miocene (11.71 Ma, 95% HPD: 8.88-14.24 Ma). According to the phylogeny, it indicated Asia Minor was a basal taxon of Mediterranean lineage, and the Eastern African was a basal taxon of EA-SA lineage. The reconstruction of ancestral areas using Bayesian Binary BBM method indicated that *S. aspera* might originated from southern Himalaya region.

**Key words:** intercontinental disjunction, phylogeography, population genomics, *Smilax*, Tethys relic.

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