



An overview and abstracts of the First World Congress on Bromeliaceae Evolution

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Abstracts of the Conferences, Symposia, Oral Presentations and Poster Presentations performed during the 1st World Congress on Bromeliaceae Evolution, March 2015 (Brazil):

Reactive oxygen species and antioxidant enzyme activities in leaves of *Guzmania monostachia* plants under water deficit

Abreu, Maria Elizabeth¹; Carvalho, Victória² & Mercier, Helenice¹

CAM plants have the capacity to deal with highly changing environments due to the flexibility of reversible morphological and physiological adaptations to multiple stresses. However, little is known about the signalling pathway of ROS in plants with CAM metabolism, other than the knowledge that ROS production is limited in CAM plants. In the present study, we assessed the effects of drought stress on reactive oxygen species and antioxidant enzyme activities in leaf portions of *Guzmania monostachia*. The exposure of *G. monostachia* plants to 10 days of water deficit led to a decrease in the leaf relative water content (RWC) from 75% to 50% in all leaf portions (apical, middle and basal); hence, it was concluded that plants subjected to drought produced higher levels of reactive oxygen species (ROS) when compared with control plants. Significant variations to the formation of ROS were also identified in all leaf portions during the diurnal cycle. After ten days of CAM induction, H₂O₂ concentration increased significantly in contrast to control plants during the day-night cycle. In addition, the activity of antioxidant enzymes in processes related to the elimination of ROS was also evaluated. The analysis showed that the middle and basal portions of the leaves increased the activity of superoxide dismutase (SOD) at 12pm when subjected to water deficit, while in the apical portion, this enzyme was most active at 8am in the control plants. Moreover, the plants with the water supply suspension showed greater activity of catalase (CAT) at 8am when compared to control plants, which also showed an increase in CAT activity at 8pm in the basal portion. Ascorbate peroxidase (APX) was most active in the middle portion of plants under drought. Meanwhile, as a general tendency, a higher activity of antioxidant enzymes (SOD, CAT and APX) was observed in the middle portion of the leaves of *G. monostachia* that were subject to ten days of water deficit.

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Key words: Reactive Oxygen Species; Antioxidant Enzymes; CAM Metabolism.

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Chemical and pharmacological properties of Bromeliaceae species from the Caatinga biome

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The Bromeliaceae family is predominantly Neotropical. The phytochemistry of this family is characterized by the presence of flavonoids, triterpenoids, steroids, diterpenes, cinnamic acid derivatives, lignans, alkaloids, among others. The purpose of this lecture is to present results of research carried out at the Universidade Federal do Vale do São Francisco with the species *Bromelia laciniosa*, *Encholirium spectabile* and *Neoglaziovia variegata*, typical species from the Caatinga biome (semi-arid region of Brazil). *Bromelia laciniosa* is a species which is known in the Northeast region of Brazil as “macambira” and is used in the alimentation of man and domestic animals, especially in times of drought. The main therapeutic indications are for treating child colic, diarrhea, fever, jaundice, dandruff and hepatitis. The decoction of the roots is also popularly used against hepatitis and intestinal disorders and as a diuretic, while the dried and powdered leaves are used in

cooking as a source of protein. A study conducted by our research group showed that the ethanol extract of this species (Bl-EtOH) has antinociceptive activity. The ethanol leaf extract apparently presents no significant toxicity. A flavonoid was isolated from this species that is derived from quercetin. *Encholirium spectabile* is locally known as “macambira-de-flecha” and “macambira-de-pedra”. The crude ethanol extract (Es-EtOH) of this species has antinociceptive activity, probably of peripheral origin. The mechanism involved is not completely understood but at least in part there is the participation of opioid receptors. Es-EtOH has gastroprotective activity against gastric mucosal damage induced by ethanol, HCl/ethanol, ibuprofen, ischemia and reperfusion, which suggests that the extract may activate cytoprotective mechanisms that increase the release of prostaglandins, increase the concentration of NPSH groups, increase catalase activity and activate the NOS pathway. *E. spectabile* contain phenolic compounds which can serve as natural sources of antioxidants and antimicrobial agents. Dried extracts of *E. spectabile* present antioxidant and photoprotective activities *in vitro*. This shows the possibility to use this extract as sunscreen in pharmaceutical preparations. *Neoglaziovia variegata* is popularly known as “caroá”. The acute toxicity of crude ethanol extract of *N. variegata* (Nv-EtOH) was evaluated. The extract can be considered of low toxicity. Nv-EtOH also possesses an antinociceptive effect, which probably is related with a central mechanism. It was demonstrated that the extracts from this plant contain high contents of phenolic compounds and flavonoids which could be responsible for their antibacterial, antioxidant and photoprotective activities. We have established a promising gastroprotective effect of Nv-EtOH and provided evidence for the involvement of NO, PG, mucus, sulfhydryl groups, catalase, and KATP channels, factors that play an important role in gastric cytoprotection. Pharmacological and chemical studies are continuing in order to characterize the mechanism responsible for these effects.

Funding: CNPq, FACEPE.

Key words: *Bromelia laciniosa*; *Encholirium spectabile*; *Neoglaziovia variegata*.

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Floristic and phenology of Bromeliaceae in the Ecological Reserve Guapiaçu, RJ: preliminary results

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The Atlantic Forest is the second largest forest formation in Brazil, covering originally about 12% of the East Coast. Formed by overlapping ecosystems, it is one of the richest formations in terms of flora, and it is characterized by the large number of endemic species. Moreover, it is one of the main centers of diversity of the Bromeliaceae family, comprising about 30 genera and nearly a thousand species. This work is being developed in an area adjacent to the mountain region of the state of Rio de Janeiro, located in the coastal lowlands, in the city of Cachoeiras de Macacu. The Ecological Reserve Guapiaçu (REGUA), is a private conservation area, with altitudes of up to 2,000m, near the State Park of Três Picos, and is part of the Central Fluminense Mosaic, one of the mostly well-preserved rainforest mosaics in the state, comprising the National Park of Serra dos Órgãos, which is considered an important place for ecological and genetic interactions. It is believed that the local Bromeliaceae flora may indicate similarities and possible differences in relation to adjacent areas due to geographical distances and altitude. To carry out the floristic survey, a map of the trails was made, and the samples were taken by the traversal method, where every 100m was sampled to the left, and to the right, in order to increase the collection area. The trails sampled have different successional stages and cover an altitudinal gradient between 0–1,000m. Phenology was accompanied by observing the phenofases (bud, flowering, fruiting) present at the time of each collection. So far there are over 100 collections in 11 trails, with monthly visits to the locality. A total of 11 genera and 31 species have been recorded. It has been observed that the occurrence of some species is limited to certain altitudes. Concerning phenology, most of the recorded species display a well-defined flowering period, over a short time period and relatively synchronous.

Key words: Floristics; Phenology; Atlantic Forest.

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Nomenclatural correction in *Cryptanthus* Otto & A. Dietrich. (Bromeliaceae - Bromelioideae)*Alves, Marccus¹ & Marcucci, Rosella²*

Cryptanthus is a genus endemic to eastern Brazil from Rio Grande do Norte to Rio de Janeiro and Minas Gerais. After a long search at libraries and European herbaria, a correction is presented to the author designation of a species as well as some input about synonymy and morphological variation. *Cryptanthus zonatus* (Vis.) Beer has been cited by several authors but there is a mistake that has been copied for years. Roberto de Visiani, botanist and director of the Botanical Garden of Padua (Italy) from 1837–1878, published in 1847 on the last page of an index of plants from the institution a monospecific genus called *Pholidophyllum* Vis. Under this new genus, he provided a short description of *P. zonatum* Vis. and *P. zonatum* β *fuscum* Vis. For both taxa, Roberto de Visiani also indicated as synonyms two names which had never been published but were used for cultivated specimens at “Orto Botanico Patavino”. Both names are *nomen nudum*, indicated and first published as such by Visiani and not by Otto & Dietrich, as cited later in major works in Bromeliaceae. In 1854, Roberto de Visiani published a short and poorly known paper which was found at the library of University of Padua. In this publication, he proposed a new combination for both taxa, under the genus *Pholidophyllum*, established by him 8 years previously. Two years after that, Beer in his major work about Bromeliaceae and unaware of the recent publication by Visiani, also proposed the same new combinations. No type is clearly indicated in the protologue of both taxa, except for the indication that the short descriptions were based on cultivated specimens from the botanical garden (“Orto Botanico”) in Padua. Living specimens had possibly been sent from the Botanical Garden of Genova to Roberto de Visiani (at “Orto Botanico Patavino”) and to Antonio Bertoloni (at “Horto Botanico Bolognese”). The Bromeliaceae collection at herbaria PAD, where the original collection from Roberto de Visiani is deposited, and BOLO, which also holds some specimens studied by him, were carefully searched and no specimens of *Cryptanthus* (or under the name *Pholidophyllum* or *Tillandsia*) were found. A correction on the name of the authors as well as a new synonym for *C. zonatus* are proposed here.

Funding: CNPq, NSF, Velux Stiftung, Beneficia Foundation.

Key words: Nomenclature; Atlantic Forest; Northeastern Brazil.

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Genetic structure and diversity patterns of *Tillandsia aeranthes* (Lois.) L.B. Smith in Brazil*Aoki-Gonçalves, Felipe¹; Guimarães, Thais² & Palma-Silva, Clarisse³*

Brazil is the diversity center of Bromeliaceae. Such an area with high levels of species richness and endemism is considered to be an ideal site for speciation. *Tillandsia aeranthes* is epiphytic and in Brazil it occurs mainly in the state of Rio Grande do Sul, which presents very distinctive topographic profiles. Objectives: 1) test cross-amplification of microsatellite markers developed for other bromeliads in *T. aeranthes*; 2) quantify genetic diversity of *T. aeranthes* populations; 3) evaluate genetic structure of *T. aeranthes* natural populations through different topographies in southern Brazil. Leaf samples from 8 populations (87 individuals) were collected and total DNA was extracted. Ten nuclear and four plastidial microsatellite markers were tested, genotyped and analyzed. Levels of genetic diversity were described through: number of alleles per locus, observed heterozygosity (Ho) and expected heterozygosity (He). Deviations from the Hardy-Weinberg Equilibrium were calculated through the inbreeding coefficient (FIS). Levels of genetic structure of analyzed populations were estimated using the Analysis of Molecular Variance (AMOVA). The fixation index FST was used as a genetic differentiation parameter between populations. The hypothesis of isolation-by-distance by comparing geographic and genetic distances among populations was also tested. Seven nuclear loci amplified satisfactorily and were polymorphic. All plastidial markers amplified satisfactorily and one was polymorphic. Per population, the number of alleles ranged from 39 to 59; the mean He value was 0.844 and the mean Ho value was 0.703. The mean value of FIS was 0.179 (all FIS values were significant - $p < 0.05$). The mean FST value was 0.032 and highly significant ($p\text{-val} = 0.0003$). The isolation-by-distance test did not show

correlation. These results suggest low yet significant levels of genetic structure, which indicates effective gene flow throughout the analyzed area, despite the topographic differences along it.

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Key words: Bromeliaceae; Phylogeography; Microsatellites.

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A guide to bromeliad identification in the Cantareira State Park, SP, Brazil

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Bromeliaceae has a relevant role as one of the main floristic components of physiognomies of Brazilian ecosystems. Nowadays, it represents about 3,300 species in 58 genera, figures constantly grow due to ongoing discoveries and taxonomic reviews. The family has a Neotropical distribution (except for *Pitcairnia feliciana* (A.Chev.) Harms & Mildbr., found on the African Continent) and two centers of diversity: northern Andes and eastern Brazil, which is mostly covered by Mata Atlântica, a biome in which about 900 bromeliad species are recorded. Used for a variety of purposes, for food, fibers, medicines and ornamentation, Bromeliaceae is cultivated, highly commercialized and has its diversity threatened by indiscriminate harvesting in its natural environment or by the loss of its habitat due to deforestation. The knowledge and disclosure of the high diversity of Brazilian bromeliads are extremely important, whether making the beauty of native plants available to the general public, or contributing to the preservation of the Brazilian flora. The study of Bromeliaceae from Parque Estadual da Cantareira proposed herein aims to provide subsidies for conservation policy of protected areas. The Park has 7,916 hectares, has been created with the purpose of protecting one of the remaining tracts of Mata Atlântica from the State of São Paulo and covers the municipalities of São Paulo, Guarulhos, Caieiras and Mairiporã. The objective of the proposal is to prepare a field identification guide for the Park's Bromeliaceae, which, besides servicing the general public, shall provide data for recognizing the flora from this region. The identification guide for bromeliads shall contribute to the on-going floristic studies in the Park and in other areas of Mata Atlântica. The book shall have a pocket size, in order to facilitate its use in the field and it shall contain photographs of the habits and vegetative and reproductive details, with brief descriptions, comments on distribution and phenology of species, in addition to the category of threat according to the analysis of the populations in the natural environment. Research has already begun with the surveying of herbaria and based on speciesLink, with the inclusion of new collections carried out recently in the region. The samples collected are stored in SP herbarium, being georeferenced and photographed for illustration in the guide. Until now, species of *Aechmea* Ruiz & Pav., *Billbergia* Thunb., *Canistrum* E. Morren, *Tillandsia* L. and *Vriesea* Lindl. have been registered. Among these is included *Vriesea vulpinoidea* L.B.Sm., a species with restricted distribution to the Atlantic rainforest formations of the states of São Paulo and Santa Catarina and which has very few records.

Key words: Bromeliaceae; Field Guide; Atlantic Forest.

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The monodominance of *Encholirium spectabilis* Mart. ex Schult. in inselbergs from an area of the semi-arid region in northeastern Brazil

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The semi-arid region in the Brazilian Northeast, locally called Caatinga, holds several inselbergs, for which just a few located on the states of Bahia, Ceará, Paraíba and Pernambuco have floristic inventories available. Over the last three years, the inselbergs located in the municipality of Patos, state of Paraíba were studied and 307 species of vascular plants were recorded from them. In the studied area, *Encholirium spectabilis* is

the only saxicolous species of the Bromeliaceae. The species is remarkably abundant in all nine inselbergs evaluated and is dominant at all altitudinal levels. However, the monodominance is more intense in the middle altitudinal levels. The species is ecologically very aggressive and blooms during the entire year during the rain and dry seasons. The dense clumps provide shelter for several species of small animals including lizards which are very common in the area. Bees are common visitors to the flowers but sometimes visitors such as dragonflies were also recorded in the area. In two inselbergs almost the whole area of exposed rock is covered by *Encholirium spectabilis* which also provide shade and a humid habit for the establishment other plants. It reinforces the ecological value of the species to preserve the diversity of animals and plants in the inselbergs in the semi-arid region in Brazil.

Funding: Fundação O Boticário.

Key words: ecology, inselbergs, caatinga

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Phylogeny and morphology demand a revised classification of Bromeliaceae subfamily Tillandsioideae

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Bromeliaceae subfam. Tillandsioideae is a morphologically and phylogenetically well characterized, monophyletic taxonomic group. Efforts to propose a complete, consistent, internal classification based on morphological and molecular DNA data failed mainly due to restricted sampling in individual studies. However, traditional generic concepts and morphological characters used to delimit taxa have shown to be partly unpredictable, especially in species-rich genera. Therefore, in the last decade efforts were undertaken to expand the taxonomic coverage and re-evaluate morphological characters. Hence, a revised classification for Bromeliaceae subfam. Tillandsioideae is presented based on a multi-locus molecular phylogeny (viz., plastid *rpoB-trnC-petN*, *trnK-matK-trnK*, and *ycf1*, and nuclear *PHYC*) and morphology. Traditional morphological characters (e.g., petal morphology) were critically reviewed. Phylogenies based on DNA data were then compared with traditional and new morphological evidence (e.g., stigma, ovule, anther, pollen and seed morphology). The four tribes proposed earlier are confirmed, but *Catopsidae* replaces the formerly used name *Pogospermeae* for the monotypic tribe of *Catopsis*. In addition, the two new subtribes *Cipuropsidinae* and *Vrieseinae* are proposed within tribe *Vrieseae*. *Alcantarea*, *Catopsis*, *Glomeropitcairnia*, *Guzmania*, and *Werauhia* are confirmed and *Racinaea* is expanded. Several new genera are established to render taxonomic units monophyletic and morphologically well circumscribed. They represent segregates of either *Mezobromelia*, *Tillandsia*, or *Vriesea*. Two new subgenera are established and one subgenus is resurrected within *Tillandsia*.

Key words: Tillandsioideae; Phylogeny; Morphology.

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Diversity pattern of epiphytic Bromeliaceae in the northwestern Andes of Colombia

Benavides, Ana Maria¹

The northwestern Andes of Colombia is a diverse area in species of Bromeliaceae. The Catalogue of Vascular Plants Flora de Antioquia recorded 183 species, of which 70% have an epiphytic habit. In this study we examined the *in situ* diversity and distribution of vascular epiphytes, as well environmental variables (annual rainfall, temperature, rainfall in wet and dry months and wet and dry seasons) at 10 forest stands located between 62 and 2,887 m.a.s.l. in Antioquia, Colombia. Following SVERA methodology, a total of 850 spe-

cies of vascular epiphytes were identified to species or morphospecies. The orchids were the most diverse group with 159 species, followed by bromeliads (105 species). Here, we present the results for Bromeliaceae. Bromeliaceae exhibited an elevational diversity gradient with a middle elevation richness peak at 2,100 m.a.s.l. However, correlations between elevation and environmental variables suggest that the elevational diversity gradient is affected by temperature and rainfall. For wind-dispersed species (anemochory) richness decreased with increasing rainfall, possibly because of deficient dispersal in wet environments, indicating that richness patterns differ among groups with different dispersal modes. Forest stands at middle elevations with lower rainfall present lower richness. At the forest stand level, matrix correlations between anemochory (wind dispersed) and zoochory (animal dispersed) Bromeliaceae composition and spatial-environmental variables (host tree structure and host spatial distribution) exhibited no patterns. Nevertheless, species showed vertical stratification, suggesting that mechanisms related with microenvironmental factors may lead to local distribution. In Colombia bromeliads are considered under threat and all species are protected. Legislation requires compensation strategies in the case of any intervention for species of this family. Currently, most compensation strategies involve transplanting plants to other forest. Results as presented in this study (that microenvironmental factors related with vertical stratification are key to species distribution) help improve compensation strategies.

Key words: anemochory; rainfall; vertical stratification.

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Genetic structure in *Aechmea calyculata* explained by different type of vegetations in southern Atlantic Forest

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Aechmea calyculata occurs in the southern region of the Atlantic Rainforest and presents a geographical distribution different from the remaining species of *Aechmea* subgenus *Ortgiesia*. While most species of *Ortgiesia* are found in the coastal line and nearby mountains in the eastern region of Brazil, *A. calyculata* distribution extends from the mountain regions in eastern Brazil to northeastern Argentina, characterizing an east-to-west pattern of occurrence. The objectives of the present study were to investigate if there is an east-west genetic structure among *A. calyculata* populations, and to evaluate how the genetic diversity is distributed along the geographical range of the species. For this purpose, we sampled six populations of *A. calyculata*, totaling 144 individuals, which were genotyped using 12 nuclear microsatellite markers (SSR). Added to this, two chloroplast regions (rpl32-trnL and rps16-trnK - cpDNA), and the nuclear gene phyC were sequenced for 43 and 40 individuals, respectively. Our results showed high levels of genetic structure across *A. calyculata* populations with a F_{ST} of 0.231 for SSR, a $G_{ST} = 0.866$ for cpDNA and of 0.486 for phyC. Structure analysis with SSR revealed a $K = 2$ genetic groups, with populations from the western region of distribution strongly differentiated from those of the east, with few individuals identified as migrants. The same pattern was recovered in BAPS analyses using cpDNA and phyC data. High levels of genetic diversity were found in *A. calyculata* for SSR, with the number of alleles ranging from 42 to 80, and the allelic richness from 3.25 to 4.23 per population. Observed heterozygosity ranged from 0.389 to 0.638, while HE varied from 0.504 to 0.623. For cpDNA only five haplotypes were found for *A. calyculata*, varying from one to two per population. On the other hand, more variation was observed with phyC, which displayed nine haplotypes, varying from one to five per population. The present study revealed high genetic structure across the *A. calyculata* distribution, especially between populations of western and eastern regions of occurrence. These two regions are separated by Araucaria forest, and in higher altitudes by grassland ('Campos'), which could be acting as a barrier to gene flow between populations from the east and west. Nowadays grassland is restricted to higher altitudes but during the Pleistocene this was the predominant type of vegetation covering the southern region of Brazil, and could have promoted isolation. The genetic diversity indices found for *A. calyculata* were very similar among populations with SSR and cpDNA data. However, higher diversity was

found for the eastern populations with phyC, which could indicate a long-term persistence of *A. calyculata* in this region.

Funding: CNPq, FAPERGS, FAPESP.

Key words: Phylogeography; Bromelioideae; Genetic Diversity.

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Taxonomic study of *Dyckia maritima* complex (Bromeliaceae, Pitcairnioideae): preliminary results

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Endemic to the southern Brazilian states of Rio Grande do Sul and Santa Catarina, the informal group called “complex *Dyckia maritima*” was proposed by Strehl & Beheregaray (2006) based on a morphological study of *Dyckia* seeds. That study grouped species, different from the typical *Dyckia* complex that have narrow-ovate seeds (vs. wide-oval), not flat (vs. flat, discoid) and triangular (vs. semicircular or sickle). The first species published of complex was *Dyckia maritima*, originally proposed as a monospecific genus, *Prionophyllum* K. Koch (1874), and consists of *Prionophyllum selloum* (= *Dyckia selloa* (K. Koch) Baker), this was widely accepted by Mez (1895, 1896, 1935), being diagnosed in these works mainly by dimorphic flowers (perfect or pistillate). This feature, rare in Bromeliaceae, was not subsequently observed in any other specimen of the complex. Baker, in the Handbook of the Bromeliaceae (1889), proposed a broader constituency for *Dyckia* and downgraded *Prionophyllum* to a subgenus of *Dyckia* and described *Dyckia maritima*, *D. grandifolia*, *D. macracantha* and *D. myriostachya*, species belonging to the complex. Smith (1943, 1956) and Smith & Downs (1974), in a review of the Bromeliaceae family, considered *Prionophyllum* as synonymous with *Dyckia*, which is still the most widely accepted position. Considering the few studies in relation to the *Dyckia maritima* complex today, in reason of the difficult taxonomic position and the specific delimitation of taxa, a taxonomic revision is being performed, including observations and collections of species of the complex in its habitat, a literature review and consultations in herbaria. The studies, according to morphological observations, indicate the existence of a group of closely related species within the complex (*Dyckia maritima* complex *sensu strictu*) composed of *D. maritima*, *D. myriostachya* (species considered by Mez (1935), Smith (1943, 1956) and Smith & Downs (1974) as synonymous with *Dyckia selloa*, but here considered species), *D. retroflexa*, *D. nigrospinulata* and *D. rigida*. In these species it was found that other species are also within the *D. maritima* complex *sensu lato*, or fit under the characteristics mentioned above: *D. agudensis*, *D. alba*, *D. delicata*, *D. domfelicianensis*, *D. hebdingii*, *D. polycladus*, *D. selloa*, *D. tomentosa* (species considered by Mez (1935), Smith (1943, 1956) and Smith & Downs (1974) as synonymous with *D. maritima*, here considered species). The *Dyckia retardata* species was considered a synonym of *D. tomentosa*. Phylogenetic analyzes presented by Krapp (2013), based on nuclear DNA gene sequence data phyC, suggests the monophyly of the *Dyckia maritima* complex, so in order to verify the monophyly of the group (with the highest number of species in this analysis) and better understand the interspecific relationships within the group, studies using molecular characters will be developed in future by our research group.

Funding: Colégio Politécnico da Universidade Federal de Santa Maria.

Key words: Taxonomy; Austro-brasilica Flora; *Prionophyllum*.

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***Dyckia pontesii* (Bromeliaceae, Pitcairnioideae), a new species from Rio Grande do Sul, Brazil**

Büneker, Henrique Mallmann¹; Witeck-Neto, Leopoldo¹ & Soares, Kelen Pureza¹

Dyckia Schult. & Schult.f. is a large genus of Pitcairnioideae (Bromeliaceae), consisting of about 164 species (Gouda *et al.* 2014) found as saxicolous or terrestrial plants in South America, especially in Brazilian Central, Southeast and South regions, but also in Bolivia, Paraguay, Argentina and Uruguay (Smith & Downs

1974). A recent study conducted by Krapp *et al.* (2014) presented the first comprehensive phylogeny based on molecular analysis to *Dyckia* characters, confirming the monophyly of the genus previously been reported based on morphological characters (Forzza 2001). In the state of Rio Grande do Sul (southern Brazil), among Bromeliaceae, the *Dyckia* genus is the one with the highest species diversity, with 29 species cited (Strehl 2008; Büneker *et al.* 2013; Forzza *et al.* 2014). It is seen that Rio Grande do Sul is home to a great specific diversity of *Dyckia* where new species can still be found. From morphological studies *Dyckia pontesii* sp. nov. is proposed, this morphologically close to *Dyckia elisabethae* S. Winkler and *Dyckia duseii* L. B. Smith. It differs from *D. elisabethae*, which occurs in the eastern region of Rio Grande do Sul, due to its leaves with laminar margin and slender, generally inconspicuous spines (vs. large, conspicuous spines), white sheath (vs. brown), green stalk (vs. red), oval-triangular floral bracts with acute-attenuated apex (vs. elliptical with acuminate apex), sessile flowers (vs. pedicellate), sepals with acute apex, glabrous or sparse-tomentose at the apex of abaxial (vs. rounded and tomentose on the abaxial surface) and glabrous petals (vs. villous petals). It differs from *D. duseii*, which occurs in the states of Paraná and Santa Catarina according to Forzza *et al.* (2014) due to white sheath (vs. brown), oval-triangular floral bracts with acute-attenuated apex (vs. ovate with acuminate apex), sessile flowers (vs. pedicellate) and free filaments above the ordinary tube with petals (vs. connate above the ordinary tube with petals). The species also differs from the others by having a slightly zygomorphic cup, an unusual characteristic for the genus, this because their adaxial sepals are crenated and closer to each other than with the abaxial, which is non-crenated or slightly non-crenated. *D. pontesii* grows in outcrops and escarpments of conglomeratic rocks in the Pampa Biome in the city of Pinheiro Machado, in southern Rio Grande do Sul state, a region known as the Serra do Sudeste.

Funding: Colégio Politécnico da Universidade Federal de Santa Maria.

Key words: Taxonomy; Austro-brasilica Flora; Pampa Biome.

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Optimization of *in vitro* seed germination of *Vriesea minarum* (Bromeliaceae), a vulnerable species from Serra da Piedade

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Vriesea minarum L. B. Smith is a rupicolous species and endemic to the Iron Quadrilateral region in Minas Gerais state. It grows in places frequently disturbed by the process of extracting iron ore, which has had a large impact on their habitat. Its occurrence inside only a few protected areas of Campos Rupestres of the Serra da Piedade has led to its categorisation as a vulnerable species. The aim of the work was to establish a protocol for *in vitro* seed germination that can be used to maintain genetic variability. Seeds were germinated and grown in water (T0) and Murashige and Skoog (MS) medium in the presence of light at 25°C. *In vitro* germinated seedlings were multiplied in MS medium supplemented with BAP (6-benzylaminopurine) and IAA (indole-3-acetic acid): control – MS (T1); MS + 2.22 µM BAP (T2); MS + 0.57 µM IAA (T3). The seed disinfection was effective for all treatments. The percentages of seed germination obtained were 60.1 % in water (T0) and 55.9 % in the MS medium (T1). After three months in MS medium, the largest shoot height and fresh matter were obtained in T3- IAA (115.3 ± 2.1 mm and 22.0 ± 0.6 mg respectively), while statistical difference was not observed between the seedlings which grew in MS medium and water (T0). The concentration of pigments (total chlorophyll) was similar between T2 and T3, but significantly higher in MS medium (T1 = 1.07 ± 0.32 mg g⁻¹) and lower in water (T0 = 0.36 ± 0.09 mg g⁻¹). The results of these experiments demonstrated the viability of *in vitro* multiplication. When initiation of the culture is done through seeds obtained from a wide range of capsules, many different genotypes can be conserved. The development of micropropagation protocols from seeds is an alternative for conservation of this species in *in vitro* germplasm banks.

Funding: Fapemig.

Key words: *Vriesea minarum*; *In Vitro* Multiplication; Seed.

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Drought affects antioxidant enzyme activities in *Guzmania monostachia* (L.) Rusby ex Mez atmospheric plants

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During exposure to environmental stresses, plants undergo an excessive production of reactive oxygen species (ROS), which could lead to cell death. Cells have an antioxidant defense mechanism that prevents imbalance in ROS concentration, composed by enzymes and non-enzymatic substances. Recently, ROS have been associated with signaling to activate defense mechanisms against abiotic stress. For instance, crassulacean acid metabolism (CAM) pathway allows for protection against drought stress. It is hypothesized that CAM also prevents excessive production of ROS by dissipating accumulated energy in chloroplasts, generated during drought stress. The bromeliad *Guzmania monostachia* (L.) Rusby ex Mez is a C3-CAM facultative species, which performs CAM after 7 days of exposure to drought during both atmospheric and tank-form stages. The objective of this study was to assess the diurnal variation of antioxidant enzymes activities and of ROS production in atmospheric plants of *G. monostachia* after exposure to drought. Plants of *G. monostachia* were cultured *in vitro* for 6 months in a grow chamber. The plants were subsequently transferred to trays containing substrate, which were kept in a controlled environment chamber until they reached approximately 4–5 cm in height. Plants were then submitted to drought, in which watering was suspended during 10 days. Control plants were watered daily during the same period. Plants were harvested every 4 hours, starting at 8:00 and ending at 4:00. Total ROS production and activities of superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and glutathione reductase (GR) were quantified from leaf samples of 10 plants. Relative water content was 76% and 40% in control and drought plants, respectively. ROS production was stable between 12:00 and 24:00 in both treatments. An increase in ROS started at 4:00 and peaked at 8:00, which may be related to the fact that the light period began two hours before the sampling time (6:00). Still, at 8:00, plants under drought stress showed significantly lower levels of ROS than control plants. At that same period, drought plants had higher activity of SOD, APX and GR compared to control, which may indicate that the stress conditions induced higher enzymatic action of the antioxidant system. Therefore, these enzymes may be protecting cells against the increased production of ROS in this period.

Funding: Fapesp.

Key words: Superoxide Dismutase; CAM; Stress.

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Unraveling the *Tillandsia capillaris* complex: two species coexisting in sympatry?

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We performed phylogenetic analyses on different populations of eight taxa from the *Tillandsia capillaris* complex within subgenus *Diaphoranthema*, a polymorphic and (tetra-) polyploid group of highly specialized *Tillandsia*, distributed in mountainous arid regions from central Peru and Bolivia to northern and central Argentina and Chile. We aimed to investigate the evolutionary relationships and phylogeographic patterns among microtaxa of the complex with chloroplast and low-copy nuclear markers. We obtained sequences from almost 100 individuals from 69 populations of the complex in addition to more than 20 outgroup taxa. We edited and aligned two matrices, one made of three plastid markers (rpoB-trnC-petN, ycf1, matK-trnK) with 8100 bp, and another of the nuclear marker (phyC) with 1200 bp. We constructed Bayesian phylogenetic trees and only unrooted phylogenetic networks for the nuclear marker. For the phylogeographic analyses, chloroplast markers were used and haplotype networks using parsimony and Bayesian trees were rebuilt. Both datasets, the plastid and nuclear, indicated the existence of two main evolutionary lineages, which could be recognized in the two species proposed by Till in 1984: *T. capillaris* and *T. virescens*, this last group includes *T. kuehhasii* (described in 1995 as outside the complex). The nuclear data had less resolution but still two main groups can be distinguished; only a few individuals identified morphologically as “Capillaris” appear in the “Virescens” group, results that hint at hybridization. Regarding phylogeographic patterns, the distribution

of 63 cpDNA haplotypes in 69 populations also resulted in two main lineages co-occurring from northern Peru to southern Argentina and Chile, with high haplotype diversity and a set of haplotypes defined for each species. Despite the geographic similarities, including the presence of both species in some localities, we found some differences between the two main lineages. *T. capillaris* is molecularly and morphologically a homogeneous group and comprises populations with enough genetic variation to seemingly have the ability to expand into new habitats but maintain enough cohesion to be recognized as part of the same reproductive unit. *T. virescens* is genetically and morphologically more divergent with most of the forms growing in high and arid mountains and a recent subclade “Cordobensis” growing in low and moist montane habitats from central Bolivia and Argentina. Results suggest evidence of ancient speciation for the two main clades of the *T. capillaris* complex, and a complex evolutionary history involving geographic expansion, adaptive radiation and probably polyploidization events.

Funding: CONICET-UNC, MINCyT, BMFW.

Key words: *Tillandsia capillaris* Complex; Phylogenetics; Phylogeography.

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Leaf anatomy of *Guzmania* Ruiz & Pav. (Bromeliaceae) occurring in Ceará, Brazil

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In Brazil there are nine species of *Guzmania* Ruiz & Pav. (Tillandsioideae), three of them in the Northeast: *G. lingulata* (L.) Mez, *G. monostachia* (L.) Rusby ex Mez and *G. sanguinea* (André) André ex Mez. Ceará is the only state in the Northeast where the three aforementioned species exist. This work aimed to characterize the anatomical structures, in cross section, of leaves of *Guzmania* spp. occurring in Ceará, focusing on the characters useful for taxonomy. For the histological slides, central and margin of the middle third of the leaf and central and edge of the sheath region were selected, followed then by the usual procedures in plant anatomy; the permanent slides were stained with Alcian blue and Safranin (4: 1) and set in synthetic resin. Qualitatively, the species were compared by cluster analysis. We identified 59 states of discriminative characters standing out in the middle region of the leaf: the margin format, straight with projection toward the abaxial in *G. sanguinea* and sharp curved abaxial face in the others; cell number of trichomes stalk 2 to 3 in *G. sanguinea* and in the remaining 3 to 4; fiber cap on the vascular bundles in *G. lingulata* and *G. monostachia*; circular 1st order bundle in *G. lingulata* and elliptical in the other species; internerval distance more than 12 cells in *G. sanguinea* and up to 12 cells in other species. The sheath was considered the most distinctive feature: the number of vascular bundles in the margin were 2 in *G. lingulata* and 3 in the other species; water storage parenchyma facing the adaxial side is larger than that facing the abaxial in *G. lingulata*, in other species they are the same size; vascular bundles are equidistant in *G. lingulata* and *G. sanguinea* and slightly facing abaxial epidermis in *G. monostachia*. From the cluster analysis, it was verified that, anatomically, *G. lingulata* and *G. monostachia* are closer to each other than *G. sanguinea*.

Key words: Taxonomy; Leaf Characters; Tillandsioideae.

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Anatomy of the rhizomes of *Aechmea*, *Nidularium* and *Vriesea* (Bromeliaceae)

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Bromeliaceae is included in the basal clade of the Poales, and is characterized by having leaves in rosettes, water-storage tissue in the mesophyll, lepidote indumentum, and stigma spiral-conduplicate. The family is presently organized into eight subfamilies, 58 genera and about 3,248 species, which are predominantly Neotropical. There are few anatomical studies of their flowering rhizomes, as well as, no information that

allow an evaluation of their taxonomic potential. The rhizomes of five species, belonging to two subfamilies of Bromelioideae (*Aechmea pectinata* Baker, *Nidularium innocentii* Lem., *Nidularium procerum* Lindm.) and Tillandsioideae (*Vriesea incurvata* Gaudich, *Vriesea rodrigasiana* E. Morren) were analyzed, with three replicates. The species were collected in the Restinga area of Cananéia (25°01'35"S and 45°57'43"W), State of São Paulo, Brazil. The rhizomes were conserved in 70% ethanol, set in plastic resin and stained with Tolu-dine Blue after cross sections were obtained using a rotary microtome. All the species showed one-layered epidermis, internal cortex with conspicuous intracellular space, internal cortex with conspicuous intracellular spaces and presence of raphid idioblasts, and cortical and medullar vascular bundle. However, characters such as a thick epidermis, two or three cortical levels, presence or absence of a continuous fiber cylinder (= sclerotic cylinder) and intercortical roots aided in the differentiation of the species. The present study established that the anatomy of the rhizomes has taxonomic potential, making it possible to differentiate the species.

Key words: Bromelioideae; Tillandsioideae; Atlantic Rainforest.

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Morphological characters in Bromeliad phylogenies: why use them?

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The proposition of phylogenetic hypotheses based on molecular data brought a new paradigm for the evolution of living organisms at all taxonomic levels. In Bromeliaceae, since the first hypotheses were proposed, while using one or a few plastid markers, the monophyly of the family and the polyphyly of Pitcairnioideae were highlighted. Due to the large number of characters generated by such a set of data, unambiguous coding, speed and ease of access them, the external nodes and the major lineages could be identified. More recently, nuclear markers have brought more resolution to the trees. On the other hand, morphological data have been little used for such reconstruction mainly due to high levels of homoplasies, low resolution and low levels of support of the branches. However, molecular data have lower resolution in more external nodes of the trees due to lower nucleotide substitution rates. In this sense, the morphological data have shown to be powerful in more closely related taxa, both isolated or when combined with molecular data. This is evidenced in the phylogenies proposed for different genera of Bromeliaceae, where data from the external morphology (and in some cases leaf anatomy) were used, especially in *Lymania*, *Pitcairnia*, *Puya*, *Quesnelia* and *Vriesea*. Moreover, the inclusion of morphological data in the matrices allows the identification of synapomorphies that can be used in classification.

Key words: Morphological Data; Phylogeny; Classification.

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Molecular infrageneric relationships and genome size evolution in *Cryptanthus*

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The genus *Cryptanthus* is a distinct group within Bromeliaceae, comprising about 67 species endemic to eastern Brazil occurring in the Atlantic Forest, restinga, campos rupestres and caatinga. Due to high endemism, narrow geographic distribution, and loss of natural habitats, the genus presents many endangered species. Here we present the first molecular phylogeny of *Cryptanthus* based on AFLP analysis of 48 species together with genome size data in a phylogenetic framework. For the phylogenetic reconstruction the AFLP banding pattern was scored manually, considering presence or absence of each band at a particular position. Phylogenetic trees were generated using Maximum Parsimony (MP), Maximum Likelihood (ML) and Bayesian Inference. The genome sizes were estimated by flow cytometry and compared with a selected phylogenetic tree. In the phylogenetic tree generated, the subgenera *Cryptanthus* and *Hoplocryptanthus* appeared as non-monophyletic. The proposed morphological groups of the genus presented homoplastic characters, with the exception of the inconclusive group *Lacerdae*. Phylogenetic relationships

among the species remained ambiguous due to low resolution. Furthermore, some of the clades that received good support – mainly in the subgenus *Cryptanthus* – did not follow the morphological species delimitation. Atlantic Forest and campos rupestres showed a defined pattern of occupation within subgenus *Hoplocryptanthus*, which may also be evidence of the polyphyletic condition of this lineage at the habitat level. Regarding genome size, the 1C DNA values differed up to 2.13-fold among species (varying from 0.38 pg up to 0.83 pg). The analyses of the genome size distribution on the *Cryptanthus* phylogenetic tree matched the two defined major lineages. Therefore, there was a significant difference in DNA content between the subgenera *Cryptanthus* and *Hoplocryptanthus*. This study provided the first insights on the molecular phylogenetic relationships of the genus *Cryptanthus*. Our phylogenetic reconstruction indicated that both subgenera *Cryptanthus* and *Hoplocryptanthus* form a polyphyletic group. Several nodes within the genus *Cryptanthus* remained unresolved maybe because they concern recent evolution trends within Bromeliaceae. Moreover, the data at hand showed no clear phylogenetic position of the group *Lacerdæ* and it is necessary to improve the phylogenetic resolution to get a more conclusive answer about the position of this group. Regarding genome size, our findings suggest that various evolutionary forces and processes have shaped the observed genome size variation. Nevertheless, apparently the phylogenetic relationships were the most determinant factors of the observed divergence in genome size at the basal nodes.

Funding: CNPq, Capes, DAAD.

Key words: Bromelioideae; *Hoplocryptanthus*; Nuclear DNA Content.

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Influence of light, temperature and storage time on seed germination of *Vriesea crassa* Lindl. (Bromeliaceae)

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Seeds storage has been suggested as an efficient, long-term method for conservation of plant genetic resources. For successful seed conservation, knowledge of seed storage behavior is a prerequisite. This work studied the effects of storage time, light and temperatures on seed germinability of *Vriesea crassa* Lindl., from campos rupestres of Serra da Piedade, Brazil. Seeds were stored in a black plastic box at room temperature for 6, 12 and 18 months. The effects of light and temperature on germination of stored seeds were evaluated under constant temperatures (15, 20, 25 and 30°C) for both light (photoperiod 12-hr) and dark conditions. The germinability of seeds, after 6 mo., varied from 56.0% (15°C) to 76.0% (30°C) in light and from 36.0% (15°C) to 72.0% (20°C) in dark, while after 12 mo., it varied from 34.7% (15°C) to 62.7% (25°C) in light and from 21.3% (35°C) to 50.7% (25°C) in dark. However, the germinability of seeds after 18 months varied from 2.67% (15°C) to 8.0% (20°C) in light and from 5.3% (30°C) to 10.67% (25°C) in dark. Seed germination was not observed for *V. crassa* at 15°C in dark. The preliminary results obtained for these bromeliads suggest the storage of seeds does not contribute to their *ex situ* conservation.

Funding: FAPEMIG.

Key words: Storage Time; Seed Germination; *Vriesea crassa*

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Taxonomy of the genus *Cryptanthus* Otto & A. Dietr. (Bromeliaceae) in septentrional northeastern Brazil

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The genus *Cryptanthus* (Bromeliaceae) comprises 76 species distributed in eastern Brazil and growing as terrestrial and/or saxicolous plants. Its species occur in open or shaded areas of Atlantic Forest, Caatinga and Cerrado, especially in the physiognomy of the campos rupestres, from sea level to 2,000 meters of elevation.

The goal of this study was to produce a taxonomic treatment of the species of the genus *Cryptanthus* occurring in a section of the northeastern region of Brazil. The studied area includes the Brazilian states of Rio Grande do Norte, Paraíba, Pernambuco, Alagoas and Sergipe. Morphological study was based on collections of UFRN, JPB, UFP, IPA, HST and ASE herbaria. The species were identified using specialized literature, as well as by comparison with type specimens. In the present study ten species were found in the area as follows: *C. alagoanus* Leme & J. A. Siqueira, *C. bahianus* L. B. Sm., *C. burle-marxii* Leme, *C. diana* Leme, *C. felixii* J. A. Siqueira & Leme, *C. fosterianus* L. B. Sm., *C. pickelii* L. B. Sm., *C. reptans* Leme & J. A. Siqueira, *C. sergipensis* I.Ramírez and *C. zonatus* (Vis.) Beer. Herein a taxonomic treatment with an identification key, descriptions, illustrations and comments are presented.

Funding: Capes, CNPq, NSF.

Key words: Inventory; Floristics; Bromelioideae.

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Contrasting patterns in reproductive phenology of a monocarpic perennial bromeliad (*Puya nitida*) in a paramo of Colombia

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In the paramo of National Natural Park Chingaza, in the Eastern Mountain Chain of Colombia (04°44'15.1"N and 73°50'28.52"W), the reproductive phenology of bromeliad *Puya nitida* was studied at the population, patch, rosette and flower level, between February 2013 and February 2014, at 3,400 m altitude. The phenological patterns of duration, frequency and synchrony of the following phenological stages: floral primordia, buds, flowers, unripe fruits and ripe fruits were analyzed. At flower level, different phases according to differences found in the development and maturity of reproductive structures and nectar production were defined. *P. nitida* presented at the population level a synchronous phenological pattern, with an annual frequency and long duration (> 5 months) for fruit ripening, and an intermediate duration (4 months) for flowering. Fruit ripening coincided with the rainy season (May-July), and flowering showed a peak in the period of lower rainfall (November). At the patch level, the pattern was asynchronous, due to infertile, fertile and dead rosettes being recorded simultaneously. At the level of rosette, flowering was also asynchronous, with a duration of two months; one acropetal flowering type was recognized, finding flowers at various stages of development at the same time. At the flower level, development of reproductive structures was asynchronous, since temporal separation between these (dichogamy) was present, first mature female organs and then the male organs (protogyny). Seven floral phases were recognized; the full development lasted about 18 days, in which flower expanded phase was the longest one (4 days). The coincidence of maturation and seed dispersal with the rainy season promotes seed germination and the possibility of the establishment of seedlings of sexual origin. Likewise, different components of reproductive phenology of *P. nitida*, such as the coincidence of flowering with the dry season, flowering asynchrony between patches and inside rosettes, prolonged duration of expanded flower phase along with high-calorie nectar production (26 calories per flower), and the temporal separation of the floral reproductive organs may favor sexual reproduction mediated by pollinators.

Funding: Colciencia.

Key words: Paramo; Phenology; *Puya nitida*.

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Cytogenetic diversity, banding patterns and B Chromosomes in *Cryptanthus*

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Bromeliaceae are generally known for conservation in chromosome numbers, with a prevalence of 2n=50, considered the diploid level, and different ploidy levels or still associated dysploid numbers as 2n=48. The

genus *Cryptanthus* is an exception, since it presents lower numbers that are not multiples of the base number $x=25$ and is supposed to occupy a basal position within the family, being classified in the Bromelioideae subfamily. The unusual chromosome numbers $2n=34$ and 36 are observed and in some species the presence of B chromosomes has been reported. In the present study, chromosome counts are presented for 10 species and first reports using fluorochrome staining CMA/DAPI (Chromomycin A3 and 4',6-Diamidino-2-Phenylindole) and also FISH (Fluorescent *In Situ* Hybridization) with 45S rDNA probes is reported for the first time for the genus. The chromosome numbers found were: $2n=32$ in *C. dineae* and *C. marginatus*; $2n=34$ in *C. acaulis*, *C. bahianus*, *C. burle-marxii*, *C. aff. correia-araujoi*, *C. schwakeanus* and *Cryptanthus* sp. 09/2013, while B chromosomes were observed in *C. bahianus* ($2n=34 + 1-3B$) and *C. praetextus* ($2n=32 + 1-2B$). Fluorochrome staining was carried out in *C. burle-marxii*, *C. aff. correia-araujoi*, *C. diana* and *Cryptanthus* sp. 09/2013, evidencing CMA+/DAPI (GC-rich) terminal bands in two chromosome pairs and in one pair of *Cryptanthus* sp. 4972. FISH with 45S was performed in *Cryptanthus* sp. 4972 and (different from the observation with fluorochrome staining) revealed two pairs associated with the NOR regions. For some species genome sizes were also estimated: [i] $2n=34$: *C. acaulis* = 0.69 pg, *C. bahianus* = 0.38 pg, *C. burle-marxii* = 0.66 pg, *C. aff. correia-araujoi* = 0.83 pg, *C. schwakeanus* = 0.36 pg; [ii] $2n=32$: *C. diana* = 0.68 pg and *C. marginatus* = 0.70 pg; [iii] $2n=34$ with B-chromosomes: *C. praetextus* ($2n=32 + 1-2 B$) = 0.68 pg. No association between genome size and chromosome number was evident. Considering the low content of satellite DNA (based on CMA/DAPI staining) the observed genome size variation (from 0.36 up to 0.83) is probably due to dispersed repetitive sequences. Variation in chromosome number and also in the number of NOR-bearing chromosomes indicate that dysploidy (possibly associated with hybridization processes) may be important processes in the evolution of the genus. Financial Support: CNPq and CAPES.

Key words: DNA Content; B Chromosome; Chromosome Banding.

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Genetic diversity of *Bromelia hieronymi* Mez (Bromeliaceae) from humid chaco, Mato Grosso do Sul, Brazil

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The Bromeliaceae family have ca. 3,000 species, which are exclusively distributed in Americas. Bromeliads have been threatened by anthropic pressures due to predatory gathering and habitat destruction, being used for ornamental, forage, food and medical uses. Chaco or "Gran Chaco" is the term applied to seasonal vegetation, which is divided into an eastern sector, with a more humid climate, and a western, drier sector, whilst the submeridional lowlands include a large depression between these two areas. In Brazil, Chaco vegetation is restricted to the western border of Mato Grosso do Sul State (MS). In this study, we analyzed the genetic diversity of natural populations of *Bromelia hieronymi* Mez (Bromelioideae), which occur in humid Chaco, in Fazenda Retiro Conceição, Porto Murtinho city in the southwestern part of MS. We cross-amplified five primers described for other species: Ac01 and Ac25 (*Aechmea caudata*, Bromelioideae), VgA04 and VgC01 (*Vriesea gigantea*, Tillandsioideae), and PaD07 (*Pitcairnia albiflos*, Pitcairnioideae). Based on those microsatellite nuclear molecular markers, we described the basic genetic diversity parameters: number of alleles per locus (N), observed (HO) and expected heterozygosities (HE), index of fixation (FIS); and to test data for Hardy Weinberg equilibrium (HWE). We conducted DNA extractions using the CTAB protocol in a sample of 28 individuals from a natural population. We performed PCR reactions following the protocols previously developed by our research group. The PCR products were analyzed on 1% agarose gels stained with GelRed (Invitrogen) and visualized on an ultraviolet light transilluminator. In this natural population of *B. hieronymi*, we observed 5 to 10 alleles per locus, with a mean of 7.2 per locus. Our set of markers revealed high levels of genetic diversity, the HO and HE heterozygosities ranged from 0.277 to 0.958 and 0.627 to 0.911, respectively. On average HO and HE were 0.668 and 0.798, respectively. On average, FIS was low (0.166), and ranged from -0.053 to 0.651, deviations from HWE were observed only in the locus Ac01 ($p = 0.00001$). High levels of genetic diversity have been observed in many other bromeliad species from different

genera and biomes. A good set of polymorphic microsatellite loci are necessary to perform different studies concerning population genetics and conservation. The cross-amplification of microsatellite loci seems to be a great tool for research in natural populations of native bromeliad species, regarding different areas of knowledge, such as population genetics (genetic diversity and structure) and gene flow, which will produce very important data for the diagnosis of natural population viability and their conservation.

Funding: FUNDECT, CNPQ.

Key words: Bromelioideae; Conservation; Wetlands.

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Diversification of Bromelioideae in the Atlantic rainforest: a case study of *Aechmea* subgenus *Ortgiesia*

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Aechmea subgenus *Ortgiesia* comprises ca. 17 species which occur in Brazil, Argentina, Paraguay, and Uruguay. In Brazil the species are found in the south and southeastern regions, with most of them endemic to the Brazilian Atlantic rainforest. Interspecific relationships in *Aechmea* subgenus *Ortgiesia* are so far unclear and the lack of a phylogenetic framework has hampered the evaluation of morphological characters and their taxonomic value. Therefore, the objectives of the study were to reconstruct interspecific relationships in *Ortgiesia* based on AFLP (Amplified Fragment Length Polymorphism) data; to examine the evolution of key morphological characters and discuss their taxonomic value; and to explore biogeographic patterns in *Ortgiesia*. A total of 96 accessions belonging to 14 species of *Ortgiesia* were sampled and genotyped with 11 AFLP primer combinations. Our results confirmed previous studies based on a smaller sampling that *Aechmea* subgenus *Ortgiesia* is a natural group. Two main groups were recovered in the neighbor joining (NJ) tree, one formed by all accessions of *A. blumenavii* and *A. calyculata* and the second encompassing the remaining 12 species. In the second group four main subgroups were recovered: 1) formed by *A. gamosepala*, *A. gracilis*, and *A. recurvata*; 2) united *A. coelestis*, *A. organensis*, and *A. sp.*; 3) clustered *A. comata*, *A. kertesziae*, and *A. kleinii*; 4) mainly formed by *A. caudata* and *A. winkleri*. Mapping the petal color (yellow, blue, pink, or white), inflorescence type (simple or compound), and shape (ellipsoid, subcylindric, cylindric, or pyramidal) onto the NJ tree, revealed that yellow petals and simple inflorescences are the ancestral state in *Ortgiesia*. Compound inflorescences evolved at least three times independently, while high levels of homoplasy were observed for inflorescence shape. So, only petal color was confirmed as a valuable diagnostic character for *Aechmea* subgenus *Ortgiesia*. Biogeographic characterization indicated ombrophilous forest as the ancestral area for *Ortgiesia*. Most *Ortgiesia* occur between latitudes 25° and 27° S, in the southern region of the Brazilian Atlantic rainforest, which indicates this area as the center of diversity for the subgenus. In this study we presented the first insights into interspecific relationships in *Aechmea* subgenus *Ortgiesia*, revealing the main groups and subgroups of species, which thus now could be the focus of population genetics and phylogeographic studies to further shed light on their relationships. Ancestral state reconstruction analysis revealed different levels of homoplasy of inflorescence branching pattern, a character that is normally used as diagnostic in *Aechmea* subgenus *Ortgiesia*. Petal color, another character used in species identification of *Ortgiesia*, was confirmed as valuable by our analysis. The biogeographic pattern of *Ortgiesia* indicates that the subgenus diversified at the southern portion of the Brazilian Atlantic rainforest and this process may have been affected by Pleistocene climatic oscillations, leading to geographical isolation and thus promoting allopatric speciation.

Funding: CNPq, FAPERGS, FAPESP.

Key words: Interspecific Relationships; Aflp; Character Evolution.

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Phylogeography and population differentiation of seven *Aechmea* species from the Atlantic Rainforest

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Aechmea subgenus *Ortgiesia* has ca. 17 species, which possess yellow, blue, pink, or white petals. Yellow-flowered *Ortgiesia* are almost all restricted to the southern region of the Brazilian Atlantic Rainforest, where some of them overlap in geographical distribution. They blossom mainly during the autumn (March – June), some species share pollinators, and taxa boundaries are difficult to identify, especially where they are found in sympatry. Therefore the objectives of the present study were to examine if morphologically identified species are genetically differentiated; to investigate the occurrence of hybridization between taxa; and to quantify genetic diversity and its distribution to identify areas of value for conservation. Samples from the seven yellow-flowered *Ortgiesia* were collected in 26 populations throughout the entire geographical distribution of the species. Two plastid regions (rpl32-trnL and rps16-trnK – cpDNA) and the nuclear gene phyC were sequenced for 204 and 179 individuals, respectively. Thirty-two haplotypes were found for all seven *Ortgiesia* using cpDNA, of which three were shared among species. This haplotype sharing was not geographically structured. Only one possible ancient hybridization event was detected by our cpDNA analyses, which involved *A. comata* and *A. caudata*. Less genetic structure was observed with phyC, with 11 out of 54 haplotypes shared among all the seven *Ortgiesia*. Considering all the populations sampled, the highest number of haplotypes were found at latitudes of ~27° S, the northeast region of the Brazilian state of Santa Catarina, for both cpDNA and phyC data. Our results suggest that the haplotype sharing found for the seven yellow-flowered *Ortgiesia* is probable due to the persistence of ancestral polymorphism. However, localized hybridization events may also have occurred during the diversification of the group. The results found in this study, together with morphological and ecological data, support the contention that five of these species are distinct lineages (*A. blumenavii*, *A. calyculata*, *A. caudata*, *A. kleinii*, and *A. winkleri*). *Aechmea comata* and *A. kertesziae* did not present clear genetic boundaries and also do not present clear morphological characters or ecological data that can be used to differentiate them. Therefore more studies involving these species are needed to identify if hybridization between them is going on, as individuals with intermediate morphology are observed, or if they represent only one taxon. The northeast region of Santa Catarina state (latitude of ~27° S) was identified as harboring the highest levels of genetic diversity, and thus is of conservation value for yellow-flowered *Ortgiesia*.

Funding: CNPq, FAPERGS, FAPESP.

Key words: cpDNA; Haplotype Network; phyC.

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Microsatellite data reveal unexpected patterns on *Encholirium spectabile* population genetic structure

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Brazilian dry forest Caatinga occupies most of the Brazilian Northeast region, which is known for its extreme environmental conditions, supporting a distinct flora composition as a result of influences from soil, altitude and climatic factors. In this region *Encholirium spectabile* (Bromeliaceae, Pitcairnioideae) presents striking morphological variation, mainly concerning populations from the northern portion of the ‘Espinhaço’ Range that include individuals with short pedicels, differing from the long pedicel observed in specimens found mostly on the Caatinga depression, Borborema plateau and Raso da Catarina. The species is almost exclusively found on rocky outcrops and less frequently on shallow and rocky soils, conferring an island-like geographic distribution. In order to assess the population genetic structure of *E. spectabile*, eight nuclear and four chlo-

roplast microsatellite markers were applied to 240 individuals collected from 21 Caatinga populations. The results were analyzed using the statistics programs STRUCTURE, ARLEQUIN, HAPLOTYPE, GENEPOP and FSTAT. In general terms, populations presented patterns in accordance to other bromeliads from rocky outcrops (F_{st} nuclear = 0.3604; F_{st} chloroplast = 0.9055). No correlation between genetic and geographic distances was recognized. For the genetic nuclear data, Bayesian clustering by STRUCTURE revealed two groups ($K=2$), clustering populations from western Caatinga and Borborema plateau together and isolating them from the central-eastern limit of Raso da Catarina, contrasting to the expectations considering morphological features. Similar patterns were also observed for chloroplast data, where shared haplotypes occurred between northern and southern populations whereas central-eastern populations presented distinct haplotypes. Limited gene flow between these regions was also predicted ($Nem \sim 0.1182$). The differentiation between these two areas was clear when population pairwise F_{st} values were compared, since values between these two groups were higher. It was also possible to compare chloroplast and nuclear genetic structure, revealed by AMOVA, uncovering a more efficient nuclear gene flow (5.1 times) than chloroplast, suggesting that pollination plays the main role in gene flow. The dissimilarities between clusters were not expected for this species, as the most important morphological differentiation is between north and south. Since this species presents other variable morphological features, the circumscription of subgroups within this species remains difficult. In this case, environmental factors seem to be responsible for speciation in progress, whereas the observed genetic structure suggests that these two groups represent cryptic species.

Funding: CAPES/DAAD/PROBRAL, FACEPE, CNPq.

Key words: *Encholirium*; Ssr; Cpsr.

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Morphometric analyses of the *Dyckia saxatilis* complex (Bomeliaceae)

Guarçoni, Elidio¹ & Costa, Andrea²

Dyckia is the second largest genus of the subfamily Pitcairnioideae, with 163 species occurring throughout Brazil and neighboring countries (Argentina, Bolivia, Paraguay and Uruguay). Brazil retains 140 species, of which 127 are endemic and mostly grow in cerrado areas (80 spp.). Recent molecular work split the genus into four ecological groups, although infrageneric molecular data are presently poorly defined, with paraphyletic or polyphyletic groups. This situation is probably due to taxonomic problems in the delimitation of its species, as *Dyckia* shows only a limited range of morphological variations, contributing to a number of uncertainties in the delimitation of its species. While some species of *Dyckia* can be easily distinguished morphologically, others require additional tools to assist in their delimitations due to the absence of clear morphological discontinuities between some groups of species in terms of their vegetative and reproductive characters. In this context, analyses of morphological variations in natural populations have proven useful in distinguishing species from different genera in the family. In order to delimit the species of the *Dyckia saxatilis* complex, we sampled 256 individuals representing 14 populations occurring in areas of rocky field vegetation in Minas Gerais State. Discriminant analysis of 30 morphological variables showed clear distinctions between species occurring in the Iron Quadrangle and those occurring in the central region of the Espinhaço Range. The populations occurring in the municipalities of Ouro Preto (type locality of the species) and Mariana were separate from the others. The lengths and distances between the leaf spines and the extent of filament connation were the variables that most contributed to grouping the populations. Eleven species (two of which were restored from synonymy with *Dyckia saxatilis*, and two new ones) were identified. These results demonstrate that morphometric analyses associated with intensive fieldwork are fundamental to defining species delimitations.

Key words: Taxonomy; Poales; Discriminant Analysis.

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Leaf anatomy of species of the *Dyckia* (Bromeliaceae): *D. saxatilis* complex*Guarçoni, Elidio*¹; *Azevedo, Aristéia*² & *Costa, Andrea*³

Dyckia Schultes & Schultes f. comprises 163 species occurring in all regions of Brazil as well as neighboring countries such as Argentina, Bolivia, Paraguay, and Uruguay. One hundred and forty species have been recorded in Brazil, 127 being unique to that country and distributed in five phytogeographical regions: Cerrado (80 species), Atlantic Forest (36), pampa (20), Caatinga (10), Amazon (4), and Pantanal (3). The genus shows uniformity of its floral characters but intraspecific variability of its vegetative characters – making the delimitation of species and their correct identification difficult. The hypothesis of a recent explosive radiation of *Dyckia* may explain some of the difficulties encountered in distinguishing consistent morphological characters that are taxonomically useful for distinguishing its species, even with complete and fully documented specimens in hand. We analyzed 15 populations with a total of 11 species plus an additional morphospecies of the genus *Dyckia* occurring in different phytogeographical formations. The vouchers were deposited in the VIC herbarium at the Federal University of Viçosa, in Minas Gerais State, Brazil. The anatomical analyses of the mid-regions of the leaves from at least three individuals of each species in each natural population, which were collected in the outer region of the rosette; these were fixed in FAA50 and preserved in 70% ethyl alcohol. Cross-sections of the mid regions of the leaf blades were made and the sections clarified using a 20% sodium hypochlorite solution, washed, and subsequently stained with 1% astra blue and 1% aqueous safranin. Semi-permanent slides were prepared using 50% glycerin as a mounting medium. Observations and photographic documentation were made using a light microscope (Olympus model AX70TRF, Olympus Optical, Tokyo, Japan) with a U-Photo system with a coupled digital camera (Zeiss AxioCam HR3, Zeiss, Göttingen, Germany) using Axion Vision software, at the UFV Plant Anatomy Laboratory. The leaf structures of all of the *Dyckia* taxa analyzed in this study followed the general pattern described for the family, with: peltate scales on the leaf surfaces (sometimes both surfaces), epidermal cells with silica bodies on both sides, stomata restricted to the abaxial surface, the hypodermis differentiated into mechanical and water-storage tissues on both leaf surfaces, and vascular bundles distributed over a range. Some taxa showed variations in these anatomical structures, indicating important anatomical characters that can be used for distinguishing the species: the number of sclerenchymatous hypodermal layers, the positions of the chlorophyll parenchyma cells, type of channel cells in the aerenchyma, idioblast positions, arrangements of the vascular bundles, undulating adaxial and abaxial surfaces, numbers of cells in the pedicle, and the proportion of the adaxial face in relation to the abaxial face.

Key words: Poales; Anatomy; *Dyckia*.

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Tillandsia usneoides*: why is the species so morphologically variable?Heller, Sascha; Paule, Juraj & Zizka, Georg*

Tillandsia usneoides is the most widely distributed species of the family Bromeliaceae (Poales). It ranges from the southeastern United States to Argentina and Chile. *Tillandsia usneoides* grows as an epiphyte with missing aerial roots and is dispersed by seeds as well as by fragments of the plant. Within the species, striking morphological differences can be observed as far as size characters are concerned and previous authors recognized up to five varieties or subspecies. Morphotypes have been shown to be stable in cultivation while growing under same conditions, excluding phenotypic plasticity. In order to investigate possible reasons for the variation, the relative genome size of 75 specimens covering the whole distribution range was measured and combined with morphological, distributional and climatic data. Significant variation in the relative genome size which corresponded to the morphological differences (i.e. five morphotypes) and reflected the north - south distribution gradient was detected. However, observed variation could not be attributed to any particular climatic factor.

Funding: DFG, DAAD.

Key words: Genome Size; Morphotype; Geographical Distribution.

Goethe University & Senckenberg Research Institute, Germany.

Molecular contribution to the taxonomical reorganization of the eu-Bromelioideae

Heller, Sascha¹; Leme, Elton²; Forrza, Rafaela Campostrini³; Paule, Juraj¹ & Zizka, Georg¹

The Bromelioideae is the second largest subfamily with more than 900 species in 33 genera within the family Bromeliaceae. Based on molecular and morphological evidence the species are divided into the early diverging lineages and the eu-Bromelioideae. The genera in the early diverging lineages are well defined with good phylogenetic support. On the contrary, many eu-bromelioid genera, originally defined on the basis of morphological characters, are para- or polyphyletic. Furthermore, patterns and processes behind the bromelioid diversity are only poorly understood. We present phylogenetic trees based on a taxonomically comprehensive sampling with up to 650 accessions representing more than 550 bromelioid species out of 32 genera. The phylogenetic analysis (maximum likelihood performed with RAxML) is based on nuclear (Agt1, ETS, PHYC) and plastid (matK, ycf1) data. The para- and polyphyly of currently recognized genera, with emphasis on the species rich and highly polyphyletic genus *Aechmea*, are presented. *Aechmea* species were found to be scattered all over the eu-bromelioid diversity. We identified 30 monophyletic groups and discussed them from the biogeographic and morphological point of view in order to contribute to a new generic circumscription. For instance, so called *Portea-Gravisia*-group is composed by *Aechmea*, *Canistrum* and *Portea* species, but the phylogenetic support and biological features such as facultative epiphytism, restriction to the Atlantic Forest and polyporate pollen grains suggest a separate taxonomic rank. Furthermore, the highly variable nuclear marker Agt1 is presented as a potential genetic barcode for identification of eu-bromelioid genera and species.

Funding: Goethe University, Senckenberg Institute, DAAD, DFG.

Key words: Phylogeny; Bromelioideae; *Aechmea*.

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Patterns of hybridization in three sympatric species of *Dyckia* (Bromeliaceae) endemic from southern Brazil

Hirsch, Luiza; Zanella, Camila; Melo, Camila & Bered, Fernanda

Hybridization is a powerful evolutionary force in the process of diversification of lineages and speciation. The knowledge of this force and interspecific gene flow patterns which potentially occur in correlated species is needed to understand the process of maintenance of the different species. Natural hybridization between sympatric species can be relatively common and provides a valuable tool to study the evolution of reproductive isolation barriers. Species that have undergone recent adaptive radiation are highly informative to study reproductive isolation and to better understand the role of drift and natural selection in the cohesion of species. *Dyckia* has around 145 species and is the second largest genus of Pitcairnioideae. In Rio Grande do Sul, the southernmost Brazilian Federal state, 28 species are described, of which 17 are potentially endemic. The aim of our study is to understand mechanisms of speciation, reproductive isolation and species cohesion in three sympatric endemic *Dyckia* species, *D. elisabethae*, *D. hebbingii* and *D. choristaminea*. *Dyckia elisabethae* is currently classified as “vulnerable”, *Dyckia hebbingii* and *Dyckia choristaminea* as “endangered”. To evaluate the pattern of interspecific gene flow, we sampled two sympatric populations and one allopatric population of *D. hebbingii*, totaling 250 individuals. We performed DNA extraction from all samples and we achieved sixteen nuclear microsatellite loci successfully tested for the three populations. Three regions that were previously sequenced exhibited polymorphisms among all three species, suggesting differences among them. This research will elucidate the role of such differences in hybridization and the dynamics of isolation barriers. Our prospective plan is to collect another sympatric population and two allopatric populations, referring to *D. elisabethae* and *D. choristaminea*, in order to be used as reference samples of pure individuals of each species.

Funding: CNPq, FUNDECT, PPGBM-UFRGS.

Key words: Bromeliaceae; Endemism; Microsatellites.

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Nectar and pollinators in species of *Puya*

Hornung-Leoni, Claudia¹; González-Gómez, P.^{2,3} & Troncoso, A.³

Five Andean *Puya* species (*P. alpestris*, *P. chilensis*, *P. coerulea*, *P. raimondii* and *P. venusta*) were studied to determine the relationship between their avian visitors and plant morphology and nectar characteristics. Our results showed significant relationships between nectar concentration, presence of sterile apex and avian pollinator species. In contrast, nectar composition was not related to the frequency of avian visits. We found that *Puya* species are mainly visited by specialist nectarivorous birds such as hummingbirds (e.g., *P. coerulea* and *P. venusta*), lacked a sterile apex and produced high nectar concentration in low volumes. In contrast, species mainly visited by generalist passerine birds (e.g., *Puya chilensis* and *P. alpestris*) were characterized by the presence of a sterile apex and production of highly diluted nectar in large volumes. In a mono-specific group we found that *P. raimondii* produces highly concentrated nectar in large volumes, and its flowers were visited by hummingbirds and passerine birds. We found no effect of nectar composition on avian visits. Our study highlights the interplay between morphological traits, nectar characteristics and ecological framework to explain specialist and generalist bird pollination systems.

Key words: Pollination; *Puya*; Hummingbirds.

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Morphometric analysis of natural populations of the *Billbergia amoena* (Lodd.) Lindl. complex (Bromeliaceae, Bromelioideae)

Jacques, Suara Souza & Costa, Andrea

Billbergia amoena (Lodd.) Lindl. belongs to *B.* subg. *Billbergia* Thunb.; this is a monophyletic group that includes many species of difficult delimitation. Featuring the broadest geographical distribution among all of the subgenera, and the consequent morphological variation and plasticity, this species has 16 synonyms and seven varieties. Herbarium collections have indicated wide morphological variation. The fact that there are other closely related taxa reinforces the importance of testing their delimitation. In this context, the study aimed to evaluate the existing variation between varieties through morphometric study in natural populations. We sampled 110 individuals from six natural populations, including the location of some types. 29 variables were used, four vegetative and 25 reproductive. Were performed the Kruskal-Wallis test (KW), Principal Component Analysis (PCA) and discriminant analysis (DA), with the software STATISTICA 8.0 and PAST v. 2.04. Normality and homoscedasticity data were tested respectively by the Shapiro-Wilk and Levene tests. The result of the comparison of the characters in the KW test showed significant differences among the natural populations. In PCA, the first three components explained 62% of variation between individuals. Analyzing components 1 and 2, the samples form two groups, separating the population of Santa Teresa (ES2) from the others that overlapped. With DA performed, the samples form four groups, there is a clear separation of the ES2 population. The second group by the population of Praia das Neves (ES1), the third the population of Marica (RJ1), and the fourth by the other populations. The results of these analyses point out characteristics that were thought to be important for the separation of the populations of Espírito Santo from those of Rio de Janeiro. The ES2 population differs from the others both in vegetative characters as well as reproductive ones. The ES1 population is distinguished by reproductive characters. The populations of Rio de Janeiro are still together in the analysis. The populations sampled to date suggest the existence of two species and the maintenance of five varieties.

Funding: CNPq.

Key words: Bromeliaceae; Morphometric; Taxonomy.

Museu Nacional, Universidade Federal do Rio de Janeiro, Brazil.

Identification of diazotrophic bacteria associated with leaves of the epiphytic bromeliad *Guzmania monostachia*

Kleingesinds, Carolina Krebs¹; Rodrigues, Maria Aurineide¹; Aidar, Marcos Pereira Marinho² & Mercier, Helenice¹

Since epiphytic plants are subjected to intermittent supply of water and nutrients, association with microorganisms may be very important for the survival of these species. This study aimed to verify whether nitrogen-fixing (diazotrophic) bacteria are present in distinct leaf portions of the epiphytic-tank bromeliad *Guzmania monostachia*, and if they are in the exterior or interior of the leaves. Fully expanded leaves were collected from adult plants grown in both their natural environment (CE, Brazil) and in the greenhouse of the Laboratory of Plant Developmental Physiology (Biosciences Institute, University of São Paulo). All harvested leaf blades were sliced into three portions: basal (comprising the leaf sheath), middle, and apical (two halves of the remaining leaf blade). The plant material was sonicated to separate bacteria from the leaf surface (epiphytic bacteria). Subsequently, all leaf samples were submitted to an antiseptic procedure for final maceration and further investigation of bacteria present inside the plant tissues (endophytic bacteria). Four different media (NFb, JNFb, LGI e LGD) were used for bacterial growth. All these culture media are free of reduced nitrogen. Selection of diazotrophic bacteria was assessed by acetylene reduction assay (ARA). The isolates were identified by 16S rRNA and sequencing with the primer pair 27F (5' to 3' GAGAGTTT-GATCCTGGCTCAG) and 1512R (5' to 3' TACGGTTACCTTGTTACGACTT). The results revealed two genera that are usually used in agriculture: *Burkholdeira* and *Paenibacillus*. The only culture medium which provided growth from both the natural habitat and the greenhouse was the JNFb. In addition, a higher number of ARA+ bacteria were isolated from the natural environment than the greenhouse and in both circumstances we found larger number of colonies in the basal portion. These results suggest that diazotrophic bacteria are located mainly in the base of *G. monostachia* leaves, where they might fix atmospheric nitrogen and provide it to this bromeliad mainly during periods of nutrient starvation.

Funding: CNPq, Fapesp.

Key words: Nitrogen-Fixing Bacteria; Bromeliaceae; 16s rRNA.

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The group *Vriesea platynema* Gaudich. (Bromeliaceae: Tillandsioideae) in Paraná State, Brazil

Kowalski, Vanessa Koza¹ & Tardivo, Rosângela Capuano²

Vriesea Lindl. is the second largest genus in Tillandsioideae with 281 species, distributed mainly in South America. In Brazil, it occurs in such biomes as the Cerrado, Amazon, Caatinga, Pampa but mainly in the Atlantic Rain Forest, the major center of diversity for the genus. Currently, *Vriesea* is divided into two sections: *Vriesea* and *Xiphion*. *Vriesea* sect. *Vriesea* is characterized by diurnal flower anthesis, red or yellow floral bracts, ligulate petals and exerted stamens. *Vriesea* sect. *Xiphion* presents night anthesis, green or brownish floral bracts, obovate petals and included stamens. *Vriesea* sect. *Xiphion* is the largest section of the group *V. platynema*, with 41 species, occurring from Central America to Argentina. The high polymorphism of this group leads to misidentification of the lower taxa. This study aims to describe the *V. platynema* group occurring in Paraná, to map its geographical distribution and to indicate the conservation status of the lower taxa. This study was based on specialized bibliography, herbarium specimens and botanical expeditions conducted all over the phytogeographical units of the Atlantic Forest in Paraná State. Three taxa are recognized to Paraná State: *Vriesea platynema* var. *variegata* and *V. tijucana*, found in Dense Ombrophilous Forest on the Atlantic Coast; and *V. platynema* var. *platynema*, found in Mixed Ombrophilous Forest on the Second and Third Parana Plateau. The morphology of the *V. platynema* group is characterized by sigmoid stamens, flattened dorsoventrally and extended towards the apex. The lower taxa can be differentiated by the number of flowers: 40–70 in *V. tijucana*; up to 36 in *V. platynema* var. *platynema* and *V. platynema* var. *variegata*.

Vriesea platynema var. *platynema* has leaves irregularly arranged, winding, green, with dark green transverse lines, and urceolate corolla. On the other hand, *V. platynema* var. *variegata* has green leaves, with white longitudinal striations on the adaxial surface and purple-reddish macules on the apex, and a campanulate corolla. According to the IUCN criteria, *V. platynema* var. *platynema* is not endangered, whereas *V. platynema* var. *variegata* and *V. tijucana* are endangered (EN).

Key words: Parana Flora; Morphology; *Vriesea*.

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Growth *in vitro* and *ex vitro* of bromeliad *Alcantarea imperialis* (Carrière) Harms in different concentration of ammonium

Kurita, Flavia Maria Kazue; Santos, Soraya Graciliano & Tamaki, Vívian

Alcantarea imperialis is a bromeliad used in landscaping and is endemic of Serra dos Orgãos/RJ/BR, making conservation measures are necessary, for which *in vitro* and *ex vitro* cultures could be tools. An important aspect of *in vitro* culture is the mineral supply of the culture medium. Nitrogen (N) is the main component of amino acids, nucleic acids, chlorophyll and coenzymes and one of its sources is ammonium (NH_4^+). There are no studies for this plant comparing *in vitro* and *ex vitro* culture with different NH_4^+ concentrations. This study aimed to compare the *in vitro* and *ex vitro* growth of plants of *A. imperialis* with four NH_4^+ concentrations. Plantlets germinated *in vitro* and *ex vitro* were transferred to the culture conditions (culture medium or *Pinus* sp. bark substrate). The culture medium used was Murashige and Skoog (MS) modified with different concentrations of NH_4^+ (5, 15, 30 and 60 mM), supplemented with 3% sucrose, 0.1 mg L⁻¹ of thiamine-HCl, 100mg L⁻¹ of myo-inositol and 5 g L⁻¹ of agar with pH adjusted to 5.8. Each *in vitro* treatment had five vessels of 10 seedlings each. In the *ex vitro* conditions, plants were fertilized weekly with of MS medium solution (50 ml) described above (but without sucrose and agar). Cultures were maintained in a growth chamber at 26±2°C and 30 μmol m⁻² s⁻¹ light photons provided by cold white lamps during 12 hours of light regime. After three months, length of the longest root, number of leaves and length of the shoots were determined, besides amounts of fresh and dry weight of both roots and shoots, as well as the quantity of photosynthetic pigments in both group of plants. The results showed that the *in vitro* cultivated plants grew more than the *ex vitro* ones, but at a concentration of 60 mM ammonium plants did not show favorable growth *in vitro*. In conclusion, ammonium at high concentrations proved to be toxic to this species under *in vitro* culture.

Funding: Fapesp.

Key words: Bromeliaceae; Nitrogen; Threatened with Extinction.

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Endogenous ammonium and nitrate in *Alcantarea imperialis* cultivated *in vitro* at different concentrations of nitrate

Kurita, Flávia Maria Kazue & Tamaki, Vívian

Alcantarea imperialis (Carrière) Harms is an endangered bromeliad used in landscaping and is endemic of the Serra dos Orgãos/ RJ, so conservation measures are necessary. *In vitro* culture may be a tool in nutrition studies and thus for the preservation of the species. An important aspect of this technique is the mineral supply of the culture medium. Nitrogen (N) is the main component of amino acids, nucleic acids, chlorophyll and coenzymes. A major source of nitrogen found in the environment is the nitrate (NO_3^-), which is assimilated into amino acids and proteins. This study aimed to evaluate the endogenous ammonium and nitrate content in *A. imperialis* cultured *in vitro* with different concentrations of nitrate. Seedlings (80 seedlings) germinated *in vitro* for 30 days were transferred to culture media with different concentrations of nitrate ranging from

5, 15, 30 and 60 mM (5 seedlings per vessels of 4 vessels per treatment) and after six months evaluated the endogenous content of ammonium and nitrate and photosynthetic pigments, as well biometric parameters. The results showed that endogenous ammonium content in plants grown in different treatments did not show statistical differences, however the endogenous nitrate content of plants grown in 60 mM showed a significant increase of this compound, about twice as much compared to plants grown at the other concentrations. In this treatment, however, the plants showed the lowest average biometric parameters and photosynthetic pigments, suggesting some nitrate toxicity at this concentration. *In vitro* cultivation plants is recommended for *A. imperialis* lasting six months with no more than 30 mM nitrate.

Funding: Fapesp.

Key words: Bromeliad; Conservation; Nutrition.

Instituto de Botânica de São Paulo, Brazil.

Genomics of speciation and species cohesion among tillandsioids in Neotropical mountains

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The adaptive radiation of bromeliads is one of the most diverse and enigmatic of the Neotropics. The Tillandsioideae subfamily represents a pertinent system for studying adaptation, speciation, and clade diversification in Neotropical plants. Our main research aim is to unravel the drivers and limits of diversification at micro-evolutionary time scales, i.e. at the level of diverging populations, species, and species complexes. Tillandsioid taxa were sampled along elevation gradients of various Central and South American mountain regions, including Mexico, Ecuador and Brazil. Common species (geographic replicates) and local endemics were defined based on their natural distribution ranges and ecological preferences. Geographic replicates were chosen preferentially to disentangle the likely evolutionary mechanisms responsible for speciation and species cohesion. A genomic approach, combining RNA-sequencing and restriction site Associated DNA sequencing (RAD-seq), will generate genetic data for both neutral and non-neutral regions of the genomes of these taxa. The genomic information yielded by this approach will be employed to characterize gene flow and allelic diversity within and among parapatric tillandsioid populations and species along elevation gradients. Great emphasis will also be given to analyses of population divergence to shed light on the genetic mechanisms of speciation and species persistence. Finally, parallel genotypic and phylogenetic patterns of evolution will be evaluated jointly and ecological correlates of population and species divergence will be identified to provide a better understanding of the diversification process. Current results and key points from this research will be presented, including spatial distributions of the sampled taxa, proof-of-concept data from RAD-seq, and available experimental/sampling designs towards reaching our research goals.

Funding: Swiss National Science Foundation.

Key words: Clade Diversification; Population Genetics; Tillandsioideae.

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Phylogeography, population genomics and adaptive variation in the *Pitcairnia lanuginosa* (Bromeliaceae) species complex

Leal, Bárbara Simões Santos; *Chaves, Cleber Juliano Neves* & *Palma-Silva, Clarisse*

The understanding of the processes leading to the diversification of lineages and the appearance of new species is particularly important in the world's biodiversity hotspots. Genotyping-by-Sequencing (GBS) approaches, such as restriction site associated DNA sequencing (RAD-Seq), have shown promise for studying such processes, even

when it involves non-model organisms. In this work, we will use the *Pitcairnia lanuginosa* complex (Bromeliaceae), which is widely distributed in tropical regions of South America, as a model to study the processes underlying the diversity in the Neotropics. First, we aim to sample populations covering most of species' geographic range and, employing nuclear and plastid markers, to provide a first insight into the phylogeographic structure and evolutionary history of the *P. lanuginosa* complex. We will also employ a large amount of SNPs markers isolated by RAD-Seq, as well as ecological niche modeling (ENM), to determine the role of historical demographic processes on the neutral and adaptive genetic variation and to test the boundaries between species of the group. In an attempt to aggregate essential biological knowledge to interpret the patterns of diversification, we will also collect data about morphological variation and reproductive biology, which are still unknown for the species of the complex. Our results about the divergence among the lineages of the *P. lanuginosa* complex occupying the Central Andean Yungas and the Brazilian Cerrado will provide insights about the patterns and processes that govern the high biodiversity of the South American continent. This study will also assist the understanding of demographic responses of herbaceous plants adapted to drought stress during the Quaternary climatic oscillations.

Funding: Fapesp.

Key words: Phylogeography; Next Generation Sequencing; Diversification; Species Delimitation.

Universidade Estadual Paulista Julio de Mesquita Filho, Brazil.

The contribution of microorganisms and metazoans to mineral nutrition in tank bromeliads

Leroy, Celine¹; Carrias, Jean-François²; Cereghino, Regis³ & Corbara, Bruno³

One critical challenge for plants is to maintain an adequate nutrient supply under fluctuating environmental conditions. This is particularly true for epiphytic species that have limited or no access to the pedosphere and often live in harsh climatic conditions. Bromeliads have evolved remarkable adaptations (e.g., terrestrial or epiphytic and tank or atmospheric forms, and C3 or CAM photosynthetic pathways) that facilitate water and inorganic and organic nitrogen (N) uptake to cope with their various, diverse and distinct habitats. We provide evidence, by the use of N-stable isotope, that bromeliads have also evolved multi-faceted nutritional strategies that allow them to respond to fluctuations in natural N supply. Bromeliads differ significantly in their reliance on insect-assisted nutrient supply, and there is a clear increase in insect-assisted N supply for tank-bromeliads compared to atmospheric ones. Bromeliads have developed mutualistic associations with many different and functionally diverse terrestrial and aquatic microorganisms that contribute substantially to their mineral nutrition and, thus, their fitness and survival. In addition to the autonomous pathway, the main strategies used, separately or combined, by bromeliads to acquire nutrients are bacterial and fungal microbiota-assisted N, digestive mutualisms, and protocarnivorous and myrmecotrophic pathways. We believe that further research is needed to study the nutritional ecology of bromeliads, and, more generally, epiphytic taxa, especially in a context of climate change.

Key words: Insect-assisted Nutrients; Digestive Mutualism; Myrmecotrophy.

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Speciation genomics and the (dis-) continuum between speciation and radiation in bromeliads

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Rapid recent developments in ecological and evolutionary genomics have facilitated novel insights into the mechanisms of population divergence, speciation, and species diversification. These issues are not only considered to be among the most relevant topics in biology of our time, they are also of great relevance beyond

fundamental science, because research in these topics is expected to further our understanding of the origin, maintenance, and functioning of biological diversity, which is a prerequisite for informed conservation and management efforts. A powerful approach to tackling the complex issues surrounding diversification is to explore genomic and biological data along the entire ‘speciation continuum’, that is, the transition from continuous variation to differentiation among local populations, ecotypes, and biogeographic lineages, and on to complete speciation and post-speciational divergence. To date, most in-depth knowledge of these topics stems from a small number of model groups. Bromeliads represent a highly relevant study group for tackling these important issues among Neotropical plants, because (1) they represent a highly species-rich adaptive radiation present throughout the New World tropics, (2) many species and clades within the family appear to exhibit genomes of tractable complexity and size, (3) numerous well understood adaptive traits and key innovations have experienced rapid evolution within the family, (4) bromeliads are involved in diverse plant-animal interaction networks which renders them ecologically important foundation or keystone species. We will commence our talk by sketching current key concepts and approaches in speciation genomics, using published examples from our group. This will include ecological and evolutionary genomic work on ‘model taxa’ such as hybridizing temperate forest trees (*Populus* spp.; poplars, aspens, cottonwoods) and other plant groups from the world’s biodiversity ‘hotspots’. From there, we will explore recently published and unpublished work on bromeliads by our group, focusing primarily on the speciation continuum in Brazilian species of *Alcantarea* and *Vriesea* in the subfamily Tillandsioideae. We will close by presenting a recently initiated research consortium that aims to close the gap that currently exists between micro-evolutionary studies of the speciation continuum and macro-evolutionary work on entire species radiations. Closing this gap is essential for achieving a more complete understanding of the dynamics and functioning of biological diversity. Bromeliads represent an ideal study group for focusing this research, but their extraordinary species richness and complexity represent challenges that can only be met by a collaborative, coordinated effort.

Funding: Swiss National Science Foundation.

Key words: Speciation Continuum; Evolutionary Genomics; Radiation.

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Systematics of *Orthophytum* and related genera

Louzada, Rafael Batista

Over the last three decades authors have considered *Orthophytum* and *Cryptanthus* as closely related genera based mainly on morphology, including traditional taxonomic methods or under a morphological phylogenetic analysis. Recently, the genus *Lapanthus* Louzada & Versieux was described to accommodate two striking species which were previously placed under *Orthophytum* and *Cryptanthus* -with an additional species added to the genus later. The three genera occur exclusively in eastern Brazil. *Orthophytum* and *Cryptanthus* can be found growing in the Atlantic Forest, Cerrado (Brazilian Savanna) and Caatinga phytogeographic domains. In contrast, the *Lapanthus* species are found only in the Cerrado domain in the state of Minas Gerais. Molecular phylogenies support the monophyly of a group including these three genera. This group is included in the Bromelioideae subfamily, and arises as the first subclade branching from the Eu-Bromelioideae clade. Mainly over the last fifteen years, taxonomic studies including descriptions of many new species, taxonomic revisions and phylogenies have been published targeting *Orthophytum* and related genera. This talk aims to give an overview of what has been accomplished until now presenting the main advances in the systematics of the three genera. Studies in progress and perspectives of projects including traditional taxonomy, phylogenetics, phylogeography, population genetics and biogeography will be presented.

Key words: Bromelioideae; Inselberg; Phylogeny.

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The Brazilian checklist of Plants: Bromeliaceae

Louzada, Rafael Batista¹; Forzza, Rafaela Campostrini²; Costa, Andrea³; Siqueira Filho, José Alves⁴; Martinelli, Gustavo²; Monteiro, Raquel Fernandes²; Santos-Silva, Fernanda²; Saraiva, Daisy Pereira²; Paixão-Souza, Bruno² & Versieux, Leonardo Melo⁵

The first inventory for Bromeliaceae in Brazil was made by Mez (1891) in Flora Brasiliensis and accounted for 408 species in 31 genera. The Brazilian Flora Checklist, the most recent inventory of plants from Brazil, has listed 1341 species and 44 genera for the family, an increase of two times the number of species cited in Flora Brasiliensis. Brazil holds in its territory six phytogeographic domains: Amazon Rainforest, Atlantic Rainforest, Caatinga, Central Brazilian Savanna, Pampa and Pantanal. In all of them are found species of Bromeliaceae. The most diverse domain, for Bromeliaceae, is the Atlantic Rainforest with 906 species and 30 genera, also considered a diversity center for the Bromelioideae subfamily. In this phytogeographic domain, the genus *Vriesea* (184 spp.) accounts for the majority of species followed by *Aechmea* (156 spp.) and *Neoregelia* (102 spp.). Although *Vriesea* is the most diverse genus in Atlantic Rainforest, *Aechmea* species found in this domain represent 55% of all species in the genus in the country. Besides the Atlantic Rainforest, another vegetation type with a remarkable diversity is the campos rupestres (rocky fields) from the Espinhaço Range which are located in the Central Brazilian Savanna and Caatinga. For the Central Brazilian Savanna are reported 257 species and 20 genera in which 126 and 16 of each occur in the campos rupestres physiognomy. Caatinga presents 130 species and 18 genera in which 53 species and 18 genera occur in the Chapada Diamantina, the northern portion of the Espinhaço Range. In Caatinga *s.l.*, the highest diversity is in the almost limited to the saxicolous genus *Orthophytum*, with 31 species. The Amazon Rainforest presents 132 species in 22 genera and it is important to stress the diversity of some genera, such as *Aechmea* (27 spp.) and *Pitcairnia* (18 spp.), as well as the occurrence of the basal genus *Brocchinia*. Pampa and Pantanal are the two domains with lower diversity in Bromeliaceae, showing 36 species and 6 genera, and 6 species and 4 genera, respectively. General data, such as the number of taxa by state in Brazil, vegetation type, endemism, geographic distribution, type specimens citation and images are available as open access in the website of the Brazilian Flora Checklist.

Key words: Floristic Inventory; Brazilian Flora; Neotropics.

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Stigma Development in Bromeliaceae

Marcos, Dayene¹; França, W.²; Costa, Andrea³ & de Toni, Karen¹

Bromeliaceae consists of 58 genera and 3,352 species, divided into eight subfamilies; which show wide morphological and taxonomical diversity, thereby hindering generic and specific delimitation. Among the morphological characters, the floral are the most prominent, because the ontogeny of the stigmas is relevant to understanding evolution of the subfamilies. Until now, six stigma types have been described for the family: conduplicate-spiral, simple-erect, cupulate, convolute-blade, coralliform and tubolaciniate. In order to contribute to the phylogeny of the Bromeliaceae, this study aims to describe stigma development, in attempt to better classify them. For this, flower buds and flowers from 36 species of Bromeliaceae were collected. The analysis indicated four stigma types among the studied samples. For all species, the upper gynoeceum begins with the simultaneous settling of its three primordium carpels, which are initially fused only at the basal portion. Upon carpel maturation, variations in the development of stigmatic lobes were identified, typifying the different kinds of stigma. Initially, in the conduplicate-spiral, the stigmas conduplicate and then spiral among themselves. This last stage becomes even more conspicuous near floral anthesis. In this stage one can also notice some specifics among the species: existence of papillate, crenate or laciniate margins. This type was described for Pitcairnioideae and Bromelioideae species. In the simple-erect type, the stigmatic lobes are erect and with papillae during all development stages, but light twists in the lobes were noticed in some

species. This stigmatic type is specific of *Tillandsia*. In the convolute-blade, during early stages, one may observe the irregular growth of irregular stigmatic margins, and then conduplication of lobes, which becomes more prominent upon the maturation of the structure. At the intermediate stage, papillae appear on the lobes. Convolute-blade is homogenous for *Vriesea*, due to the variations observed in the density of papillae and merging of stigmatic lobes; this type was divided into 3 subtypes. In addition, a new stigmatic type is described, the Alcantarea, described for the genus with the same name, in Tillandsioideae. In earlier stages, the Alcantarea-type is similar to simple-erect, but after maturation, one may observe twists on stigmatic apices and on the apical portion of the style, which intertwine but do not merge. Furthermore, one may observe the appearance of papillae, which stretch themselves until floral anthesis. Thus, ontogenetic studies show great relevance for improving classification of the genera and species within Bromeliaceae. From the ontogeny of the stigmas, it is possible to suggest that the ancestral type in Bromeliaceae is conduplicate-spiral, which is common in all subfamilies, except Tillandioideae. A large variation regarding the types was observed, with records of simple-erect, convolute-blade and Alcantarea-type. Ontogenetic studies helped for understanding stigma evolution within subfamilies from phylogenies.

Funding: CNPq.

Key words: Bromeliaceae; Stigma; Ontogeny.

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Trying to understand *Vriesea* taxonomy with leaf anatomy

Machado, Talita Mota¹; Vale, Fernando¹; Versieux, Leonardo² & Stehmann, João Renato¹

Vriesea is the largest Bromeliaceae genus in Brazil, with 208 endemic species in this country. The Atlantic Rainforest is a center of diversity for the genus, but the Espinhaço Mountain Range is also an important region for presenting many endemic species. Due to the heterogeneity of environments occupied by *Vriesea* species and the great morphological diversification developed in the occupation of these environments, the taxonomy of the genus has problems. The aim of this study is to describe the leaf anatomy of 14 species of the genus that occur in different environments and substrates and discuss the taxonomic and ecological implications of the observed features. Samples were taken from the middle of the leaf blade and fixed in FAA 50% and then preserved in 70% alcohol. Freehand cuts were made and the material was stained with safranin and astra blue and then mounted in glycerin. The general characteristics for the genus were: hypostomatic leaves with stomata on the same plane as the other epidermal cells and stomata connected to air channels, uniseriate epidermis with the presence of scales on both sides, lignified hypodermis and groups of subepidermal fibers; parenchyma consisting of three cell types: the braciiformes that are found forming the aerenchyma of air channels, isodiametrical chlorophyllian surrounding the air channel, especially in the adaxial side forming alternating arcs with vascular bundles and isodiametrical with respect to water storage tissue. Species that occupied the same type of environment as rocky fields and high altitude grassland had similar anatomy, but can be differentiated by variations in the distribution and density of stomata and scales, variation in the shape of the air channels and height of the aquiferous parenchyma. *Vriesea apparicana* was the species with the highest variation in the general aspect, showing adaptation to extreme environments such as inselbergs or conservation of ancestral characteristics. Anatomy was shown to provide important characters that can be used in taxonomy and systematics of *Vriesea* and allow a better understanding of the environments occupied by the species.

Funding: Capes.

Key words: Rupicolous; Adaptation; Evolution.

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***Aechmea* Ruiz & Pavón from the northern portion of the Atlantic Forest**

Maciel, Jefferson Rodrigues^{1,2}; Louzada, Rafael² & Alves, Marccus²

Aechmea comprises nearly 250 species distributed in eight subgenera and is one of the most representative genera of Bromelioideae. It occurs from Mexico to southern Brazil, Uruguay and northern Argentina. In the Atlantic Forest of Brazil, 140 species have been cited which represent more than 50% of the richness of *Aechmea*. The lack of updated taxonomic keys for identification, the high rate of endemism and the recent novelties published within *Aechmea* from this region motivated this work. Herein we present a taxonomic synopsis of *Aechmea* species from Alagoas, Pernambuco, Paraíba, Rio Grande do Norte and Ceará states. Samplings were conducted in areas of all states studied between 2010 and 2014. Morphological descriptions were made using terminology from the literature and DELTA EDITOR 1.04 software. Two names were excluded from the species list compiled from the literature, thus *A. conifera* and *A. marauensis* are now confirmed as restricted to Bahia. We documented a total of 27 species of *Aechmea* in the region: distributed in Alagoas (17 spp), Pernambuco (19 spp), Paraíba (12 spp), Rio Grande do Norte (3 spp) and Ceará (4 spp) states. *Aechmea* subg. *Aechmea* was the most representative with 20 species, followed by *A.* subg. *Chevaliera* with four species, and *Aechmea* subg. *Lamprococcus*, *A.* subg. *Macrochordion* and *A.* subg. *Pothuava* with one species each. In the study area, the *Gravisia* complex is of particular importance and represented by ten of the 14 species that form this informal taxonomic group.

Funding: CNPq, NSF, Velux Stiftung, Beneficia.

Key words: Bromelioideae; Centro de Endemismo; Taxonomia.

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The Wallacean Shortfall in *Aechmea*

Maciel, Jefferson Rodrigues^{1,2}; Siqueira, Marinez Ferreira³ & Alves, Marccus²

Biodiversity database of hyperdiverse taxa and regions are limited for providing an accurate picture of species distributions. This problem is named the Wallacean Shortfall and represents the main difficulty for Neotropical biogeographic studies. An alternative to face this problem is ecological niche modelling (ENM). However, we do not know how Wallacean Shortfall affects ENMs assumptions, especially the sample sufficiency assumption. Thus, herein we developed a comparative analysis of environmental spaces among potential and observed distributions of 18 *Aechmea* species from northeastern Brazilian Atlantic Forest. A principal component analysis was made for four environmental datasets of each species: 1) from bioclim models, 2) from maxent models, 3) from convex hull of species distributions, and 4) from observed distributions. The two first axes were used in a kernel density analysis to compare the environmental spaces these four datasets. All analyses were performed in the R software environment and its associated packages. Patterns of geographic distributions of the 18 species can be classified as wide distribution, occurring continuously from Central America to Brazil (1 species) and disjunctly between Amazonia and Atlantic Forest (3 species); Atlantic distribution, occurring on the East Coast of Brazil (4 species); and endemic to the northeastern Atlantic Forest (10 species). Species with wide and Atlantic distributions show higher adjustment between observed and expected distribution, as opposed to endemic species that show low adjustment. In general, species with low adjustment also show fewer occurrence points than species with higher adjustment. The evidence suggests that the Wallacean Shortfall affects the quality of *Aechmea* species models differently, but widely distributed species show good environmental sampling and might be good biological models for ENMs studies, while endemic species require customization of the approaches used.

Funding: CNPq, NSF, Velux Stiftung, Beneficia.

Key words: Ecological Niche Modeling; Distribution; Bromelioideae.

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The genus *Vriesea* Lindl. *Vriesea* section in the state of Paraná: taxonomic and molecular aspects*Malagón, Maria & Tardivo, Rosângela*

The genus *Vriesea* Lindl. *Vriesea* section in the state of Paraná: taxonomic and molecular aspects. *Vriesea* Lindl is the most differentiated genus in the Bromeliaceae, with a total of 207 species reported for Brazil being the genus with the largest specific diversity found in the Atlantic Forest. It is estimated that 38 species of the *Vriesea* occur in southern Brazil, and of these 34 are found in Parana State, 23 of which fall under the *Vriesea* section. In the recent publication of the Vascular Plant List of Paraná were cited 27 *Vriesea* species. The aim of this study is to know the diversity of the genus *Vriesea* section *Vriesea* found in the State of Paraná, emphasizing the morphological and taxonomic aspects and conservation status of the taxa. This work is part of a master's thesis in progress. Field trips to localities and other sites in different areas, ecosystems and altitudinal levels in the State of Paraná were carried out to collect specimens of the taxa for the study. These localities include the phytogeographic domains: Cerrado, Atlantic Forest, Araucaria forest and grasslands. The collected material was herborized according to plant taxonomy methodology and the specimens were deposited in the Herbarium HUPG. Morphological analyses and geographical distributions were based on herbarium specimens from the following herbaria: HUPG and MBM. To identify the material we studied original descriptions, photos and herbarium specimens. The conservation status for each species was evaluated using the World Conservation Union (IUCN) criteria, and was based on the herbarium material and field observations. Given the difficulty in identifying some species, molecular analysis will be conducted to support the identification of these species. As a partial result, 11 species belonging to the genus *Vriesea* section *Vriesea*, ten of which were collected in Atlantic forest areas (*V. carinata* Wawra, *V. ensiformis* (Vell.) Beer, *V. erythrodactylon* E. Morren, *V. flava* A. Costa, H. Luther & Wand., *V. inflata* (Wawra) Wawra, *V. incurvata* Gaud., *V. heterostachys* (Baker) L. B. Sm., *V. phillipocoburgii* Wawra, *V. procera* (Mart. ex Schult. & Schult. f.) Wittm. and *V. rodigasiana* E. Morren) and five species in areas of Araucaria forest (*V. friburgensis*, *V. procera*, *V. phillipocoburgii* and *V. rodigasiana*). The most representative species of the Atlantic forest areas were: *V. phillipocoburgii*, *V. ensiformis*, *V. incurvata*, *V. procera* and *V. rodigasiana*, which were observed in different environments of this phytogeographic domain. Species such as *V. rodigasiana* and *V. procera* were more frequently observed in the coastal areas of the state while *V. carinata*, *V. erythrodactylon*, *V. flava*, *V. heterostachys* and *V. inflata* have only been found so far in the Serra da Graciosa. We elaborated morphological descriptions, identification keys, geographical distribution maps and illustrations of the studied taxa.

Key words: *Vriesea*; Taxonomy; Paraná State.

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A combined molecular and cytogenetic approach to study *Pitcairnia azouryi* (Bromeliaceae)*Manhães, Vitor Cunha; Miranda, Fábio Demolinari; Clarindo, Wellington Ronildo & Carrijo, Tatiana Tavares*

This study reports the discovery of new areas of occurrence of *Pitcairnia azouryi* Martinelli & Forzza, and the study of their populations regarding the species' genetic diversity, the amount of nuclear DNA and chromosome number. Seven new areas of occurrence for this species were located between northern Rio de Janeiro (RJ) State and southern Espírito Santo (ES) State, and georeferenced to the other studies. Leaf samples of 13–15 individuals were collected in five of these populations (PLB - Pedra Lisa Burarama, PA - Pedra das Andorinhas, PTI - Pedra Três Irmãs, PPC - Pedra Parada Cristal and PLC - Pedra Lisa Campos), totaling 66 individuals. Nine SSR markers were used in PCRs. Genetic polymorphisms were identified from polymorphism between individuals sampled, detected by electrophoresis on an 8% acrylamide gel. The parameters of diversity and genetic structure of populations were calculated using the software Fstat, PopGene and Structure. The flow cytometry analysis was performed using leaf samples obtained from adult individuals of five populations. Cytogenetic analyses were performed with roots obtained from germinated seeds collected in two populations. The nine SSR primers produced satisfactory amplification products, and proved to be polymorphic. The number of alleles per locus ranged between two and eight. The values of gene diversity index (H') and the fixation index (F) in populations ranged from 0.440 to 0.552 and 0.0046 to 0.1718, respectively. The G_{st}

values observed showed that 87.33% of the total genetic variation is found within populations and the occurrence of gene flow (Nm) 1,073 (average number of migrants per generation). Bayesian analysis indicated that a model of $k = 3$ populations is able to better capture the variation in the data on the genetic structure. The high variation of fixation index added to the large genetic variation within and moderate genetic differentiation among populations suggest the occurrence of the founder effect in the dispersion of new individuals into new locations, followed by genetic drift, especially for the population at Campos dos Goytacazes. The flow cytometry analysis showed that the nuclear DNA content is $2C = 1.16$ picograms in all populations analyzed, except on PLC population, whose DNA content ($2C = 2.32$ picograms) was measured twice. This result was explained by cytogenetic analysis which revealed individuals with $2n = 50$ chromosomes in ES and individuals with $2n = 100$ chromosomes in RJ. This result may be due to PLC's geographical isolation, the environmental characteristics of the local or even evolutionary processes.

Key words: Atlantic Forest; Conservation Biology; Rocky Outcrops.

Universidade Federal do Espírito Santo, Brazil.

Intraclonal physiological specialization in the endemic nurse-plant *Neoregelia cruenta*

Mantuano, Dulce; Neves, Tatiana Campos & Rocha, Lawrence da Silva

Clonal plant species can be considered as populations of interconnected ramets which are basically identical in form and function, and potentially independent from each other. Experimental studies suggest that an intraclonal specialization of ramets with different roles can increase the performance of clonal systems under heterogeneous condition. This study explores structural and functional variation of rosettes of *Neoregelia cruenta* (R. Graham) L.B. Smith (Bromeliaceae), a CAM tank bromeliad, under three environmental conditions in sandy coastal plain vegetation in Southeast Brazil. The main question asked is if heterogeneous habitats are able to induce different transpiration rates on connected ramets. It would allow water source sharing among connected ramets. We assumed it is an essential mechanism to sustain exposed ramets. *N. cruenta* plays an important ecological role, increasing soil nutrient content and initiating vegetation islands in sandy soils, which are related to species diversity enhancement. In field conditions, clonal clumps can be found shaded or fully exposed to sunlight (homogeneous microhabitats), and partially exposed to full sunlight (heterogeneous microhabitat). We compared morphological and physiological parameters among intraclonal and interclonal rosettes in all microhabitats. Leaf area and length of leaf blade vary strongly, decreasing with increased light exposure. Leaf succulence and specific leaf weight were similar between connected ramets but lower in the exposed microhabitat. Chlorophyll and carotenoid contents decrease as the light gradient increases. This result suggest that, although acclimation features could be observed, exposed ramets (entire clonal clump of exposed microhabitat and half clump of partially exposed microhabitat) are under light stress conditions. Stomatal conductance was different between rosettes of heterogeneous habitat, contributing support to the contention that directional xylem flow between ramets is likely to occur.

Key words: Clonal Specialization; Physiology; *Neoregelia cruenta*.

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Transcriptomic analyses of *Vriesea carinata*: diversity of cysteine proteinases and identification of microRNAs

Margis, Rogério

Small RNA and RNA-seq libraries were constructed to identify microRNAs and their putative targets in *Vriesea carinata*, a native bromeliad species from the Brazilian Atlantic Rainforest. Illumina technology was used to perform high throughput sequencing and data was analyzed using bioinformatics tools. We obtained 2,191,509 mature miRNAs sequences representing 54 conserved families in plant species. Further

analysis allowed the prediction of secondary structures for 19 conserved and 16 novel miRNAs. Potential targets were predicted from pre-miRNAs by sequence homology and validated using RTqPCR approach. This study provides the first identification of miRNAs and their potential targets of a bromeliad species. This transcriptomic analysis also allowed the identification of 28 members of the papain/bromelain-like family and more than a hundred other cysteine proteinases.

Funding: CNPq.

Key words: Next Generation Sequencing; Microma; Cysteine Proteinase.

Universidade Federal do Rio Grande do Sul, Brazil.

Embryological and anatomical features in Bromeliad Systematics

Mariath, Jorge Ernesto

Plant anatomy, especially of reproductive organs, has been very important in Bromeliaceae. The analysis of ovary and ovule development in the Nidularioid complex identified traits that can assist in the general delimitation of the complex and compare the characters found with the current taxonomic proposals. The number of cell layers of integuments, format of the outer integument cells and cell morphology of the nucellar epidermis in the micropyle zone, gametophyte format and the presence or absence of a chalazal appendage were highlighted as important morphological features. As to the ovary, the number of columns of ovules per locule, the orientation of the ovules in relation to the ovary axis, as well as the analysis of the mesophyll morphology, where the formation of aerenchyma was observed, were considered important. This feature was a synapomorphy found for the species of the complex, and is related to the hypoxic condition that these ovaries are subjected to. The parsimony analysis separated *Canistrum* from the other species of the complex. This result agrees with the latest phylogenies. And while *Witrockia* and *Edmundoa* are used by some authors as a synonym for *Canistrum*, in relation to the ovary and the ovule they share a greater number of characters with other species in the complex than with *Canistrum*. The analysis of five genera of Tillandsioideae demonstrated the variations in the number of columns of ovules per locule, the presence or absence of chalazal appendages and their dimensions and details of the development of integuments, as important taxonomic characters. Similarly the details of the ovule development of *Billbergia nutans* of the Bromelioideae subfamily underscored useful characters for taxonomy and identified the evolutionary scenario of such embryological characters in the family. We highlight the first, three-dimensional analysis of a female gametophyte in monocots hoping that this approach will be further explored to assist the interpretation of reproductive structures and detection of new characters. Fruit anatomy has been used to define or solve taxonomic problems among species, genera, tribes and families in different plant groups. The anatomy of the dehiscent fruit is closely related to the type of dehiscence. Details such as wedged macrosclereids and striking structures of the pericarp of Bromeliaceae capsules and the U-shaped thickness of the cell wall, reported only for eudicotyledonous fruit, are also shown as occurring in monocots. Based on phylogenetic proposals, the evolution of the fruits shows a tendency to indehiscence, with a probably plesiomorphic capsule character. Finally, a partial analysis of mature seeds of 14 species of *Tillandsia* and *Vriesea* established structural patterns of seed coat, endosperm and embryo, allowing one to distinguish between these two genera. The information presented here reinforces the need for further morpho-anatomical analyses.

Key words: Anatomy; Morphology; Taxonomy.

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The Brazilian Red-List of Flora: Bromeliaceae

Martinelli, Gustavo.

Jardim Botânico do Rio de Janeiro, Brazil.

Morphological characterization of the ovarian wall, stigma, stylus and ovules in two species of *Dyckia* (Bromeliaceae)

Martins, Merielem; Dorneles, Mariane; Oliveira, João Marcelo & Nicoloso, Fernando

The complexity and large structural variability of the carpel, stigma, style and ovule, make such characters very important in morphological, taxonomic and phylogenetic analyses. The number of undetermined species or inaccurate identifications of *Dyckia* species is considered high, due to a lack of consistent and useful taxonomic characters. The objective of the present work is to comparatively describe the ovary wall, style, stigma and ovule by histology and light microscopy in *Dyckia racinae* L.B.Sm. and *Dyckia polycladus* L.B.Sm. in order to increase knowledge of the species and family and see if the characters studied are consistent. Flowers at various stages of development were collected in São Pedro do Sul, and Santa Maria, RS, Brazil. For the structural analysis, the usual plant microtechniques and light microscopy were performed. *D. polycladus* and *D. racinae* have perfect, trimerous, heterochlamidous flowers, surrounded by a bract. *D. racinae* presents larger flowers. The ovary is superior and syncarpous, trilobular, plurispermic. The stigma is the spiral, conduplicate type, with three carpels unfused along their margins, separate and spiraling together. In *D. racinae* the style is long, with a length approximately twice the ovarian length, differing from *D. polycladus*. In both species, the ovules are anatropous. The ovule in *D. racinae* presents provascular tissue in the outer integument, unlike *D. polycladus*. The inner integument of *D. racinae* is longer than the outer integument, while in *D. polycladus* they are the same length. *D. polycladus* presents an outer integument with intercellular spaces in the upper part, differing from *D. racinae*. *D. racinae*, which have a chalazal appendix developed from cells recruited from dorsal and ventral region in the chalaza. In *D. polycladus*, the chalazal appendix cells are recruited only from the dorsal region, including the funiculus. Among the characters presented here for *Dyckia racinae* and *D. polycladus*, the most informative are: ovarian size relative to the style, vascularization of the outer integument, chalazal appendix position, and integument length. Thus, the morphological analysis of the ovules shows differences between taxa. It is believed that the expansion of studies, with emphasis on the ovules can contribute to the understanding *Dyckia* and Bromeliaceae phylogeny.

Funding: FAPERGS, CNPq.

Key words: Bromeliaceae; *Dyckia Polycladus*; *Dyckia Racinae*.

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Monograph of the genus *Catopsis* Griseb. (Tillandsioideae)

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Catopsis (Tillandsioideae) comprises epiphytic and rupicolous herbs distributed throughout Mexico, Central America, the Caribbean, and northern South America. The genus is more diverse in Mexico, where more than 85% of its species exist. *Catopsis* is characterized by its seeds with an apical appendage formed by numerous hairs born at the chalazal end. Most of the species are dioecious, although some taxa are monoecious or trioecious. The objectives of the present revision were to perform a taxonomic treatment of the genus, as well as to propose a hypothesis of phylogenetic relationships among the species. The study was based on a review of 1,900 herbarium specimens corresponding to 1,548 collections, from 16 herbaria, as well as examining live plants. Phylogenetic analyses were conducted using 70 morphological characters and DNA sequences of the plastid genes rps16 and matK. *Catopsis* is a monophyletic group that comprises 27 species, five of which are new to science and are in the process of being described. *Catopsis compacta*, *C. occulta*, *C. paniculata*, *C. pendula*, *C. wawraneana* and one new species are endemic to Mexico, whereas *C. pisiformis* is endemic to Panama and *C. werckleana* to Costa Rica. The citation of the original publication, as well as the lectotypification of the genus are clarified. The monograph presented here provides a broad synthesis of previously published information and includes data on nomenclature, morphology, geographic distribution, and phylogeny, which complete the descriptions and synonymies of the species. It also includes an identification key, as well as descriptions and distribution maps for each species.

Key words: *Catopsis*; Phylogeny; Taxonomic Treatment

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Floral biology of *E. erectiflorum* L. B. Sm. (Bromeliaceae-Pitcairnioideae) at Sete Cidades National Park, Piauí

Masrua, Mariana Lenara¹, Sousa, Gardene¹; Lopes, Ariadna Valentina²; Aguiar, Bruno Ayron²; Soares, Elda Simone² & Silva, Ana Claudia²

In the Americas, the Bromeliaceae family performs a valuable functional role as it offers resources continuously which ensures the dynamics in ecological processes of different groups biological, such as hummingbirds, bees and bats. The genus *Encholirium* is exclusively distributed in Brazil. It occurs associated with inselbergs, in the regions of Brazilian scrub and savanna vegetation, and the Brazilian Atlantic Rainforest. The aim of this study was to analyze the floral biology of *E. erectiflorum*, a species of large abundance at Sete Cidades National Park (PI). We recorded the flowering period as between May to July (2012), and also performed an analysis of the floral morphology, duration of anthesis and as well the volume of nectar produced, stigmatic receptivity, viability pollen, the ratio of pollen/ ovule and observation of pollinators at the maximum peak flowering of the species under study. We selected 30 flowers for each test, among them, pollen viability using carmine staining method 2% acetic acid, the stigma receptivity by 0.25% permanganate solution potassium (KMnO₄) and for the determination of the ratio of pollen / ovule. Analyzing *Encholirium erectiflorum* morphologically, the species was found to present orange flowers; ovate, obtuse, green sepals with brown spots; oblong petals, orange; isodynamous stamens, longitudinal dehiscence; a tricarpellate, trilocular ovary; spiral-duplicated stigmas. Flower opening begins at 17h, when flowers initially expose the stigma, which is already receptive. However, anther dehiscence comes only from 7 am the next morning to early flower opening. The production of nectar starts at about 5pm, though not continuously, and the volume decreases as time passes, ceasing at approximately 11am. Pollen viability is considered high (96.86%). Based on the pollen / ovule ratio being considered high (1256.86), it is believed that the reproductive system of individuals is by cross breeding. Analyzing the ecological processes, *Apis mellifera* L. is considered an effective pollinator species, followed by *Trigona spinipes* Fabricius, as an occasional pollinator and *Chlorostilbon lucidus* Shaw, rated as a nectar thief, because it collects nectar without contacting reproductive structures. The species has the aid of pollinators, but there is the possibility of self pollination. Thus, the species presents a dichogamous, protogynous reproductive system which there is early maturing of the female reproductive whorl.

Funding: UFPI.

Key words: Floral Biology; *Encholirium*; Bromeliaceae.

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Pollen and pistil interactions and hybridization in sympatric Bromeliaceae species in southeastern Brazil

Matallana, Glória¹ & Wendt, Tânia²

Pollen flow between sympatric species may result in hetero-specific crosses allowing hybridization. In these cases reproductive pre- and post-zygotic isolation barriers could be selected for the maintenance of species as entities. This study evaluates the inhibition of pre-zygotic pollen tube growth in intra-specific, inter-specific and inter-generic crosses in sympatric species of Bromeliaceae using fluorescence microscopy techniques. The possibility of hybridization between target species was tested and pistil length and speed of pollen tube growth in hetero-specific crosses were measured. Patterns of pollen tube growth inhibition and incongruity involving reactions of unilateral incompatibility in inter-generic and inter-specific crosses were the most frequent. However, egg penetration occurred in 26% of the crosses, pointing to the possibility of hybridization. The high frequency of egg penetration in inter-specific and inter-generic crosses in species such as *Quesnelia quesneliana* and *Aechmea araneosa* suggest weak pre-zygotic barriers. Egg penetration, in hetero-specific crosses, was more frequent in crosses where both parents had short pistils (48%) and in crosses where the recipient had a short pistil and the pollen donor had long pistil (33%). Pistil length seems to be significant in pollen tube growth and egg penetration.

Funding: FAPES, CNPq, FAPERJ.

Key words: Pre-zygotic Isolation; Atlantic Forest; Pollen Tube Growth.

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***Ananas* (Bromelioideae, Bromeliaceae) – Phylogenetic relationships based on nuclear, plastid and AFLP data**

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Ananas Mill. is native to South America and well known due to the cultivated pineapple *Ananas comosus* (L.) Merr. (Leme and Siqueira Filho 2007). Since description of the genus in 1754, the taxonomy of *Ananas* has varied substantially (Coppens d'Eeckenbrugge and Leal 2003). The latest classification of Smith and Downs (1979), corrected by Leal (1990), recognized seven *Ananas* species and accepted *Pseudananas sagenarius* (Arruda) Camargo as a monotypic distinct genus. This concept has been brought into doubt based on morphological, biochemical and molecular diversity data as well as reproductive behaviour (Coppens d'Eeckenbrugge and Leal 2003). Therefore, Leal *et al.* (1998) suggested a simplification by merging the seven species of *Ananas* into a single species with *Pseudananas sagenarius* classified as a second species of *Ananas*. In the latest study concerning the taxonomy of *Ananas*, phylogenetic relationships have been inferred by examining Chloroplast DNA diversity using PCR-RFLP data (Duval *et al.* 2003). There are two ways of interpreting of the results of Duval *et al.* (2003): (1) follow the biological concept and lump the *Ananas* species as suggested by Leal *et al.* (1998), or (2) consider monophyly as an essential criterion for classification and maintain the seven species because of observed paraphyly. We present a fully resolved phylogenetic tree comprising all seven *Ananas* species and also *Pseudananas sagenarius* based on three nuclear markers (*agt1*, *ETS*, *phyC*), five plastid markers (*atpB-rbcL*, *trnL-trnF*, *matK*, *ycf1* (part 1 and 6)) and AFLP data. Genetic distances in the *Ananas* clade are low, which can be interpreted in favour of the classification of Coppens d'Eeckenbrugge and Leal (2003). However, we follow Leme and Siqueira Filho (2007) by maintaining the species level, because (1) genetic distances in all Bromelioideae are comparatively low and gene flow seems to be possible even between genera, and (2) a thorough morphological study of the *Ananas* taxa is lacking.

Funding: DFG.

Key words: *Ananas*; Phylogeny; Species Level.

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Bromeliaceae from a submontane Atlantic Forest area in northeastern Brazil

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Bromeliaceae is one of the richest families in the Atlantic Forest with a high number of endemic species. The Serra do Urubu comprises a well-preserved and continuous, submontane (600 to 800 m) forest fragment in the southern part of the state of Pernambuco (northeastern Brazil). Several studies with Orchidaceae, ferns and birds have confirmed the value of the area for conservation because of its biological diversity. A floristic inventory was carried out with field trips from August 2010 to December 2014. Local herbaria (UFP, PEUFR, HST, IPA, and JPB) were also visited to study specimens previously collected in the area. Bromeliaceae is represented in Serra do Urubu by 42 species and 17 genera. The family is one of the most diverse among the Angiosperms in the study area, which holds almost 40% of all species cited so far to the state of Pernambuco. *Aechmea* and *Vriesea* with nine species each, and *Tillandsia* with four species comprise more than half of the species found in the area. Twenty-three species are endemic to the Atlantic Forest, and 11 of them are restricted to the “Centro de Endemismo Pernambuco” (Pernambuco Endemism Center). Twenty species are endemic to the states of northeastern Brazil, including *Aechmea gustavoi*, which is considered Critically Endangered;

Canistrum pickelii and *Guzmania monostachia*, Vulnerable; and *Canistrum auratiacum*, Endangered. *Vriesea wawraana* Antoine is Endangered and restricted to the states of Espírito Santo, Bahia and Pernambuco and finally, *Aechmea serragrlandensis* Leme & J.A. Siqueira which was known only from a few populations in the state of Alagoas and was recently found in the area. The diversity of bromeliads found in the Serra do Urubu reinforces its value for conservation of submontane forests in northeastern Brazil.

Funding: CNPq, NSF, Velux Stiftung, Beneficia.

Key words: Submontane Forest; Conservation; Pernambuco.

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Phylogeography of *Dyckia* species (Bromeliaceae) endemic to the Brazilian Cerrado biome: evolutionary implications

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In South America the number of phylogeographical studies is scarce when compared with other regions, despite having one of the highest levels of biodiversity on Earth. The genus *Dyckia* belongs to the subfamily Pitcairnioideae (Bromeliaceae) and its species usually inhabit dry, rocky outcrops with little water supply and high sunlight. The diversity center of *Dyckia* occurs in montane regions of the central Brazilian Cerrado biome (BCB), which is delimited in the east by the Atlantic Forest and Caatinga, and by Chaco in the west. The BCB is the most diverse savanna in the world, mainly with high levels of endemism and species adapted to stressful environments. However, the original area has been reduced by more than 50% in recent years, making this biome one of the main global hotspots for biodiversity conservation. Here we studied a group of four species with restricted geographical distribution (*Dyckia grandidentata*, *D. pottiorum*, *D. excelsa* and *Dyckia* sp.), which are endemic to the BCB, and one associated species (*D. leptostackya*), with a wide distribution. The collection of samples was realized in February of 2014 in Mato do Grosso do Sul Federal state (Brazil), comprising seven localities and eighteen populations, totaling 368 individuals. Aiming to understand the evolutionary history of this group and also contribute to the better understanding of the BCB history, we tested three plastidial sequences (rpl32-trnL, rps16-trnK, trnD-trnT) from populations throughout the known distribution of all five species in order to perform a phylogeographical analysis. The preliminary results showed differences in molecular sequences among species. The analyses are in progress and certainly will help us understand the process of speciation of this group in the microhabitats of BCB, and may provide valuable insights into the historical processes underlying diversification in this region.

Funding: CNPq, FUNDECT, PPGBM-UFRGS.

Key words: Phylogeography; *Dyckia*; Cerrado.

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Flexibility in use of inorganic and organic nitrogen sources by bromeliads: a key adaptative response

Mercier, Helenice; Takahashi, Cassia & Matiz, Alejandra

The leaves of bromeliads have specialized trichomes that can absorb distinct nutrients, including nitrogen (N). Nitrate (NO₃⁻), ammonium (NH₄⁺) and organic N (amino acids, peptides, urea, etc.) may be provided to the epiphytic environments through atmospheric N deposition. Besides that, organic N may be provided by animal excretions (e.g., frogs), and some tank bromeliads are known to grow better when urea is available in the tank. Urea utilization may be an important feature for some bromeliads to colonize and survive in oligotrophic environments. We have shown that leaves of the tank bromeliad *Vriesea gigantea* can absorb different N forms present in the tank (NO₃⁻, NH₄⁺, amino acids, urea). However, urea uptake is more efficient

than inorganic N sources and apparently aquaporins may be involved in its transport across cellular plasma membranes. This organic N source can be hydrolyzed to NH_4^+ and CO_2 by urease. It was very interesting to observe that urease in leaves of *V. gigantea* showed its greatest activity in the basal portion, which is in close contact with the tank solution. We have obtained evidence that urease may also be secreted in the tank. This fact might give some advantages to the bromeliads for N acquisition during competition with microorganisms inhabiting the tank solution. We have also found that *V. gigantea* can use CO_2 generated by the hydrolysis of urea. Cytochemical CO_2 detection has allowed us to observe the presence of CO_2 in the cell wall, intercellular spaces and around the chloroplasts, suggesting that urea may be both N and carbon sources for these plants. To confirm this hypothesis, we provided ^{13}C -urea to detached leaves of *V. gigantea*, and we looked for ^{13}C -labeled malate. Interestingly, we have found a great incorporation of ^{13}C in that organic acid, indicating that CO_2 was assimilated when urea was available to this bromeliad. Similar results were observed for *Guzmania monostachia*, another tank epiphyte bromeliad. Thereby, these results suggest that the use of CO_2 from urea is not exclusive to *V. gigantea*. The use of a N source like urea to obtain carbon seems to be a relevant strategy to cope with N-deficient environments and it might be significant for managing some specific situations, such as drought, when stomata closure impairs atmospheric CO_2 uptake. Therefore, during water shortage we might expect assimilation of CO_2 from urea.

Funding: CNPq, Fapesp.

Key words: Nitrogen Sources; Urea; Nitrogen Nutrition.

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PEPC and MDH activity in *Alcantarea imperialis* (Carriere) Harms cultured *in vitro* with polyethylene glycol – PEG 6000

Mollo, Luciana & Nievola, Catarina

Plant species from rupicolous environments are adapted to temperature variations and to low water availability, as is the case of the ornamental bromeliad *Alcantarea imperialis*, known as imperial bromeliad, native to the Serra dos Órgãos (RJ, Brazil), a montane tropical rainforest area with “Campos de Altitude”. It grows on rocky outcrops (inselbergs), in shallow and stony soils and is exposed to episodes of water shortage. Under this condition, plant species can express the CAM pathway, induced by the absence of water. The goal of this study was to analyse the activity of phosphoenolpyruvate carboxylase (PEPC) and malate dehydrogenase (MDH) enzymes in *A. imperialis* plants cultured *in vitro* under water-deficit conditions, simulated with different polyethylene glycol (PEG 6000) concentrations, in order to verify if this species can express the CAM pathway when exposed to such conditions. Plants were obtained through seed germination in Murashigie & Skoog culture medium with macronutrient concentration reduced to half, 30 g L⁻¹ of sucrose and 7 g L⁻¹ of agar. After 6 months, plants were transferred to the same media, without agar and with PEG in concentrations of 0% (T1, with -0.5 MPa); 7.5% (T2, with -1.9 MPa); 30% (T3, with -4.7 MPa); 45% (T4, with -8.5 MPa); and 60% (T5, with -17 MPa) during 7, 15 and 30 days in a growth chamber under 26 °C ± 4 °C and 12 h photoperiod. Plants were analysed in terms of biometric parameters, PEPC and MDH activities. No differences were observed in biometric parameters between treatments. However, results indicated that longer exposure to water-deficit conditions led to a gradual increase in PEPC and MDH activities, which could be involved with this species adaptation to water-deficit conditions, although no expression of CAM could be confirmed in plants cultivated at any of the PEG concentrations applied.

Funding: CNPq.

Key words: Photosynthesis; C3; Pepc.

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Leaves of Bromeliaceae from the Brazilian Chaco: morphological and anatomical features of six species

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The Bromeliaceae family comprises about 3,140 species distributed in eight subfamilies with epiphytic, saxicolous or terrestrial species, inhabiting different vegetation types in South America. Some species live in humid forests and others are found in arid zones and present anatomical and morphological frameworks that enable them to occupy the various microenvironments. In this study we investigated the leaf anatomy of six species of Bromeliaceae - *Aechmea distichantha* Lem., *Bromelia balansae* Mez, *Dyckia ferruginea* Mez, *Encholirium lymanianum* E. Pereira & Martinelli, *Tillandsia duratii* Vis. and *Tillandsia tenuifolia* L., aiming to identify the anatomical features associated with different Bromeliaceae habitats in Brazilian Chaco Forest fragments in Mato Grosso do Sul state (Brazil), where the plants undergo extensive water limitation due to seasonal rains. The leaves are rigid, succulent and have an opaque surface due to the presence of scales; some of them have rigid spines on the margin. All species investigated presented anatomical features important in water retention. A thin, external cell wall and cuticular layer was observed, but internally, the epidermal cell walls are thick and strongly lignified as in *Bromelia balansae* and *Dyckia ferruginea*. The anticlinal cell wall is extremely sinuous; the epidermal cell lumen is reduced and has a spherical silica body, most remarkably in *Dyckia ferruginea*, *Aechmea distichantha* and *Bromelia balansae*. Peltate multicellular trichomes (scales) were observed on both leaf surfaces, but are more frequent over stomata apertures. The stomata are in rows, and could be located in grooves, under the level of the other cells, thus avoiding water loss by transpiration, as in *Encholirium lymanianum* and *Dyckia ferruginea*. Under the epidermis a mechanical hypodermis, consisting of lignified and rigid cells are detected in all species, less developed only in *Tillandsia* species. The leaves are strongly succulent and the water storage parenchyma could be colorless or present chloroplasts. Collateral vascular bundles surrounded by a parenchymatous sheath and fiber caps constitute the vascular and support system. The anatomical features recognized could be related to different biotic and abiotic stresses acting on Chaco vegetation, such as extreme temperature range, intense light, herbivory, nutritional deficiency and seasonal availability of water playing an essential role in allowing species to occupy a dry environment.

Funding: CAPES-casadinho.

Key words: Sclerified Tissues; Succulent Leaves; Xeromorphics.

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Reproductive isolation between incipient outcrossing and selfing *Vriesea* species (Bromeliaceae)

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Speciation requires the evolution of reproductive isolation mechanisms that provide barriers to hybridization between diverging lineages. Patterns of gene flow in hybrid zones are often observed to be asymmetrical and these asymmetries can arise from variations in the strength and direction of isolation barriers in the species involved. Incipient species can be reproductively isolated effectively by one or multiple prezygotic or postzygotic mechanisms as long as they provide a strong barrier to gene flow. We aim to study the consequences of hybridization in the process of speciation-with-gene-flow between *Vriesea simplex* and *Vriesea scalaris*. These species have similar morphology, overlap in flowering phenology, and share hummingbird pollinators. Putative hybrids are reported when bromeliads occur in sympatry. Here, we will present: 1) a description of the mating systems of both species and results of inter-specific manual-crossing; 2) genetic structure of sympatric and allopatric populations and genetic composition of hybrids, using 15 nuclear SSR loci. The intra-specific manual-crossing, revealed that *V. simplex* is predominantly outcrossing, whereas *V. scalaris* is predominantly of selfing. Seed viability from selfing treatments in *V. simplex* (4.0 ± 7.34) was much smaller than in *V. scalaris* (64.57 ± 43.56), indicating that *V. simplex* is partially self-compatible and pollinator-dependent. The inter-specific manual-crossing treatments revealed higher fruit ($F=3.597$, $p=0.064$) and significantly seed set ($F=5.702$,

$p=0.022$) for *V. simplex*, when receiving pollen of *V. scalaris*, (93.02%, and 581.43±235.56, respectively) than for *V. scalaris* when receiving pollen of *V. simplex* (74.07% and 422.43±180.35, respectively). These results may suggest hybridization is asymmetrical from *V. scalaris* to *V. simplex*. However, the percentage of seed germination was very low but similar among species (*V. simplex* = 26.60±38.24 and *V. scalaris* = 26.75±37.71), suggesting that postzygotic barriers can act at different stages. The analysis of genetic admixture, based on nuclear microsatellites, in three sympatric populations identified a total of 30 hybrids. The genetic diversity parameters in the outcrosser *V. simplex* were higher than in the selfer *V. scalaris*. *V. scalaris* species is more highly structured ($F_{ST}=0.483$) than *V. simplex* ($F_{ST}=0.120$). Furthermore, the inbreeding coefficient (FIS) of *V. scalaris* was higher (0.526) than in *V. simplex* (0.170). These data are consistent with the species' mating system. The AMOVA analysis showed low genetic differentiation between species (7.12%). Collectively, the results indicate mating system is a potential prezygotic barrier between these two species, and pointed to the important role of postzygotic barriers to maintain species integrity.

Funding: FAPESP, FAPERJ.

Key words: Hybridization and Introgression; Speciation-with-gene-flow; Mating Systems.

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***Aechmea* and allied genera (Bromelioideae, Bromeliaceae) from Serra dos Órgãos National Park, Rio de Janeiro, Brazil**

Neves, Beatriz¹; Uribbe, Fernando¹; Jacques, Suara¹; Paixão-Souza, Bruno¹; Moura, Ricardo¹; Barros, Ana Angélica² & Costa, Andrea¹

The Serra dos Órgãos National Park (PARNASO) constitutes one of the largest remnants of the Brazilian rainforest in Rio de Janeiro state. In this biome, Bromeliaceae has one of its main centers of diversity, with 31 genera and 803 species, being more than 500 belonging to the subfamily Bromelioideae. The imprecision in the generic limits within Bromelioideae is evident, especially in those groups related to *Aechmea*, such as *Billbergia*, *Hohenbergia* and *Quesnelia*. As a part of the project Bromeliaceae Organensis in development in the Department of Botany of the Museu Nacional- UFRJ, the present work aims to inventory the species of *Aechmea*, *Billbergia*, *Hohenbergia* and *Quesnelia* on PARNASO, providing their morphological description and an identification key, in addition to data about their distribution, in general and inside the park. The taxonomic study was conducted through the analysis of dried specimens, collections and field observations. Six species of *Aechmea*, six of *Billbergia*, one of *Hohenbergia* and four of *Quesnelia*, were revealed. Although well represented in number of species, *Billbergia* is represented by a small number of herbarium records, with four species referenced by one or a few more samples in the collections. The locality is also home to populations of endemic species of Rio de Janeiro state, like *A. wellbachii*, *A. fasciata*, *Q. lateralis* and *Q. edmundoi* var. *edmundoi*.

Funding: CNPq, Capes.

Key words: *Billbergia*; *Hohenbergia*; *Quesnelia*.

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Species boundaries in *Vriesea incurvata* complex: a morphometric and taxonomic study reveal a new taxon

Neves, Beatriz & Costa, Andrea

Bromeliaceae stands out in Brazil for its species richness and high rates of endemism. The genus *Vriesea* constitutes a polyphyletic group that encompasses a lot of species of difficult delimitation, including *Vriesea incurvata*, the object of this study, which shows great morphological variation through its distribution in the

Brazilian Atlantic Forest. Another six taxa are morphologically closely related to the species: *Vriesea atrococcinea*, *Vriesea joyae* (with two varieties), *Vriesea sucrei*, and *Vriesea taritubensis* (with two varieties), that comprise the species complex studied. Our aims were to evaluate morphological variation applying a morphometric study of natural populations; to investigate the taxonomic relationships among these taxa, establishing their limits and validity; and to perform a taxonomic treatment, providing descriptions, key for identification, illustrations and data on geographical distribution. For the morphometric analysis, 241 individuals from 15 populations related to *V. incurvata* were sampled, in which 23 variables were measured. The taxonomic study was performed by the analysis of herbarium specimens, collections, field observations and results of the morphometric analysis. Five species were recognized: *Vriesea incurvata*, *Vriesea sucrei*, *Vriesea taritubensis*, *Vriesea* comb. & stat. nov., and *Vriesea* sp. Four synonymies were proposed: *Vriesea rostrum-aquilae*, *Vriesea atrococcinea*, *Vriesea joyae* var. *joyae* and *Vriesea joyae* var. *parvula*. A lectotype was designated for the synonym *Vriesea duvaliana* sensu Alexander. This review resulted in species being better circumscribed by considering a better sample of the taxa, illustrations and provided more detailed data of their geographical distribution.

Funding: CNPq, Capes.

Key words: Inflated Inflorescence; Multivariate Analysis; Morphology.

Museu Nacional, Universidade Federal do Rio de Janeiro, Brazil.

Ovary and ovule anatomy in the nidularioid complex and its taxonomic utility (Bromelioideae: Bromeliaceae)

Nogueira, Fernanda Mayara¹; Fagundes, Natividade Ferreira²; Kuhn, Sofia Aumond²; Fregonezi, Jeferson Nunes² & Mariath, Jorge Ernesto²

In Bromeliaceae the relationships and monophyly of subfamilies are well supported by morphological and molecular data, but delimitation among genera and species seems to be the most problematic in the Bromelioideae subfamily. The so-called nidularioid complex contains intimately related genera, the circumscription of which has been problematical. The search for morphological features, mainly regarding reproductive characters, has helped enlighten evolutionary processes among different groups. The goal of this work was to describe the ovary and ovule anatomical structures of six species of the nidularioid complex belonging to *Canistropsis*, *Canistrum*, *Edmundoa*, *Neoregelia*, *Nidularium*, and *Wittrockia* and to use these characters in a parsimony analysis of relationships. In all analysed species, schizogenous-type aerenchyma are present within the ovarian mesophyll. *Edmundoa lindenii* has trichomes on the ovary surface, and this character appears as an autapomorphy for the species. *Canistrum aurantiacum* exhibited some autapomorphies, such as four cell layers in the outer integument, non-elongated cells in the nucellar epidermis, and three or four layers of cells in the parietal tissue. In the remaining species of the complex, the presence of transversally oriented ovules and anticlinally elongated cells in the outer integument at the micropylar region appear as synapomorphies. *Canistrum aurantiacum* exhibits only a few characters in common with the other species, and our results, as well as the latest phylogeny, show that *Canistrum* species do not belong to the current nidularioid clade.

Key words: Plant Embryology; Aerenchyma; Trichomes.

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Diversity of species, altitudinal gradient and flowering season of *Vriesea* in Bahia, Brazil

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Vriesea is the third largest genus of Bromeliaceae, more than 300 species are found in the Neotropics and 219 species in Brazil, with 208 (~ 95%) endemic to the country, focused mainly in the rainforests of eastern Brazil, which is one of the diversity centers of the genus. Recent studies indicate that mountainous areas, such

as the Espinhaço Range, act as the western boundary of distribution, in addition to being an additional center for diversification of the genus. With a large variety of soils, topography and climate, the state of Bahia has different and diverse phylogeographic areas such as savanna, cerrado, grasslands with rock outcrops, tropical deciduous forests, rainforests, presenting also two major centers of endemism: the Chapada Diamantina in Espinhaço Range domain and Atlantic Rainforest of eastern Bahia. Knowledge of *Vriesea* diversity in Bahia has increased in recent decades, with publications of new species year after year, however, a large number of undetermined collections or doubtful are still observed in the herbaria. Due to its great ecological importance and high number of species found in Bahia, this study focused on increasing knowledge of the taxa from *Vriesea* in Bahia and understanding of a possible relationship between flowering season and the distribution of species. A total of 52 species of *Vriesea* was recognized, with two pikes in season flowering, probably related to the two majors centers of occurrence. The species were observed, collected, and the altitudinal gradient was registered to compare the two centers. The rainy season can be related to flowering, which showed first and second peak of blossoming. Tables with flowering season, altitudinal gradient and similarity of species are provided.

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Key words: Bromeliaceae; Tillandsioideae; Northeast Brazil.

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Phylogeny of Nidularioid Complex (Bromelioideae) based on plastid and nuclear DNA sequences and morphological data

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Bromeliaceae (Poales) is one of the most important Neotropical epiphyte families. Although this family is considered a monophyletic group, the relationship between some genera is confusing, especially in Bromelioideae. In this subfamily, the Nidularioid complex, which comprises the genera *Nidularium* Lem., *Wittrockia* Lindm., *Canistropsis* (Mez) Leme, *Canistrum* E. Morren, *Neoregelia* L.B.Sm. and *Edmundoa* Leme, presents a confusing circumscription. A recent phylogeny shows that some of these genera are not even monophyletic in their current circumscription. Thus, this work aims to provide morphological and anatomical characters of the vegetative and reproductive organs that can help understanding of the relationship between these, and to propose a new phylogeny based on molecular and morphological data. We sampled 28 species, including all genera of the Nidularioid Complex plus *Aechmea*, and *Quesnelia* Gaudich. as outgroups. DNA sequences of *atpB-rbcL* spacer, *trnL-trnF* spacer, *MatK* and *PhyC* were obtained from GenBank and aligned with Muscle. We also described 96 characters from the morphological and anatomical data. Bayesian analysis was made with Mr. Bayes using combined molecular and morphological data and the resulting phylogeny was summarized in a consensus tree, rooted in *Aechmea bromeliifolia*. For morphological ancestral state reconstruction, we used maximum parsimony method in Mesquite. Our analysis shows that no genera in the Nidularioid complex are monophyletic in their current circumscription. It also shows that the Nidularioid clade comprises *Nidularium*, *Neoregelia*, *Wittrockia* and *Edmundoa* excluding *Canistrum*. On the other hand, *Canistrum* appears in our analyses as sister group of *Aechmea*, sharing the presence of asymmetric sepals. *Aechmea* and *Quesnelia* appears as the sister group of Nidularioid clade. Our results also show that *Aechmea* is not a monophyletic group, as described by other studies. The Nidularioid complex may be considered monophyletic if we exclude *Canistrum*, corroborating what previous taxonomic studies have suggested. In this case, the Nidularioid clade is supported morphologically by the absence of isolated extra-fascicular fibrous strands in sheath mesophyll. Another interesting feature is the presence of elongated wing cells in paradermal view of trichomes in species of the Nidularioid complex s.l. (including *Canistrum*), that are rounded in the other genera studied.

Funding: CNPq.

Key words: Nidularioid Complex; Phylogeny; Morphological Data.

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Insights into the biodiversity of *Dyckia* from Brazilian Pantanal and chaco of Mato Grosso do Sul

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We present some insights of selected, collaborative recently published or unpublished research into the biological and genetic diversity of bromeliad species, most of which focus on *Dyckia* (Pitcairnioideae), occurring mainly in the diagonal of dry areas from the Brazilian Pantanal to Chaco, in Mato Grosso do Sul state (MS), Brazil. The checklist of MS showed 11 genera and 41 spp, with the subfamily Pitcairnioideae being the richest in species (5 genera / 18 spp.). *Dyckia* presented 13 species, four of them endemic to MS. The second richest genus was *Tillandsia* with 12 species, with only *T. bonita* Versieux & Martinelli endemic to MS. Because of the richness observed in these two genera, and some inconsistencies observed in the identification of some taxa, it was suggested that a taxonomic study of *Dyckia* and *Tillandsia* from MS should be done. The second work involved an improvement in the knowledge of the biodiversity of *Dyckia* species, as *Dyckia excelsa* Leme was rediscovered, a species known only from the description of one individual in a private collection. In this work, we described three natural populations of *D. excelsa* located in ironstone outcrops or “cangas” from Corumbá city, MS. It was the first record of *D. excelsa* in nature; apparently, it is also endemic to MS, as it was first described as occurring in Mato Grosso State (MT), but with no precise local. Recently we started to study the population genetics of *D. excelsa* and *D. leptostachya* Baker, using cross-amplification of nuclear microsatellite loci. Also, we are studying the phylogeography of *Dyckia* species from MS. Preliminary results in population genetics of *D. leptostachya* wild populations, based on four nuclear microsatellite loci, have shown high genetic diversity in this self-compatible species (observed and expected heterozygosity varied from 0.192 to 0.941 and from 0.491 to 0.677, respectively). For *D. excelsa* we were able to amplify eight of the ten microsatellites loci tested, using touchdown amplification protocols; a complete population genetic analysis is ongoing for this species. For the initial phylogeographical studies we chose four species endemic to MS (*Dyckia grandidentata*, *D. pottiorum*, *D. excelsa* and *Dyckia* sp.), aiming to understand the evolutionary history of this group and contribute to a better understanding of the Brazilian Cerrado Biome (BCB) history. The samples were collected at seven localities, totaling 18 populations and 368 individuals, in which we tested three plastidial sequences (rpL32-trnL, rps16-trnK, trnD-trnT) from populations throughout the known distribution of all four species. The preliminary results showed some differences in molecular sequences among species. The analyses are in progress and will provide valuable insights into the historical processes underlying the diversification of those species in the dry diagonal region of Brazil, a poorly known region in terms of biodiversity and conservation.

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Key words: Conservation; Endemism; Genetic Diversity.

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Pollination biology of *Deuterocohnia meziana* kuntz ex. Mez (Bromeliaceae) from ironstone outcrops, Mato Grosso do Sul, Brazil

Paggi, Gecele Matos; Arruda, Kelly Conceição Rondon; Finati-Alves, Diego & Lenzi, Maurício

Bromeliads are perennial herbs that play important ecological functions in different environments. The family comprises approximately 3,000 species in 58 genera and eight subfamilies. In this family the mating systems vary widely, from autonomous to obligate outcrossing species. Also, studies on phenology are important as they describe the stages of life cycles or activities in a timeline throughout a year; especially because the reproductive period is a very important phase for population dynamics and reproductive success of a species. In Bromeliaceae, ornithophily is the predominant syndrome, but melittophily, psicophily and chiropterophily

also occur. *Deuterocohnia meiziana* Kuntz ex. Mez (Pitcairnioideae) is distributed on ironstone outcrops, where environmental characteristics impose inhospitable conditions on the vegetation. The plants are aggregated forming islands of vegetation, leading to increased substrate volume, which can help to reduce soil exposure allowing water conservation and mitigating the severe environmental conditions. In this study, we evaluated the pollination biology of *D. meiziana*, considering observations of anthesis, stigma receptivity and floral visitors. To describe anthesis, two flower buds were marked at pre-anthesis in five individuals, the flower buds were observed for 12 hours to verify anthesis morphology and duration. Stigma receptivity was tested in four flowers per individual at different intervals of time during anthesis, five plants were used as replicates (n = 20). The flowers were bagged and the stigma receptivity was tested observing the catalysis activity of H₂O₂ (10% by volume). We observed pollinator visits for two days. Results showed that *D. meiziana* has a period of anthesis lasting, on average, three days, from bud to complete flower opening. Stigma receptivity occurs when the flower is completely open by 7:00 am, until its closing, in the evening at 7:00 pm. Pollen is already collected in the early hours of anthesis. Hummingbirds are the most frequent visitors, followed by bees. *D. meiziana* possibly need pollinators for sexual reproductive success, for this reason, it supplies floral resources (pollen and nectar) throughout anthesis. To prevent predation of flower buds, flowers and fruits, the species offers extra-floral resources, as do other species of bromeliads.

Funding: FUNDECT, CNPq.

Key words: Reproductive Biology; Conservation; *Bromelia*.

Universidade Federal de Mato Grosso do Sul.

How important is hybridization for speciation in Bromeliaceae?

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Hybridization is a common and widespread phenomenon, and a larger proportion of extant non-hybridizing species are likely to have hybridized in the past. Hence, understanding the importance of hybridization as a mechanism in the evolution of species biodiversity is extremely important. How much gene flow occurs during the early stages of divergence is also essential for understanding the evolution of reproductive isolation barriers. The degree of reproductive isolation among related species is an important factor influencing species genetic integrity and hybrid formation. Here we do not intend to debate species concepts or to discuss whether species are real entities. Rather we aim to investigate the consequences hybridization and introgression actually have on the diversification process and in structuring biodiversity in the large, adaptive radiation of the Bromeliaceae family. We will debate: 1) the potential of hybridization for generating adaptive variation, functional novelty and new species, and 2) the evolution of reproductive isolating barriers (pre-versus post-zygotic barriers) that prevent hybridization between diverging lineages. Bromeliads are largely cross-species and -genera compatible and easily form artificial hybrids, records of natural hybridization are available mainly for the genera *Tillandsia*, *Vriesea*, *Pitcairnia*, *Fosterella* and *Puya*. We have studied intra- and inter-specific gene exchange among species from two bromeliads genera (*Vriesea* – Tillandsioideae) and *Pitcairnia* (Pitcairnioideae). *Vriesea* are usually epiphytes and forest dwelling species, while *Pitcairnia* are saxicolous, adapted to rock outcrops. Population structure, demography and hybridization among closely related species from these ecologically contrasting genera will be presented and discussed.

Funding: Fapesp.

Key words: Hybridization; Speciation; Neotropics.

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Genome size evolution in Bromeliaceae

Paule, Juraj; Heller, Sascha & Zizka, Georg

Bromeliaceae is an important Neotropical family mainly due to its distinctive biological and ecological features such as epiphytic life form and the presence of “tank habit”, which are considered key to the fast adaptive radiation of the family. However, evolutionary forces causing chromosomal speciation and adaptation are still enigmatic. Hence, Bromeliaceae might be considered as model and an example for the effect of adaptive radiation on chromosomal diversification. To fully understand the diversity, function, and evolution of plant genomes, an approach synthesizing information from several disciplines is desirable. In addition to chromosome counts, genome size estimations using flow cytometry represent a comprehensive insight into genome evolution. Published and newly collected data from the family Bromeliaceae were analyzed and interpreted in a phylogenetic framework. Preliminary data show significant differences between the subfamilies. The highest overall diversity found in the subfamily Tillandsioideae could be attributed to the activity of transposable elements, translocations or inversions. Moreover, polyploidy seems to be much more common than previously assumed and was found across the whole family. This is documented in more detail in a case study of the subfamily Bromelioideae as well as in two genera from the subfamily Pitcairnioideae (*Fosterella* and *Deuterocohnia*). Finally, for *Fosterella*, geographical and ecological patterns of the ploidy distribution have been recognized.

Funding: DFG, DAAD.

Key words: Genome Size; Polyploidy; C-value.

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The effects of nutritional and water deficiencies on CAM induction in the leaves of the bromeliad *Guzmania monostachia*

Pereira, Paula Natália & Mercier, Helenice

Leaves of *Guzmania monostachia*, an facultative C3–CAM, epiphytic bromeliad, when subjected to a water deficit, have been shown to perform stronger CAM photosynthesis in the apical compared with the basal portion of the leaf blade. To investigate the possible interaction of nutrient deficiencies and water deficits in CAM induction, detached leaves of *G. monostachia* plants (2 years old) were subjected to treatment for seven days using 30% PEG 6000 plus modified Knudson solution deficient in N, P, K, or Ca. After determining that nitrogen had the greatest influence on the CAM pathway, a further experiment was conducted to test the influence of alternative nitrogen sources on this metabolism. This experiment consisted of supplying the detached leaves with either 5 mM NO₃⁻ or NH₄⁺ in modified Knudson solution, in the presence of 30% PEG 6000. To assay CAM activity, nocturnal malate and citrate contents were measured together with extractable PEPC and PEPC activities in the apical, middle, and basal portions of the leaves, in combination with determinations of relative water content. We observed that nitrate deficiencies, plus the presence of ammonium, combined with a water deficit, were the most important factors affecting CAM induction in the apical portion of the leaves of the *G. monostachia*. Furthermore, the activity of antioxidant enzymes (glutathione reductase, GR; superoxide dismutase, SOD; catalase, CAT and ascorbate peroxidase, APX) were verified in order to test whether or not NH₄⁺ nutrition improves the capacity of *G. monostachia* leaves to limit oxidative damage by stimulating the activities of the antioxidant enzymes. These results would suggest that the CAM photosynthesis was maintained due to an increased PEPC activity provided by the presence of ammonium.

Funding: Capes, Fapesp.

Key words: Bromeliad; Cam; Nutrients.

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Phylogeny of *Tillandsia* subg. *Tillandsia* (Tillandsioideae) II: Evidence from nuclear DNA regions

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Previous results from plastid DNA regions showed that *Tillandsia* subg. *Tillandsia* is not monophyletic, with its characteristics (exserted stamens and style) appearing at least twice in the course of evolution of genus *Tillandsia*. Moreover, some of the species of *Tillandsia* subg. *Allardtia* were nested within the main clade of the subgenus *Tillandsia* s. str. Some internal structure was evident, standing out the Mexican Clade, the *T. utriculata* clade, the *T. limbata* clade, the *T. circinnatioides* clade and the Core Group I Clade. However, species-level relationships were far from being resolved; especially within the Core Group I Clade, which showed a large polytomy of more than 50 species. To test the hypothesis that rose from plastid DNA and to try to get more internal phylogenetic structure, we explored four nuclear DNA regions from which we selected PhyC (Phytochrome C gene) and PRK (Phosphoribulokinase exons 2–5) and performed Bayesian inference analyses, Maximum Parsimony analyses and Bootstrap resampling to test clade support. The genus *Tillandsia* resulted as paraphyletic, with *Racinaea* and some species of *Vriesea* inserted. In contrast with plastid DNA results, deep relationships of *Tillandsia* were not resolved, and the status of subgenus *Tillandsia* was not possible to establish. However, some previously internal clades of subgenus *Tillandsia* s. str. were confirmed and some other unknown clades were recovered: *T. utriculata* and the *T. limbata* clades form a monophyletic group, including *T. fuchsii* and *T. filifolia*; some lytrophic large species represented by *T. parryi* were grouped together. The Core Group I Clade was also obtained although with low support. Nevertheless, some species appeared forming clades: the neotenic-like *Tillandsia kammii* and *T. hondurensis*, as well as *T. plagiotropica* and *T. velutina*; the grass-like *T. remota* and *T. pseudosetacea* (with *Allardtia*-like flowers); the silver leaved *T. weberi* and *T. circinnatioides* and *T. pueblensis*-*T. schatzlii* were also grouped together. According to these results it can be predicted that the combination of plastid and nuclear DNA evidence should help to better understand the phylogeny and evolution of subgenus *Tillandsia*, the former resolving the deeper clades and the latter helping to elucidate more species-level relationships.

Key words: Phylogeny; *Tillandsia*; Nuclear DNA.

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The diversity of the Bromeliaceae of Mexico from a phylogenetic perspective

Pinzón, Juan¹; Ramírez-Morillo, Ivón² & Carnevali, Germán²

Bromeliaceae is composed by 3,400 species, 398 of which occur in Mexico, accounting for the 11.6% of total. However, not all groups of bromeliads are represented; of the eight subfamilies, only four are found in Mexico, being Hechtioideae and Tillandsioideae the most representative, but also Pitcairnioideae and Bromelioideae are present. In the case of Hechtioideae, which only genus is *Hechtia*, 20 new species have been discovered since the last checklist of bromeliads from Mexico in 2004, to make a total of 76, (97% of the total); *Hechtia* represents the most basal bromeliads found in Mexico, mainly distributed in arid habitats and probably originated in Central America but radiating in Mexico. Pitcairnioideae s. str. is only represented by two genera: *Pitcairnia* (including *Pepinia*) with 48 species (12.3% of the total of this genus), and *Fosterella* (only *F. micrantha*); in contrast with *Pitcairnia*, *Fosterella* is well derived within Pitcairnioideae, both originating in South America. Bromelioideae is relatively poorly represented in Mexico with only 22 species. Of the basal Bromelioideae, only four species of *Bromelia* and two of *Greigia* are found. The Core Bromelioideae Clade is also poor in Mexico; however all species of the genera *Androlepis*, *Hohenbergiopsis*, and *Ursulaea* are distributed in Mexico, and along with *Billbergia viridiflora*, *Hohenbergiopsis guatemalensis*, and two species of *Aechmea*, form a clade originated in Mexico. *Billbergia pallidiflora* is the only member of true *Billbergia* in Mexico. Subgenera *Aechmea* and *Platyachmea* are only represented by one species each;

these and *A. matudae* are probably the result of three independent colonizations of the polyphyletic *Aechmea* from South America. The recent discovery of *Hohenbergia mesoamericana* accounts for the only register of this genus in Mesoamerica, invading probably from the Antilles. The most diverse subfamily in Mexico is Tillandsioideae with 251 species. *Catopsis* is the most early-divergent member of this subfamily in Mexico, with uncertain origin, but radiating in Mexico (with 79% of the total species). Tribe Vrieseae is represented by seven species of *Werauhia*; the two species of Mexican *Vriesea* are nested within Tillandsieae. There are only two species of *Guzmania* and *Racinaea* each, but *Tillandsia* is the most diverse genus in the country with 223 species (35.5% of total). There are four independent lineages of *Tillandsia* in Mexico: two species of subg. *Diaphoranthema*, the mesic *Tillandsia* of Group III, three species of subg. *Pseudalcantarea*, and the diverse subg. *Tillandsia* s. str. of which more than 20 species have been described since 2004. According to the species discovery rate there are probably still undescribed species in *Tillandsia* and *Hechtia*. Plus, some species need to be reassigned in other genera, so more phylogenetic analyses are needed to clarify the nomenclature and phylogenetic diversity of this family in Mexico.

Key words: Bromeliaceae; Diversity; Mexico.

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New records in *Tillandsia* L. (Bromeliaceae, Tillandsioideae) in Rio Grande do Sul, Brazil

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The genus *Tillandsia* L. is the largest genus in Bromeliaceae, with about 700 species (Gouda *et al.* 2014). In Brazil 87 species of *Tillandsia* are cited, and of these 50 are endemic (Forzza *et al.* 2014). For the state of Rio Grande do Sul (southern Brazil) 28 species of *Tillandsia* are cited, corresponding to about 32% of the species of this genus in all of Brazil, being the Brazilian state with the highest species diversity in *Tillandsia* (Forzza *et al.* 2014; Büneker *et al.* 2014). Based on the field collections, literature review and specimens deposited in herbaria, two new records of *Tillandsia* for Rio Grande do Sul are presented, *Tillandsia loliacea* Mart. ex Schult.f. and *Tillandsia pohliana* Mez, cited previously only to other Brazilian states and other countries. Furthermore, the occurrence of *Tillandsia bandensis* Baker to Brazil is confirmed after 57 years of its exclusion from the flora by Smith (1956). *T. bandensis* occurs in Argentina, Bolivia, Paraguay, Uruguay, and has been documented again in Brazil. This is restricted to the western region of Rio Grande do Sul state, in the Pampa Biome, where the species is saxicolous or rupicolous. *T. bandensis* can be recognized by its conspicuous, fragrant flowers, blue petals and vinaceous floral bracts. *T. loliacea* occurs according to Smith & Downs (1977) in Argentina, Bolivia, Brazil and Paraguay. In the list of species of Flora of Brazil (Forzza *et al.* 2014) its occurrence is recorded for the Federal District and the states of Alagoas, Bahia, Ceará, Piauí, Rio Grande do Norte, Sergipe, Mato Grosso do Sul, Mato Grosso, Espírito Santo, Minas Gerais, São Paulo and Paraná. In Rio Grande do Sul it was found as an epiphyte in clumps of forest, in trees and open vegetation associated with cornices and hills in the western region of the state. *T. loliacea* can be easily recognized as having densely cinereum-lepidote leaves and distichous flowers. *T. pohliana* occurs, according Tardivo (2002), in the Andes region of Peru, Bolivia, Argentina and Brazil. In Brazil it occurs in the Federal District and in the states of Ceará, Paraíba, Pernambuco, Goiás, Mato Grosso do Sul, Mato Grosso, Minas Gerais, São Paulo and Paraná (Forzza *et al.* 2014). In Rio Grande do Sul two populations were located: one as an epiphyte in clumps of forest, in trees and open vegetation associated with cornices and hills in the western region of the state; and the other, atypically as an epiphyte in riparian canopy in the central region of the state. *T. pohliana* can be easily recognized by its pink-orange to yellow-green floral bracts, broadly oval-elliptical and inflated, white petals, and robust capsules.

Funding: Colégio Politécnico da Universidade Federal de Santa Maria.

Key words: *Tillandsia bandensis*; *Tillandsia loliacea*; *Tillandsia pohliana*.

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The flora and vegetation of tropical inselbergs: results and perspectives

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Mixed pollination system in *Encholirium spectabile*: a Bromeliaceae from inselbergs pollinated by bats, opossums and hummingbirds

Queiroz, Joel¹; Quirino, Zelma²; Lopes, Ariadna¹ & Machado, Isabel Cristina¹

Plant species primarily pollinated by bats but with anthesis period extended beyond the nighttime may also be pollinated by diurnal animals, such as hummingbirds. In the present study, we analyzed nectar traits, frequency, behavior and effectiveness of day- and nighttime pollinators of *Encholirium spectabile*, a bromeliad common on rocky outcrops of the semiarid of northeastern Brazil. Nectar production began at 1700 h, and was continuous and lasted for 18 h. The total sugar amount produced in the lifetime of a flower was not affected by removals. Those values were similar to other species of chiropterophilous bromeliads, and were also compatible with the requirements of hummingbirds. We also recorded, for the first time, pollination by opossum in a bromeliad and in Caatinga vegetation. Observations on frequency and behavior of *Didelphis albiventris* revealed the effectiveness of this marsupial in promoting pollen flow between individuals of *E. spectabile* during most of the flowering period. These results suggest a mixed pollination system in this bromeliad, which can be extremely advantageous in Caatinga inselbergs, a dry seasonal environment, which can be affected by fluctuations in the richness and abundance of pollinators.

Key words: Chiropterophily; Ornithophily; Nectar Secretion Pattern.

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Morphological variability in Chilean *Puya* species: preliminary results and implications for future climatic scenarios

Quezada, Iván

Mediterranean climate regions will be under heavy pressure in the forthcoming years due to global climate change. In this context, the ability of plant species to tolerate climatic variations will be of key importance for the maintenance or expansion of their distribution ranges under the new climatic scenario. The Mediterranean climate zone of central Chile spans approximately from 29° to 37°S. In this region precipitation increases and temperature decreases towards south. This zone is recognized as a biodiversity hotspot and is of key importance as home to significant endemic species and as the probable center of origin of important taxa. Six of the seven Chilean *Puya* species are distributed within this area. All of these species are adapted to face arid and semiarid conditions, permanent or seasonal, and have different latitudinal ranges, with some spanning along a few degrees of latitude and others through the entire Mediterranean region. Future climate projections show an increase in mean temperatures and a decrease in precipitation rates in this area. This will directly affect the distribution of plant species, with those better suited to respond to the new conditions probably maintaining or expanding their ranges. An important feature to counter environmental changes is the flexibility in the expression of key traits. In this work I evaluated morphological variability in 4 Chilean *Puya* species: *P. gilmartiniae*, *P. venusta*, *P. chilensis* and *P. alpestris* subsp. *zoellneri*, aiming to correlate morphological variability -or the lack of it- with the extension of the geographic range of each species and, by extension, with the climatic variability these species face within those ranges and the flexibility they might show under possible future climatic scenarios. I measured and compared a small set of leaf and whole plant attributes between populations distributed along the geographic range of each species. For all traits, variability was significantly higher in the species with broader latitudinal ranges (*P. chilensis* and *P. alpes-*

tris subsp. *zoellneri*). Lack of variability could, on the other hand, explain the reduced geographical range of *P. gilmartinae* and *P. venusta*, and predict a reduced response to climate change, leading to catastrophic consequences for both. These results, however, are preliminary and should be taken cautiously. It would be advisable to increase the number of populations per species to account for any environmental variation within geographic ranges, especially for the species with small distribution areas. Lack of morphological variation could also be explained by phenomena such as countergradient variation, which should be evaluated. Furthermore, the detected variation, if any, might not be heritable, thus losing any potential influence over selection. Nonetheless, these preliminary results give valuable insight on intraspecific variation for *Puya* species, and are important as a starting point for future research.

Funding: CONACyT.

Key words: Mediterranean Climate; *Puya*; Variability.

Universidad de Concepción, Chile.

Phylogeny, evolution and biogeography of *Hechtia* Klotszsch (Hechtioideae: Bromeliaceae)

Ramírez, Ivón¹, Pinzón, Juan²; Carnevali, Germán¹ & Castillo, Amílcar¹

Hechtia comprises more than 79 species restricted to dry or seasonally dry ecosystems of Megamexico III but with most species occurring in tropical Mexico, particularly in Oaxaca, Guerrero and Querétaro; fewer species occur in north-central Mexico or south of the Tehuantepec isthmus into northern Nicaragua. Species range from sea-level to up to 2500 m elevation. Most *Hechtia* species are saxicolous or terrestrial in well-drained, rocky soils where they are a conspicuous element of xerophytic shrublands, along with other rosetophilous plant groups. Plants are succulent, sympodial to pseudomonopodial rosettes with spiny leaves and terminal or lateral inflorescences, unisexual flowers and dioecy. We tested the following hypotheses using morphological and molecular data: 1. Based on a unique set of characters in the genus (unisexual flowers, sessile stigma, and dioecy), *Hechtia* is monophyletic. 2. The genus is tentatively composed of seven alliances defined by morphological characters associated with geographical distribution; these alliances should be monophyletic as well. 3. If physiographic accidents in Mexico played an important role in the diversification of the genus, the seven proposed alliances should be confined each to a particular biogeographically region. 4. The ancestor of *Hechtia* invaded Megamexico III from the south, and it featured a strictly sympodial growth habit (central inflorescence) and, therefore, the clade including species with these features and distributed in the southern portion of Megamexico III, should be basal in the resulting phylogeny. Preliminary results based on cpDNA (regions *ycf1*(6) and *rpl32*) of 63 *Hechtia* species, representing ca. 80% of the genus, and 18 species as outgroups, indicate that *Hechtia* is monophyletic. Internally, the analysis resulted in a trichotomy with a clade including *Hechtia guatemalensis* and *H. dicroantha* from Tierras Altas de Chiapas and Tierras Bajas del Pacific provinces; a second clade with species characterized by a strictly sympodial growth pattern, leaves minutely serrate, flowers pedicellate, with lilac or white petals, and found in the provinces of Sierra Madre Oriental, Provincia Veracruzana, and Eje Volcánico Transmexicano (*H. tillandsioides* Alliance); and a third, poorly resolved clade, including species from the other five alliances.

Funding: CONACyT.

Key words: *Hechtia*; Phylogeny; Dioecy.

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Reproductive biology in Mexican Tillandsias

Ramírez, Ivón¹ & Mondragón, Demetria²

Genus *Tillandsia* L. is the largest bromeliaceous genus in the family with ca. 622 species (representing ca. 19% of the family). In Mexico, *Tillandsia* is also the largest bromeliaceous genus with ca. 175 species (53% of ca. 400 species reported in the country). Most of the species of Mexican *Tillandsia* belong to subgenus

Tillandsia and few to subgenera *Allardtia*, *Diaphoranthema*, *Pseudoalcantarea*, and *Pseudo-catopsis*. Species in the subgenus *Tillandsia* are characterized by their epiphytic habit, inflorescences mainly racemes, odorless flowers with diurnal anthesis, tubular corollas, these lilac, yellow, purple or blue, with stamens and stigma exerted, and abundant nectar, suggesting xenogamy and pollination by hummingbirds. *Tillandsia* is one of the most important genera in horticulture in the world. We have data (from literature and our own) on breeding systems for more than 20 *Tillandsia* species native to Mexico, that show that even though floral morphology suggests xenogamy, based on fruit and seed production from experimental crosses, more breeding systems could be operating in nature. Breeding systems range from agamospermy (e.g. *T. prodigiosa*), self-incompatibility and pollinator dependent (*T. streptophylla*) to species with combined potential breeding systems such as selfing, unassisted pollination, and cross-pollination (*T. dasyliirifolia*). In order to know what breeding system(s) is (are) operating in self-compatible species, further genetic analysis should be performed as floral morphology and results on experimental crosses do not always support predictions. Examples on studies that integrate information from different fields on Mexican *Tillandsia* show that other factors besides breeding system play a more important role in the genetic composition of populations, such as seed dispersal distance (in *T. elongata* var. *subimbricata*), and clonal propagation (in *T. brachycaulos*).

Funding: CONACyT.

Key words: Breeding System; *Tillandsia*; Genetics.

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Reproductive biology of *Aechmea blanchetiana* (Baker) L.B.Sm (Bromeliaceae) in Restinga of Guriri, São Mateus/ES, Brasil

Ribeiro, Izabela Ferreira; Menezes, Luis Fernando Tavares & Matallana, Gloria

Aechmea blanchetiana, found in Restinga of Guriri, São Mateus/ES Brasil presents self-compatibility and mixed reproductive strategies from the index of self-compatibility (1.49) and self-incompatibility (1.40), considered almost five times the number limit for the self-compatibility. The Kruskal Wallis statistical test confirms the indices with a $p > 0.05$. The species is considered ornithophilous, with a nectar volume of 5.6 μ l and sugar concentration above 33%, expected for species visited by hummingbirds. In 20 hours of observation the following animals were recorded as pollinators: hummingbirds, *Thalurania glaucopis* (Gmelin 1788), *Eupetomena macroura* (Gmelin 1788), *Amazilia fimbriata* (Gmelin 1788) and *Amazilia* sp.; moths, *Lychnuchoides oazis oazis* (Hewitson 1878); and butterflies, *Heliconius erato* (Linnaeus 1758) and *Phoebis* sp. All are considered effective pollinators, pointing that the species present secondary pollination syndromes. The viability of the pollen in *A. blanchetiana*, above 88%, suggests that the species has more viable pollen grains per flower bud. The penetration of eggs occurred in all treatments of either 24 or 48 hours and highly successful germination of seeds occurred in all treatments, with no observable statistically significant difference between the treatments ($p > 0.05$). From these results the species appears able to persist in the Restinga de Guriri regardless of the reproductive strategy used.

Funding: FAPES.

Key words: Bromeliaceae; Pollination Syndromes; Self-compatibility.

Universidade Federal do Espírito Santo, Brazil.

Pollination ecology of *Lymania azurea* (Bromeliaceae): an endemic and threatened species from the Atlantic Rainforest

Rocca, Márcia¹ & Marinho, Felipe²

Lymania azurea is an epiphyte or a terrestrial species, occurring aggregately in the Atlantic Rainforest understory in southern Bahia. This species flowers from September to November, displaying mostly an intermediate annual pattern of flowering. But surprisingly, one individual (genet) produced two inflorescences (each one

on a different ramet) in an anthropogenic forest edge (a road) during the same year. It may open from two to four flowers per individual. Its inflorescence may bear 30 to 208 floral buds, with plants in the understory bearing fewer flower buds than plants near the forest edge. Flowers are tubular, the effective corolla is 1.2 cm long, petals are white to blue, and the calyx is green. There is no marked scent. By the end of the morning (1100–1200h), nectar volume and sugar concentration were 13.3 μ L (\pm 3.5) and 29.0% (\pm 4.5), respectively. Anthesis begins at 0230h and it takes about 14 h. *Lymania azurea* is predominantly self-incompatible, therefore it is highly dependent upon pollinators to ensure its reproductive success. A few hummingbird species were observed visiting its flowers, and *Phaethornis ruber* (Trochilidae) was the principal pollinator, making 77% of legitimate visits and taking pollen loads on the bill. Bees were also observed visiting flowers: the long-tongued bee *Bombus (Fervidobombus) morio* was considered a secondary pollinator if present and short-tongued bees were always pollen thieves. Mites were also observed and could interfere in exclusion experiments, transferring some pollen and producing a few seeds in bagged flowers. Fruits without seeds were also produced (probably at the very beginning of the reproductive season when few plants are in flower and available as mates) and they may improve the overall dispersal of seeds in the understory. Even though, almost whole infructescences were observed, with berries left behind and ignored, already dry near the forest edge. More light at forest edges gives more energy to plants and individuals of *L. azurea* may transfer this input into reproductive output, producing more buds, flowers, and seeds, allowing for cross pollination to occur. In spite of this, limitation in dispersion may occur in this species in forest edges.

Funding: FAPESB, FAPITEC/SE.

Key words: Reproductive Phenology; Floral Biology; Floral Visitors.

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HPLC analysis of Bromeliaceae species with therapeutic activity for the development of herbal medicines

Rolim, Larissa Araújo

Some species of the Bromeliaceae family are widely distributed in the Brazilian semiarid and are used by the local population as an excellent source of hard fiber. Such uses include that by artisans of the San Francisco Valley region for the manufacture of wicker baskets, by the textile industry and by landscapers as ornamental plants. In addition, these species are quite often fed to goats, which with the predatory extraction of man, leads to the condition of endangered species. It was decided, therefore, to investigate *Bromelia laciniosa*, *Encholirium spectabile* and *Neoglaziovia variegata* before they become extinct without their chemical and pharmacological properties being known. Considering the large number of species of this family, few of them have been studied chemically yet. Nevertheless, there is a considerable amount of identified compounds, which mainly belong to the classes of flavonoids and triterpenoids related to various pharmacological activities. Work developed by the Universidade Federal do Vale do São Francisco showed that crude extracts of species of bromeliads have antinociceptive activity, peripheral and central analgesic activity, antimicrobial activity and sunscreen. Therefore, it is extremely important to qualitatively and quantitatively study the secondary metabolites by high performance liquid chromatography (HPLC -DAD), in order to identify possible chemical and pharmacological markers. Chemical assessment with the development of analytical methods by HPLC - DAD of some species of Bromeliaceae plants, identified and quantified the presence of bioactive substances, seeking the use of these extracts with therapeutic activity. The goal to this research is to contribute scientifically to the industrial production of herbal medicines from Bromeliaceae species.

Funding: FACEPE.

Key words: Analysis; HPLC; Activity.

Universidade Federal do Vale do São Francisco, Brazil.

Cross-amplification of 10 microsatellites loci in bromeliad species from Pantanal and Chaco, Mato Grosso do Sul, Brazil

Ruas, Renata & Paggi, Gecele

The Bromeliaceae family have ca. 3,000 species, traditionally subdivided into the subfamilies Pitcairnioideae, Tillandsioideae and Bromelioideae, which are exclusively distributed in Americas. Bromeliads have been threatened by anthropic pressures due to predatory gathering and habitat destruction, being used as ornamental, forage, food and medical uses. Ironstone outcrops may be considered a peculiar feature of Corumbá and Ladário cities, which are located in the Pantanal wetlands of the state of Mato Grosso do Sul State (MS), Brazil. Chaco or “Gran Chaco” is the term applied to seasonal vegetation, which is divided into an eastern sector, with a more humid climate, and a western, drier sector, whilst the submeridional lowlands include a large depression between these two areas. In Brazil, Chaco vegetation is restricted to the western border of MS, in Porto Murtinho. The bromeliads from the genera *Aechmea*, *Bromelia*, *Deuterocohnia*, *Dyckia* and *Tillandsia*, in these wetland regions, occur as terrestrial, rupicolous or epiphytic plants in these biomes. In this study, we used nine bromeliad species, Bromelioideae - *Aechmea distichantha* Lem., *Bromelia balansae* Mez, *B. hieronymi* Mez; Pitcairnioideae - *Deuterocohnia meziana* Kuntze ex Mez, *Dyckia excelsa* Leme, *D. leptostachya* Baker; Tillandsioideae - *Tillandsia bulchlohii* Rauh, *T. loliaceae* Mart. ex Schult. & Schult.f. and *T. streptocarpa* Baker in order to evaluate the cross-amplification rate of 10 microsatellites loci developed for other bromeliad species: *Dyckia distachya*, *Dyckia marnier-lapostollei* var. *estesvii* (Pitcairnioideae), *Ananas comosus* and *Aechmea caudata* (Bromelioideae). We conducted DNA extractions using the CTAB protocol in a sample of 2 individuals of each species (n = 18). We performed PCR reactions following the protocols previously developed by our research group. The PCR products were analyzed on 1% agarose gels stained with SyberGreen (Invitrogen) and visualized on a blue light transilluminator. For Pitcairnioideae species, 80% of primers amplified satisfactorily (4 from 5 primers), without any correlation with the primer original subfamily (Pitcairnioideae or Bromelioideae). For Bromelioideae species, on average, 60% of primers amplified satisfactorily (3 from 5 primers of Pitcairnioideae primers), and 87% of primers amplified satisfactorily (4.3 from 5 primers of Bromelioideae primers). For Tillandsioideae species, none of the primers amplified adequately. In *B. balansae* nine from 10 primers amplified with expected fragment sizes. *Aechmea distichantha*, *D. meziana* and *D. excelsa* presented 80% of amplification. A good set of polymorphic microsatellite loci are necessary to perform various studies concerning population genetics and conservation. Determining cross-amplification protocols for microsatellite loci in different native bromeliad species would have great relevance for research in different areas such as, population genetics (genetic diversity and structure), gene flow, that will produced data very important for diagnosis of natural populations viability and their conservation.

Funding: CNPq, Fundect.

Key words: Bromeliaceae; Conservation; Wetlands.

Universidade Federal de Mato Grosso do Sul, Brazil.

Genetic structure of relictual populations of *Vriesea botafogensis* Mez (Bromeliaceae) inferred from two cpDNA loci

Salgueiro, F.¹; Carvalho, M.¹; Marinho, G.¹ & Menezes-Salgueiro, A.²

The Atlantic Forest is considered one of the most threatened ecosystems on Earth due to intense disturbance, having been reduced to only 7.5% of its original area. The Atlantic Forest of Rio de Janeiro State is an important remnant of this biome, presenting a high richness of endemic plants and animals. *Vriesea botafogensis* Mez. is a rupicolous bromeliad endemic to few a inselbergs within the metropolitan region of Rio de Janeiro. According to the IUCN and Brazilian Red List of Threatened Plant Species criteria, *V. botafogensis* is critically endangered (CR) because its area of occupancy is estimated to be less than 12 km² and its habitat is severely fragmented. The present study aims to investigate the phylogeography and genetic diversity of

V. botafogensis to help elucidate the evolutionary history of this threatened species. Leaves were collected from 33 samples from the three known natural populations of *V. botafogensis* located at Pão-de-Açúcar (Urca, RJ), Chacrinha (Copacabana, RJ) and Itacoatiara (Niterói, RJ) inselbergs. We also included in the analysis nine samples of *V. saundersii* (Gavea, RJ), since this species is often confused with *V. botafogensis*. DNA was extracted using the CTAB method. The rpoB and rpoC cpDNA regions were amplified and sequenced using universal primers. The sequences were aligned using the ClustalW algorithm available in MEGA6 and edited manually to avoid artifacts introduced by the program. The haplotype network was generated by the median-joining model implemented in NETWORK 4.6.1.2. Molecular diversity estimates were calculated using ARLEQUIN 3.5.1.2 and DNASP 5.1. An analysis of molecular variance (AMOVA) was performed in ARLEQUIN and a spatial analysis of molecular variance (SAMOVA) was conducted using the SAMOVA 2.0 software. The total combined cpDNA matrix presented 828 sites. Three variable characters were detected resulting in three cpDNA haplotypes. The H1 haplotype is present in Chacrinha and Pão-de-Açúcar populations, the H2 haplotype in Itacoatiara and the H3 haplotype is exclusive to *V. saundersii*. Total haplotype and nucleotide diversities were 0.6376 and 0.001540, respectively. The AMOVA analysis showed a very strong differentiation among all populations ($F_{st}=0.83562$, $P<0.0001$). Spatial analysis of molecular variance (SAMOVA) identified three phylogroups ($k=3$), one consisting of Chacrinha and Pão-de-Açúcar populations, the second consisting of the Itacoatiara population, and the third consisting of the *V. saundersii* population. Therefore, conservation strategies should take into consideration this strong genetic structure.

Funding: CNPq, UNIRIO, PPGBIO, IFRJ.

Key words: Inselberg; Phylogeography; Chloroplast.

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Pollen ultrastructure of *Dyckia distachya* (Bromeliaceae)

Santos, Rinaldo & Mariath, Jorge Ernesto

Dyckia Schult. & Schult. is the second largest genus of bromeliads within the subfamily Pitcairnioideae, comprising 159 xerophytic species, terrestrial or rupicolous. In South America, the genus occurs in all regions of Brazil, Uruguay, Argentina, Bolivia, and Paraguay. Although there are many palynological studies of *Dyckia* species, little is known about the cytology of the male gametophyte. In this work, we analyze the pollen grain ultrastructure of *D. distachya* Hassler, a rheophytic and endemic species of the Uruguay River Basin, which is listed as critically endangered. For ultrastructural analysis, sample preparation was performed using a microwave processor. Dehiscent anthers of *D. distachya* were macerated to release pollen in the primary fixative solution containing 2.5% glutaraldehyde and 2.0% formaldehyde, postfixed in a mixture of 1% OsO₄ and 0.8% K₃[Fe(CN)₆], dehydrated in an ascending acetone series, embedded in Spurr's low-viscosity epoxy resin, and polymerized in an oven at 60°C. Ultrathin sections were contrasted with a modified Thiéry reaction followed by lead solution, and observed in a transmission electron microscope at 80 kV. In addition, for the isolation of generative cells, fresh pollen grains were squashed on glass microscope slides in a drop of 20% sucrose and immediately visualized under interferential Nomarski contrast. The mature male gametophyte of *D. distachya* is bicellular. The vegetative cell is rich in dictyosomes, mitochondria, smooth endoplasmic reticulum, lipid droplets, and amyloplasts containing small starch grains. The vegetative nucleus presents an amoeboid contour and is located in the cytoplasmic zone opposite the aperture, near the sporoderm, where there is an abundance of rough endoplasmic reticulum. The vegetative nucleus partially surrounds the generative cell, giving rise to the male germ unit. The elongated generative cell is striated, delimited by a periplasmic space, and contains cortical microtubules. The generative cell is remarkably equipped with two tails, which when seen in isolated generative cells, give the cell a length of more than 70 micrometers. However, inside the vegetative cytoplasm, the tails are highly sinuous and limited to the vicinity of the vegetative nucleus. The generative nucleus also has an amoeboid shape. This is the first description of the presence of two long tails in the generative cell of the Bromeliaceae. Its functional role is still unknown as a structural association was not observed between these terminal cell portions and the vegetative nucleus. However, the behavior of the

generative cell along the pollen tube should be analyzed, as in this behavior the cell tails may be important. These early results point to the need for a wide comparative analysis of the structure of the male gametophyte in the Bromeliaceae, which should present a variety of characteristics useful in providing a better understanding of the evolution and phylogenetic relationships of the bromeliads.

Funding: Pronex-FAPERGS, CNPq.

Key words: Male Germ Unit; Generative Cell; Vegetative Cell.

Universidade Federal do Rio Grande do Sul, Brazil.

Comparative pollen morphology between *Alcantarea* (E. Morren ex Mez) Harms and *Vriesea* Lindl (Bromeliaceae Juss)

*Santos, Valéria Leobina*¹, *Wanderley, Maria das Graças Lapa*¹, *Versieux, Leonardo*², *Moreira, Bianca Alsina*¹, *Cruz-Barros, Maria Amélia Vitorino*¹ & *Luz, Cynthia Fernandes*¹

In recent phylogenetic analyses based on plastid and nuclear genome sequences, the genus *Alcantarea* emerges as a well-supported monophyletic group and sister to a clade including eastern Brazil *Vriesea* species. However, discussions involving the taxonomic position of *Alcantarea*, either as an independent genus or as subgenus of a broadly circumscribed *Vriesea* are still under debate, as well as the delimitations of some complex species with the genus. In the present work we study the pollen morphology for species of *Alcantarea* and *Vriesea*, aiming to increase the data available for taxonomic decisions and searching for diagnostic characters that can contribute to the definition of these taxa. The following species were studied: *Alcantarea acuminatifolia* Leme, *A. burle-marxii* (Leme) J.R. Grant, *A. extensa* (L.B. Sm.) J.R. Grant, *A. farneyi* (Martinelli & A. Costa) J.R. Grant, *A. geniculata* (Wawra) J.R. Grant, *A. glaziouana* (Lem.) Leme, *A. heloisae* J.R. Grant, *A. imperialis* (Carrière) Harms, *A. nahoumii* (Leme) J.R. Grant, *A. nevaresii* Leme, *A. nigripetala* Leme & L. Kollmann, *A. trepida* Versieux & Wand., *A. turgida* Versieux & Wand., *Vriesea cacuminis* L.B. Sm., *V. brusquensis* Reitz, *V. phillipocoburgi* Wawra, *V. gamba* Wawra, *V. pardalina* Mez, *V. pseudoatra* Leme and *V. simplex* (Vell.) Beer. For the pollen analysis acetolysis was used. The pollen grains were measured under optical microscopy (LM) and photographed through LM and scanning electron microscopy (SEM). The data were analyzed statistically. *Alcantarea* pollen grains are monads, the faces proximal and distal configure heteropolarity; ellipsoidal in polar view; biconvex or plane-convex in equatorial view; of large size; monosulcate; bilaterally symmetrical; with a wide sulcus, as long as the major equatorial axis, with a “*Vriesea imperialis*” margo (the diameter of the lumina decreased at the ends of the sulcus); with a reticulate heterobrochate exine (smooth simplicolumellate or duplicolumellate muri, narrow or wide, straight or curved, continuous or discontinuous muri); rounded or polyhedral lumina (in some species a narrower lumina occurs, surrounding the larger ones), psilolumina or lumina filled with bacula and pila. The diameters of the “lumina” decreased at the ends of the pollen grains, which present microreticulate or sparsely perforated sexine, with equatorial calotas, where exine ornamentation is different. Sexine thicker than nexine. The pollen morphology of *Vriesea* species was similar to that of *Alcantarea* species, which does not help in the species segregation through these characters. The monosulcate pattern was found in the four specimens analyzed of *Alcantarea nahoumii*, as well as in other species of *Vriesea*, however in this species heteromorphism was observed in the pollen grains, with extended sulcate aperture without calota and zona-aperture without calota, the latter never before described for Bromeliaceae.

Funding: FAPESP, Capes.

Key words: Vrieseaceae; Palynotaxonomy; Bromeliaceae.

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A molecular phylogeny of *Pitcairnia* (Pitcairnioideae, Bromeliaceae) - Insights from a multi-locus dataset

Schubert, Kai; Wagner, Natascha; Wöhrmann, Tina & Weising, Kurt

With 399 accepted species, *Pitcairnia* is by far the largest genus of the subfamily Pitcairnioideae (Bromeliaceae). It also occupies the widest distribution range within the subfamily. The genus is distributed in South and Central America except for *Pitcairnia feliciana* that is a local endemic on a mountain range in West Africa. No extensive molecular systematic analyses have yet been undertaken in *Pitcairnia*, and infrageneric relationships remain largely unknown. Here we present a multi-locus plastid phylogeny of the genus, based on sequences from the matK gene, the ycf1 gene and the two intergenic spacers rpl32 – trnL and rps16 – trnK. Sequences were assembled for 214 accessions from 148 species, resulting in an alignment of 4,998 base pairs. Altogether 9.23 % of all characters turned out to be parsimony informative, excluding outgroups. The data set was subjected to Bayesian, Maximum parsimony and Maximum likelihood analyses. In the resulting phylogenetic trees, *Pitcairnia* is clearly monophyletic and sister to the remainder of the subfamily. A deep basal split divides *Pitcairnia* into two large lineages. *Pitcairnia feliciana* takes a relatively early branching position in one of the two lineages. The tree topology suggests that its closest relatives are found in Venezuela. Some species form groups that reflect their geographic distribution (e.g. a Brazilian clade and a Caribbean clade), but overall geographical patterns turned out to be complex. An ancient area reconstruction done with RASP suggests the Andes as the most likely origin of *Pitcairnia*.

Key words: *Pitcairnia*; Molecular Phylogeny; cpDNA.

Universität Kassel, Germany.

Chloroplast haplotypes indicate ancient and recent hybridization in the genus *Deuterocohnia* (Bromeliaceae)

Schutz, Nicole¹, Weising, Kurt² & Zizka, Georg³

The genus *Deuterocohnia* Mez (Pitcairnioideae *s.str.*) comprises 17 species with a distribution centre in the Andes of southern Bolivia and northern Argentina. The plants grow as terrestrials on dry, rocky slopes or bare rocks. Succulent leaves and CAM photosynthesis are some of the adaptations related to their xerothermic habitat. Our ongoing project involves analyses of chloroplast haplotypes (trnS-ycf3, rps16-trnK, rpl32-trnL) from all *Deuterocohnia* species. The chloroplast haplotypes are not species-specific in *Deuterocohnia*. Thus, one haplotype was sometimes shared by several species, where the same species may harbour different haplotypes. The arrangement of haplotypes followed geographical patterns rather than taxonomic boundaries, which may indicate some residual gene flow among populations from different *Deuterocohnia* species. Phenotypic species coherence in the background of ongoing gene flow may then be maintained by sets of co-adapted alleles, as was suggested by the porous genome concept (Wu 2001, Palma-Silva *et al.* 2011). As variation among the sequences of *Deuterocohnia* was low, we could include samples of the closely related genera *Dyckia* and *Encholirium* into a haplotype network. While the two major subgroups of *Deuterocohnia* were separated by 32 mutational steps, one of the two subgroups was connected by only 11/18 mutational steps to *Encholirium/Dyckia*. If we further include *Fosterella* into the network, *Deuterocohnia* proved to be deeply paraphyletic, with five species forming a sister group to the closely related genera *Encholirium/Dyckia*. Considering that morphology as well as nuclear DNA data generated in the present study and in a former AFLP analysis (Horres 2003) all corroborate the monophyly of *Deuterocohnia*, the apparent paraphyly displayed in cpDNA analyses is interpreted to be the consequence of a chloroplast capture event. This involves the introgression of the chloroplast genome from the common ancestor of the *Dyckia/Encholirium* lineage into the ancestor of one *Deuterocohnia* subclade.

Key words: *Deuterocohnia*; Chloroplast Haplotypes; Hybridization.

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***In vitro* conservation of *Bromelia reversacantha* Mez and *Aechmea tocatina* Baker (Bromeliaceae) under minimal growth**

Sibov, Sérgio Tadeu¹, Fernandes, Fernanda de Paula Ribeiro¹ & Carneiro, Maurízia de Fátima²

Many species of native Cerrado bromeliads have strong aesthetic appeal. These characteristics make them of great interest to landscapers, decorators and collectors. However, these species are endangered by continuous change and habitat destruction by human actions. Among the species of bromeliads with ornamental potential, *Bromelia reversacantha* Mez stands out, a terrestrial species that lives on sandy soils and dry conditions and partial shade. Another one is *Aechmea tocatina* Baker, an epiphytic bromeliad found in the Cerradão phytophysognomy type. One way to ensure the germplasm preservation of these species would be *in vitro* conservation. This study aimed at establishing the best conditions for the *in vitro* conservation by minimal growth of these two species of bromeliads. To evaluate the sucrose and mannitol effect, *in vitro* germinated plants of both species were subjected to two experiments. For both species, the control treatment was MS medium with half strength of macronutrients: ½MS. Experiment 1: ½MS medium supplemented with different concentrations of sucrose (15, 30, 45 and 60 g.L⁻¹). Experiment 2: ½MS medium supplemented with different concentrations of mannitol (5, 10, 15 and 20 g.L⁻¹). The experiments were evaluated for six months and the development of plant growth was assessed after treatments. For the two species tested, the action of mannitol is more effective in inducing minimal growth compared to sucrose. The senescence of leaves after six months of treatment is also lower for both species in treatments with mannitol. For both species, the most suitable treatment for *in vitro* conservation would MS medium with half strength of macronutrients supplemented with 10, 15 or 20 g.L⁻¹ mannitol at a temperature of 25°C.

Funding: CNPq, Fapeg.

Key words: Bromeliads; Mannitol; Sucrose.

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The Bromeliad Society International: Research and Conservation

Siekkinen, Andy

From the first publication of The Bromeliad Society Bulletin—as the organization and its publication were initially known—conservation of bromeliads and their habitat and encouragement of scientific research on bromeliads were stated as primary goals of the organization. Over the years much has changed, both in terms of the Bromeliad Society International (BSI) as well as the scientific research and conservation. However, the BSI has always recognized the need to work as an international collaboration. From the founding, international experts were sought to bring together those with expertise from Brazil, Argentina, Europe, New Zealand, and the United States. As is now stated in The Journal of the Bromeliad Society International, our purpose is “to promote and maintain public and scientific interest in bromeliads through support of scientific and horticultural research, preservation, and display of bromeliads.” The primary tools the BSI has to accomplish these goals are the publication of The Journal, hosting a biannual World Bromeliad Conference (WBC), and grants. Scientific studies and taxonomic treatments have been published in The Journal alongside content of more general interest throughout the history of the BSI with The Journal serving as one of the most important publications of new bromeliad species. At the WBC events, there are presentations, tours, plant sales, and a plant auction to raise funds for bromeliad research. For a long time those funds were used for supporting the Bromeliad Identification Center that was previously managed by Harry Luther at the Marie Selby Botanical Gardens and are now devoted to a new program being developed: the HEL-BSI Scholar Program honoring the late Harry Luther. The organization is always dynamic and changing, there is always the potential for new projects for the BSI to support and work together with researchers of the Bromeliaceae.

Key words: Bromeliad Society International; Research Grants; Publications.

Bromeliad Society International.

Phylogenetic Study of the Genus *Hechtia* Utilizing Next Generation Sequencing Genome Skimming

Siekkinen, Andy & Simpson, Michael

Next generation sequencing (NGS) technology is transforming the field of plant systematics in its study of lower level taxonomic relationships and investigations of introgression and reticulation. The genus *Hechtia* (Bromeliaceae), with ~70 described species has generally been a poorly studied genus which has yet to be given a comprehensive taxonomic treatment. This project utilizes next generation sequencing (NGS) genome skimming to create a phylogenetic tree of the genus with species level resolution with the ultimate goal of proposing the first subgeneric classification. Genome skimming entails sequencing nuclear DNA at very shallow depth while recovering high copy genetic material: the plastome, nrDNA cistron, and the mitochondrial genome. This first stage analysis includes over 40% of the genus, with samples from 31 *Hechtia* species covering most of the geographic range and the natural morphological groups within the genus. Two *Tillandsia* species were included in the analysis as outgroups. The 33 samples were multiplex bar coded and accounted for ~1/3 of a shared Illumina lane. A range of 0.7–2.9 million 101bp single end reads were recovered per sample. *De novo* and reference-guided assembly methods were used to assemble a 90+kbp partial plastome, the nrDNA cistron, and several mitochondrial genes for all samples. The first diverging lineage contains the Central American species, the second group contains the ‘spineless’ species followed by a third clade with the lateral blooming species. The more recently diverging lineages will need more exhaustive sampling but look to fall into the following four groups: *H. zamudioi* group, *H. podantha*, the majority of Oaxaca/Puebla/Veracruz species, and lastly the species on the Pacific slope. The last two groups represent the largest centers of biodiversity in the genus. Using NGS genome skimming, this project successfully produced trees with the three types of genomic data showing phylogenetic relationships within the genus *Hechtia* and the large data set generated can be mined for designing primers for low-copy nuclear genes and transcriptomes for future projects.

Funding: San Diego Bromeliad Society, San Diego Cactus and Succulent Society.

Key words: *Hechtia*; Next Generation Sequencing; Genome Skimming.

San Diego State University, USA.

Flora da Usina São José, Igarassu, Pernambuco: Bromeliaceae

Silva, Júlio César da; Alves, Marccus & Louzada, Rafael Batista

Bromeliaceae is a Neotropical family including 3,472 species distributed in 56 genera. In Brazil the family is represented by 1,342 species and 44 genera with high diversity in the Atlantic Forest phytogeographic domain which is considered a center of endemism for Bromeliaceae. The devastation and fragmentation of the Atlantic Forest caused by human occupation and the expansion of agriculture, especially sugarcane plantations put the species at risk within this environment. This study aims to produce a floristic inventory of the Bromeliaceae species from a complex of six fragments of Atlantic Forest located in Pernambuco, Brasil. In this work samples of the species were collected and deposited at the herbarium UFP. The species were identified using specialized literature and/or comparison with other specimens deposited at UFP. In total, 10 species were identified in five genera: *Aechmea aquilega* (Salisb.) Griseb, *Aechmea mertensii* (G.Mey.) Schult. & Schult.f., *Aechmea multiflora* L.B.Sm., *Aechmea muricata* (Arruda) L.B.Sm., *Billbergia morelii* Brongn., *Cryptanthus zonatus* (Vis.) Beer., *Hohenbergia ridleyi* (Baker) Mez, *Tillandsia bulbosa* Hook.f., *Tillandsia incarnata* Kunth. and *Tillandsia stricta* Sol. ex Sims. Besides the list of species, an identification key, morphological descriptions, taxonomic notes and illustrations for the species are presented.

Funding: CNPq, NSF, Velux Stiftung, Benefícia.

Key words: Atlantic Rainforest; Floristics; Taxonomy.

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Growth and carbohydrate content of the bromeliad *Ananas comosus* var. *ananassoides* cultivated in different N concentrations

Silva, Priscila Primo; Carvalho, Victória; Carvalho, Camila; Braga, Márcia Regina; Gaspar, Marília & Tamaki, Vivian

Ananas comosus var. *ananassoides* (Baker) Coppens & F. Leal is a terricolous bromeliad with high ornamental value. It is endemic to the Cerrado biome, which has soil with low nutrient content. Despite being tolerant to such conditions, studies have shown that this bromeliad has optimum growth when cultured *in vitro* in nutrient medium containing sucrose and high nitrogen concentrations. It is known that carbohydrate metabolism in plants is influenced by the amount of nitrogen available in the nutrient medium. Indeed, the carbon/nitrogen ratio is determinant for plant growth. The aim of this study was to evaluate the growth and content of soluble carbohydrates and starch in *A. comosus* var. *ananassoides* plants cultured *in vitro* at different concentrations of nitrogen. Seeds were grown on Murashige and Skoog (MS) with 50% of the original macronutrient composition for two months in culture room at 26 °C, 12 h photoperiod and PAR of 30 $\mu\text{mol. m}^{-2} \cdot \text{s}^{-1}$. The obtained plants had the leaves cut 1 cm above the stem base, followed by transfer to fresh MS media with nitrogen concentrations of 15 (T1), 30 (T2) and 60 mM (T3). Plants were kept in the dark for 3 months to induce etiolation. Nodal segments were isolated and placed in MS media containing the respective nitrogen concentrations of each treatment (T1, T2 and T3), then kept for 11 months in the culture room. The limited availability of nitrogen led to lower growth in T1 and T2 plants in comparison with T3 plants. Leaves in T3 were longer, although root growth did not differ among treatments. Total carbohydrate content in T1 plants was 5 and 9 times greater than T2 and T3 plants, respectively. Therefore, nitrogen shortage led to reduced shoot growth and high content of soluble sugars in leaves, although it did not influence the root system. In contrast, the starch content increased in plants under higher nitrogen concentrations (T2 and T3). It was concluded that nitrogen deficiency affected the metabolism of carbohydrates reserve in leaves of *A. comosus* var. *ananassoides*, favoring the accumulation of soluble sugars to the detriment of starch.

Funding: CNPq.

Key words: Bromeliaceae; Micropropagation; *In vitro*.

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Chromosomal features of *Fosterella* L. (Bromeliaceae, Pitcairnioideae) species

Silva, Hévila¹; Benko-Iseppon, Ana Maria¹; Wagner, Natascha²; Pinagé, Diego Sotero¹; Gonçalves-Oliveira, Rodrigo²; Weising, Kurt² & Vidal, Ana Christina¹

The genus *Fosterella* L. comprises 31 species of terrestrial herbs with leaves in rosettes and mostly small, whitish flowers. Previous karyological studies on the genus were restricted to chromosome counts of four taxa, including diploid ($2n = 50$) and polyploid ($2n = 100, 150$) individuals. Besides chromosome counts, the present work analyzed other karyomorphological markers, including fluorochrome staining (DAPI/CMA; Chromomycin A3/4,6-diamino-2-fenilindol) and Fluorescent *In Situ* Hybridization (FISH) using 45S and 5S rDNA probes, generating data on mitotic metaphase for eight taxa. The observed chromosome numbers $2n = 50, 100$ and 150 were in accordance with literature data and also regarding the basic chromosome number $x = 25$. Staining CMA/DAPI enabled the identification of a chromosome pair with CMA+/DAPI- (GC-rich) band in all diploid ($2n = 2x = 50$) species in the terminal (*F. christophii*, *F. micrantha*, *F. spectabilis* and *F. robertreadii*), subterminal (*F. rusbyi*) or proximal (*F. gracilis*) position. Considering the tetraploid ($2n = 4x = 100$) species, in *F. hatschbachii* one chromosome pair exhibited a CMA+ proximal band, while *F. yuvinkae* presented two pairs with proximal bands. For the polyploid *F. hatschbachii* ($2n = 100$), additional CMA+ bands were expected, but a single pair was observed, indicating that this sequence was lost after polyploidization. Moreover, the presence of two CMA+/DAPI- in *F. yuvinkae* may indicate a recent polyploidization event. The application of *in situ* hybridization is unprecedented for Bromeliaceae. For *F. robertreadii*, *F. spect-*

abilis and *F. christophii* FISH using 45S rDNA probes revealed a colocalization of these sites with CMA+/DAPI- bands. In addition, a pair of 5S rDNA proximal sites was found in a second chromosome pair for *F. spectabilis* and *F. christophii*. Considering the data, it is evident that polyploidy plays an important role in the evolution of this genus. In addition, the results indicate that all species are poor in heterochromatin, except for NOR-associated sites that may vary in their position. Thus, the differences in sizes of the chromosomes can be attributed to the variation of dispersed repetitive elements.

Funding: CNPq, Capes.

Key words: *Fosterella*; Chromosome Number; Polyploidy.

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Fruit and seed morphometry of four Bromeliaceae species, São Mateus – ES, Brazil

Silva, Walace & Aoyama, Elisa

Morphometric studies contribute to species taxonomy, germination test interpretation, understanding of phenotypical variation among population in terms of seed viability and subsidizing studies about natural regeneration and management of conservation projects. This paper aimed to describe fruits and seeds of four Bromeliaceae species. The collections were performed from January to October, 2014 in the sandbanks of São Mateus city – ES, Brazil in one fragment located in Liberdade neighborhood and another in Guriri neighborhood. The studied species were: *Pseudananas sargenarius* (Arruda) Camargo, *Bromelia antiacantha* Bertol., *Vriesea neoglutinosa* Mez. and *Tillandsia gardneri* Lindl. A hundred fruits and seeds were measured per species in five different individuals from relatively far groups. The biometrical parameters used in this experiment were length and width, and the obtained values were submitted to ANOVA and the Tukey test at the level of 5% probability. Fruits were distinctly different among the species for both parameters. *B. antiacantha* and *V. neoglutinosa* also showed differences among individuals, different from *P. sargenarius* and *T. gardneri*, which showed more uniformity. Seeds of *V. neoglutinosa* and *T. gardneri* were different from the other species in terms of length. On the other hand, they had a uniform width. *B. antiacantha* and *P. sargenarius* did not present much difference in this parameter but they showed themselves different from the others in terms of width. *B. antiacantha* was the only one that was significantly different for both parameters of its structure. *P. sargenarius* develop fruits with a fibrous and hard epicarp, sorosi type and yellow-pinked color when mature, measuring 100.5 × 65.7mm. The seeds are amorphous and flat 5.72 × 3.05mm). *B. antiacantha* has berry-type fruits which turn yellow when they are mature and also have a slightly sweet smell, measuring 49.21 × 23.06mm, seeds are discoid, flat, yellow-brown in color and measure 4.70 × 4.65mm. *V. neoglutinosa* and *T. gardneri* have dry capsule-type fruits and seeds with feathered appendages. Seeds of *V. neoglutinosa* measure 31.34 × 0.17mm, while *T. gardneri* has fruits that measure 42.33 × 2.73mm and seeds, 32.46 × 0.13mm. The fruits and seeds of *B. antiacantha* were the only ones which showed difference for both parameters among the collected individuals, which can be a reflection of habitat fragmentation, as it may result from greater phenotypic plasticity or possible genetic isolation from other groups, creating low gene flow. On the contrary, *T. gardneri* showed a smaller difference in fruits and seeds for both parameters, which may indicate that they have greater gene flow, despite habitat fragmentation, and keep relations with different population.

Funding: UFES.

Key words: Fruit; Seeds; Populations.

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CAM photosynthesis as an adaptive feature in the ecophysiology and evolution of Bromeliaceae

Smith, Andrew

The Bromeliaceae represent an exceptionally diverse family of vascular plants in their range of life-forms and ecology. Nowhere is this more striking than in the variation in water availability that characterizes the habitats of different bromeliads, extending from the high-precipitation environments of true tropical rain forests to the hyperarid deserts of Chile and Peru. Associated with these habitats, bromeliads are confronted with a correspondingly wide range of temperature regimes (from lowland equatorial sites to high-elevation niches in the Andes) and light intensities (from fully exposed terrestrial sites and the tops of forest canopies to the shaded understory of lowland forests). As part of their adaptation to the more stressful habitats, especially with respect to limited availability of water, nearly half of all species of bromeliads have adopted the mode of photosynthesis known as crassulacean acid metabolism (CAM). This biochemical pathway allows plants to take up most or all of their CO₂ from the atmosphere at night, and then to process the CO₂ photosynthetically during the following daytime behind closed stomata, resulting in much reduced loss of water in transpiration compared with C3 photosynthesis. Detailed taxonomic surveys of stable-carbon-isotope discrimination within Bromeliaceae have shown that nearly half of all species possess the CAM pathway, exhibiting a strong correlation with leaf morphology (succulence) and habitat aridity. When mapped onto a well-resolved phylogeny of genera within the family, it can be inferred that CAM photosynthesis has arisen a minimum of five times within Bromeliaceae, four of which represent clades of terrestrial xeromorphic succulents and the fifth the more extreme epiphytic members of *Tillandsia*. Rate calibration of the bromeliad phylogeny indicates that the diversification of these CAM lineages took place in the late Miocene and Pliocene, coincident in part with the emergence of specific semi-arid biomes and the creation of hyperdiverse montane habitats during the final uplift of the northern Andes. More detailed work is now underway using species distribution modelling to characterize the ecological niche of the CAM bromeliads with respect to various environmental axes and to explore possible reasons for the apparent paucity of C3–CAM intermediates. The results suggest that photosynthetic pathway distribution in the family is dominated by a pattern of phylogenetic niche conservatism superimposed on a limited number of intermediate or plastic phenotypes that tend to be restricted to ecologically marginal habitats.

Key words: Photosynthesis; Water Relations; Ecophysiology.

University of Oxford, United Kingdom.

Genetic variability among populations of the *Dyckia pernambucana* complex from the Borborema Plateau, Pernambuco, Brazil

Sotero, Diego¹; Weising, Kurt²; Krapp, Florian²; Wöhrmann, Tina²; Zizka, Georg³; Wanderley, Maria das Graças⁴ & Benko-Iseppon, Ana Maria¹

In the Atlantic Rainforest located north of the São Francisco River, the humid enclaves (or continental islands), called “Brejos de Altitude”, play a significant role in the diversity dynamics of the local Flora and Fauna, due to their exclusive conditions of humidity and microclimates. The endangered species *Dyckia pernambucana* L.B.Smith and *D. limae* L.B.Smith (Pitcairnioideae, Bromeliaceae) are characterized by their narrow endemic occurrence in such “Brejos de Altitudes” of the Borborema Plateau (Pernambuco, Brazil). Furthermore, according to previous morphological inferences, the species status of these taxa remains unclear. In order to understand the patterns of gene flow and genetic variability among populations, both microsatellite loci (nuclear and plastid) and AFLP markers were employed in 50 individuals, distributed in five populations of Pernambuco Group (*D. limae* and *D. pernambucana*), as well as 37 individuals of the closest species (*D. dissitiflora*), with a total of 87 individuals. The levels of diversity found in the present work were high, despite the possible influence of genetic drift and selfing rates. Accordingly, significant inbreeding coefficient values were observed with the three markers used, indicating that the populations are notably structured. According

to the Bayesian clustering as well as phenetic analysis, *D. limae* could not undoubtedly be distinguished from the remaining populations of Pernambuco state (*D. pernambucana* populations). However, the associations found here were consistent with the historical patterns of colonization and fragmentation of the Atlantic Rainforest located north of the São Francisco River.

Funding: FACEPE, CNPq, CAPES, DAAD.

Key words: Gene Flow; Molecular Markers; Pitcairnioideae.

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A new species of *Aechmea* Ruiz & Pavon (Bromelioideae-bromeliaceae) from the Brazilian Atlantic rain forest

Sousa, Gardene Maria¹ & Wanderley, Maria das Graças Lapa²

Aechmea sp. nov., a new species from southeastern Brazil, is described and illustrated. The species belongs to the *Aechmea* subg. *Chevaliera* and shows morphological similarities with *Aechmea hostilis* E. Pereira and *Aechmea gustavoi* J. A. Siqueira & Leme, in the short-peduncled and capituliform inflorescence, but differs in the oval to oblong floral bracts, the acuminate sepals and the green colour of all perianth structures.

Key words: Brasil; Bromelioideae; *Chevaliera*.

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Morphological variation in *Vriesea procera* (Mart. ex Schult. & Schult. f.) Wittm. (Bromeliaceae, Tillandsioideae)

Uribe, Fernando Pérez; Jacques, Suara Souza Almeida; Neves, Beatriz & Costa, Andrea

The Bromeliaceae family, with 3,352 species in 58 genera, is essentially Neotropical and has in the Atlantic Forest Domain one of its main centers of endemism and diversity. One of the major problems in the systematics of the family is the difficulty of circumscription of species, especially in the most diverse genera. *Vriesea* is the richest genus of the family in the Atlantic Domain and includes numerous species complexes. The species of *V. procera* complex present geographical distribution along the South American coast between Venezuela and southern Brazil in forest formations and “restingas”, and share the same basic pattern in vegetative structure and morphology of the inflorescence. However, a great variation in dimensions, positions and shape of leaves and flowers and number and position of the branches of the inflorescence can be observed. Morphometric analyses are commonly used in botanical families whose circumscription of species is difficult, even in Bromeliaceae. In this context, this work aimed to show the morphology of taxa related to *V. procera* via morphometric study of natural populations along the Brazilian coast; establish the limits of variation and the validity of the taxa; perform a taxonomic treatment of the species, describing them morphologically, providing means to identify them by keys and illustrations and data on geographical distribution. Fourteen natural populations were analyzed along the southern coast from BA to PR states. Two hundred and seventy one individuals and 36 quantitative variables were analyzed. For the statistical analysis the STATISTICA 8.0 and v PAST software, version 2.04, were used. The Kruskal Wallis test and discriminant analysis were performed. Seven species were recognized, including four new and two new synonyms and a new combination were proposed. The resulting taxa are distinct because they have differences in the sizes of the inflorescences and leaves, but mainly in floral characteristics such as length of floral bracts, sepals, petals, stamens and pistil, in addition to the posture of the petals and relative position of the stamens during anthesis.

Funding: CNPq, Capes.

Key words: Taxonomy; Morphometry; Morphology.

Museu Nacional, Universidade Federal do Rio de Janeiro, Brazil.

Plasticity of leaf anatomy in the nurse-plant bromeliad *Neoregelia cruenta*

Vargas, Pedro da Silva; Pepe, Isabelle Soares; Silveira, Ginayan de Souza; Freitas, Hugo Dolsan & Mantuano, Dulce Gilson.

Neoregelia cruenta (R. Graham) L.B. Smith (Bromeliaceae) is a CAM tank bromeliad, endemic of southeast “restinga”, i.e., the open scrub vegetation of Brazilian coastal plains. At this location *N. cruenta* increases diversity acting as nurse-plant. Clonal reproduction is a common feature in herbaceous plants and has been shown to be important for recruitment and population maintenance in this species. *N. cruenta* populations are subject to different levels of light exposure depending on the individual position related to vegetation clumps. Connected ramets are subjected to three microhabitats: (i) exposed to full irradiance, outside vegetation islands; (ii) partially exposed to full irradiance, at the border of vegetation islands; (iii) shaded, inside vegetation islands. We examined the environmental effect on leaf anatomy of *N. cruenta* both within ramets connected in clonal clumps (intraclonal variation) and among clones (interclonal variation). Leaves of two connected ramets of five replicas in each microhabitat were sampled. Anatomical parameters were thickness of leaf blade, thickness of chlorenchyma, thickness and number of layers of water-storage parenchyma, thickness of adaxial and abaxial epidermis, density and position of trichomes, and stomatal density. All leaves of *N. cruenta* shared the same anatomical features irrespective of microhabitat. Thicker water-storage parenchyma and leaf blades were found on both connected ramets in exposed microhabitat and the exposed ramet of partially exposed microhabitat, related to the shorter time of tank water storage. Intraclonal variation was statistically significant only at the partially exposed microhabitat. Both phenotypic plasticity within connected ramets and genetic variation among connected ramets might help to explain the high abundance of *N. cruenta* along heterogeneous environment of “restinga” vegetation.

Key words: Plasticity; Nurse-plant; *Neoregelia cruenta*.

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Advances in the taxonomy and floristics of Brazilian Bromeliaceae, a collaborative perspective of selected case studies

Versieux, Leonardo¹; Lavor, Pâmela¹; Nogueira, Matheus²; Machado, Talita³; Magalhães, Átilla³; Tomaz, Eduardo³; Medeiros, Adriene; Alves, Poliana¹; Sousa, Valdeci⁴; Luz, Cynthia⁴; Santos, Valeria; Conceição, Abel²; van Den Berg, Cássio²; Oliveira, Reyjane²; Paggi, Gecele⁵; Coffani-Nunes, João⁶; Costa, Andrea⁷; Jacobi, Claudia³; Carmo, Flávio³; Stehmann, João³; Lexer, Christian⁸; Sousa, Gardene⁹; Calvente, Alice¹; Wanderley, Maria das Graças⁴

The knowledge of Brazilian Bromeliaceae is progressively improving. However, many regions are still understudied, as are some dry habitats and the Amazonian region. Here we present an overview of the advances on the taxonomy and floristics of integrative, unpublished or recently published research, primarily focused on Tillandsioideae: *Alcantarea* and *Vriesea*, as well as Bromelioideae: *Araeococcus* and *Cryptanthus*. Also, a discussion will be provided on the improvement of the floristic knowledge of Bromeliaceae in previously understudied regions, such as the northern Caatinga biome, specifically the states of Rio Grande do Norte, Ceará and Piauí, where several new occurrences are being recorded (e.g. *Aechmea muricata*, *Cryptanthus zonatus*, *Dyckia tubifilamentosa*, and *Orthophytum disjunctum*); the state of Mato Grosso do Sul (11 genera and 41 spp); the state of Bahia, which has a flora project underway, with the taxonomic treatment of *Alcantarea* published and *Tillandsia* and *Vriesea* nearly concluded; and Sergipe, where the treatment of Bromeliaceae for the Flora of Sergipe is in press. These works point toward the need to conduct further field-work and produce floras, to improve the scientific collections since many species are still poorly understood, and they suggest the necessity to consider establishing centralized and institutional living collections and DNA banks in order to take advantage of the material collected by different students in their research. The progress on the taxonomy of *Alcantarea* will also be briefly discussed using recent data on palynology and molecular

markers (*A. imperialis* vs. *A. brasiliensis*). This genus has been monographed recently and currently comprises nearly 40 species. Additional studies involving species delimitation in *Vriesea*, *Guzmania* and *Mezobromelia* will be discussed using molecular, floral, and anatomical data, indicating the necessity to collaborate at the national and international level. Opportunities and challenges for new research within the Bromeliaceae in Brazil will also be discussed, highlighting the need for more taxonomic work in the group.

Key words: Bromeliads; Epiphytes; Taxonomy.

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Breaking down all barriers - reproduction biology and speciation processes in *Fosterella* (Pitcairnioideae s.str.)

Wagner, Natascha¹; Wöhrmann, Tina¹; Schütz, Nicole²; Schubert, Kai¹ & Weising, Kurt¹

The mesophytic genus *Fosterella* (31 species) belongs to the bromeliad subfamily Pitcairnioideae s.str. Its main distribution areas are the eastern slopes of the central Andes. Recent studies suggest that *Fosterella* originated in the mid-Miocene about 10 Mya in the Andean seasonally dry tropical forests (SDTF; Wagner *et al.*, 2013). A well resolved, multilocus phylogeny of *Fosterella* based on six chloroplast DNA regions (matK, rps16 intron, atpB-rbcL, psbB-psbH, rpl32-trnL, rps16-trnK) demonstrated the monophyly of the genus and its division into six evolutionary lineages. These findings were supported by a nuclear phylogeny based on phyC. We conducted crossing experiments to study fertilization barriers within and between several *Fosterella* species. The so-called micrantha-group comprises three morphologically very similar species that show a disjunct distribution pattern, probably caused by one or more long distance dispersal events. Our results of population genetically approaches based on SSR and AFLP data show that most of the populations of the Central American *F. micrantha* are genetically separated from those of the Bolivian *F. christophii* and *F. villosula*. Species delimitation due to morphological characters is difficult within the micrantha-group. Our crossing experiments showed that all species are capable of selfing but can also form interspecific hybrids, indicating that reproductive barriers are low. Further *in-situ* crossing experiments between *Fosterella* species from several subgroups with differences in flower morphology (*F. spectabilis*, red, tubular flowers; *F. gracilis*, yellow flowers; *F. rusbyi*, small, whitish flowers with recoiled petals; *F. penduliflora*, relatively large, white flowers) were conducted. Again all treatments were successful. These results support our suggestion of weak crossing barriers within the whole genus, indicating other isolation mechanisms like geographical and ecological isolation to avoid cross-species hybridisation in the field.

Key words: *Fosterella*; Speciation Processes; Crossing Experiments.

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Phylogeographic patterns in Bromeliaceae: examples from Pitcairnioideae s.s.

Weising, Kurt¹; Wagner, Natascha¹; Schütz, Nicole²; Krapp, Florian¹; Wöhrmann, Tina¹ & Schubert, Kai¹

The present talk aims to summarize our current knowledge about phylogenetic relationships among the five genera of Pitcairnioideae s.s., the time scales in which they diversified, and phylogeographic patterns that have become apparent within the subfamily. The presented trees and networks are based on sequence data from several chloroplast DNA (cpDNA) loci and the nuclear PHYC gene. On the subfamily level, we analyzed ~30 accessions per genus covering the whole distribution area. The widely distributed genus *Pitcairnia* is monophyletic and sister to the remainder of the subfamily in all trees. Next-branching is the likewise monophyletic

genus *Fosterella*, which is sister to a well-supported xeriphytic clade consisting of *Deuterocohnia*, *Dyckia* and *Encholirium*. The Andean genus *Deuterocohnia* is paraphyletic in the cpDNA tree but monophyletic in the nuclear tree, suggesting an ancient chloroplast capture event. *Dyckia* is monophyletic and arises from within a paraphyletic *Encholirium*. The two latter genera have their diversity centres in the Brazilian Cerrado and Caatinga. Since *Pitcairnia*, *Fosterella*, *Deuterocohnia* and *Encholirium* will be covered by separate talks in this session, I will put some focus on *Dyckia* that comprises 158 species with a strongly xeromorphic habit. DNA sequence variation within *Dyckia* turned out to be extremely low, and phylogenies were hence poorly resolved. A dated cpDNA phylogeny suggests that *Dyckia* experienced a rather recent radiation beginning around 2.9 Mya. A recent expansion was also suggested by a star-like pattern of a parsimony network based on cpDNA haplotypes. Four major clades were identified that roughly corresponded to the geographic origin of the accessions. Our current working hypothesis based on molecular data and geographical distribution of extant taxa is that the common ancestor of *Dyckia* and *Encholirium* started to diversify in NE Brazil where *Encholirium* still resides nowadays. At least one lineage of *Dyckia* dispersed to S Brazil, from where a rapid colonization of suitable habitats was initiated. We hypothesize that the radiation of the genus has been triggered by the climatic oscillations of the Cenozoic.

Key words: Dated Chloroplast Phylogeography; Haplotype Network; *Dyckia*.

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Species delimitation in Bromeliaceae

Wendt, Tânia

Science has two major goals: to enhance human knowledge and to solve practical problems. One key issue often challenges a quick and easy achievement of such noble goals: the scientist's subjectivity, i.e., his own perspective on the phenomena he observes. There are several concepts already developed to define what a species is. Despite the numerous concepts proposed, species cannot be precisely defined. The different definitions of species emerged from the different objectives and purposes related to the different lines of research of its proponents. Thus, no single definition will be satisfactory enough to meet all kinds of organisms or processes. In essence, the majority of the species definitions can be grouped into three broad categories: (1) biological species concept based on reproductive isolation; (2) evolutionary and phylogenetic species concepts based on evolutionary units revealed by phylogenetic analysis; and (3) morphological species concept based on phenotypic or genotypic distinction. Despite the apparent limitation of the morphological species concept, this has been one of the most employed, especially in taxonomic revisions and floristic surveys. This is due to the fact that, in practice, usually one does not have information available on reproductive behavior or evolutionary processes, which are required for the application of other concepts. Species concepts are based on the rejection of the possibility of natural hybridization, because their theories assume that different species are reproductively isolated, and/or that new lineages cannot have polyphyletic origins. It is common to find bromeliad assemblages with similar floral characteristics, overlapping flowering periods, and sharing pollinators. These situations favor hybridization, which could be seen as a transient phase of evolution, during sympatric speciation. The role of subjectivity in scientific interpretation of species delimitation is confined by the bounds imposed by the scientific method and by the knowledge available. Thus, despite methodological soundness, science in this case remains subjective. This does not place taxonomic science into a vague or loose realm, instead it turns it into an open forum where the predominant concepts or theories of today can be invalidated tomorrow and, possibly, even rescued and revalidated the day after tomorrow. In this dynamic lie both the strength and the beauty of taxonomic science. Several new bromeliad species are described each year, and nomenclatural extinction and resurrection will continue to occur during the process of taxonomic revisions, sometimes in heated discussions. Science is not a matter of right or wrong. There is good and there is poor science and the quality can often be assessed by the quality of the journals where science is published.

Funding: CNPq.

Key words: Bromeliaceae; Species Concept; Species Delimitation.

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SSR discovery in cDNAs of *Fosterella christophii* (Pitcairnioideae) using the PacBio third-generation sequencing platform

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We used the single-molecule real-time (SMRT, Pacific Biosciences) third-generation sequencing system PacBio RS II to generate 1,590 high quality cDNA sequences of one accession of *Fosterella christophii*. After isoform-level clustering, polishing and trimming of raw data, 971 non-redundant contigs were obtained. These were screened for the presence of microsatellites (simple sequence repeats, SSRs) with motif lengths between one and six bp. Lower thresholds of 15, 7, 6, 5, 4 and 4 repeat units were used to define SSRs of the mono-, di-, tri-, tetra-, penta- and hexanucleotide repeat type, respectively. Based on these criteria, a total of 446 perfect SSRs were identified. Dinucleotide repeats were most abundant (52.9%), followed by trinucleotide (31.6%) and hexanucleotide (6.1%) repeats. Only perfect di- to hexanucleotide repeats were considered as candidates for marker development, resulting in a total of 421 potentially amplifiable SSR loci. Sufficient SSR flanking sequence of appropriate quality and length for primer design was present in 275 contigs, which together contained 335 SSR loci. Similarity searches were conducted on these contigs using Blast2Go software. A total of 20 perfect SSR loci (four di-, eight tri-, two tetra-, three penta- and three hexanucleotide repeats) were selected and tested for marker performance in several populations of the source species as well as for transferability to other *Fosterella* species. The potential of these markers to cross-amplify and to differentiate between closely related bromeliad species will be discussed.

Key words: Transcriptome; Microsatellites; *Fosterella*.

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The mode of plastid inheritance within the genus *Fosterella* (Pitcairnioideae)

Wöhrmann, Tina; Wagner, Natascha; Schubert, Kai & Weising, Kurt

The cytoplasmic inheritance of organellar DNA in land plants may occur via three different modes: uniparentally maternal, biparentally and uniparentally paternal. In most angiosperms, the chloroplast is inherited maternally via seeds, whereas in gymnosperms the paternal mode (via pollen) is prevailing. Although it is commonly assumed that plastids are inherited maternally also in Bromeliaceae, molecular evidence for this hypothesis is still lacking. To study the mode of chloroplast inheritance within the genus *Fosterella* (Pitcairnioideae) via molecular fingerprinting, we developed a novel set of 24 chloroplast microsatellite markers (cpSSRs) using 454 pyrosequencing technology. A subset of these markers was used to genotype the parents and several plants each of the F1 generation derived from seven inter- and two intraspecific crosses, involving the four species *F. christophii*, *F. micrantha*, *F. villosula*, and *F. rusbyi*. All crosses were performed by hand-pollination under greenhouse conditions. At each cpSSR locus, the banding patterns obtained after high-resolution polyacrylamide gel electrophoresis clearly indicate a maternal inheritance of plastids in each of the analysed crosses.

Key words: Plastid Inheritance; cpSSRs; *Fosterella*.

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Patterns of genetic diversity and phylogeography, insights into speciation history with Atlantic Rainforest species

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The Bromeliaceae family is one of the morphologically and ecologically most diverse flowering plant families native to the New World and is well known for its recent adaptive radiation, evolving to live in numerous

niches and occupying the most diverse types of environments. Due to its recent evolutionary history, generic boundaries often suffer changes, with incipient species not completely defined. Brazilian Atlantic Forest (BAF) is one of the diversity centers of bromeliads, being also one of the diversity centers of the genus *Vriesea*. *Vriesea carinata* and *V. incurvata* may be interesting models for studying BAF historical patterns, species cohesion processes and reproductive barrier mechanisms. These species are endemic to BAF, with wide distribution throughout this ecoregion, being found in sympatry, where they might share pollinators. We studied the phylogeographic patterns of *V. carinata* and *V. incurvata*, aiming to provide insights into the historical processes that underlined diversification in BAF. We evaluated the hypothesis that *V. carinata* and *V. incurvata* would present the same phylogeographic pattern, since they could be subjected to the same climatic changes in the past because they present similar geographic distribution. We sampled 16 populations of *V. carinata* and 11 of *V. incurvata*, aiming to describe the patterns of genetic variation in two plastid sequences (cpDNA) and 14 nuclear microsatellites. *V. carinata* and *V. incurvata* showed moderate levels of nuclear and plastid genetic diversity. Both species showed isolation by distance and present expansion towards southern margins. *Vriesea carinata* and *V. incurvata* showed similar phylogeographic patterns, with strong genetic discontinuity among north/south populations and without haplotypic sharing among these regions. The presence of two genetically distinct groups would seem to support the hypothesis that *V. carinata* and *V. incurvata* survived in more than one fragmented refugia during Pleistocene climatic oscillations. The results are consistent with records encountered in the literature for the BAF. However, more studies are required to understand the complex history of the BAF, since this pattern was probably shaped throughout the Pleistocene, but earlier events, such as uplift of the Brazilian East Coast during the Tertiary may be also have influenced the distribution and diversification of taxa.

Funding: CNPq, FAPERGS, FAPESP, PPGBM-UFRGS.

Key words: *Vriesea*; Population Genetics; Phylogeography.

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Natural hybridization between two sympatric species of bromeliads from the Atlantic Forest: evolutionary implications

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Hybridization and introgression are important for understanding the process of speciation, the movement of genes across species boundaries and the maintenance of species cohesion. The degree of reproductive isolation among related species is an important factor influencing the genetic integrity of species and hybrid formation. Multiple pre- and postzygotic components are responsible for reproductive isolation among plant species pairs. *Vriesea carinata* and *V. incurvata* are two bromeliad species of the Tillandsioideae subfamily (Bromeliaceae) with high ornamental appeal, suffering impacts from illegal extraction. Both species are endemic and distributed along the Brazilian Atlantic Rainforest (BAF), with populations occurring in sympatry. These bromeliads showed sequential flowering along the year, with a short period of flowering overlap, and share the same floral visitor; both were pollinated by hummingbirds. Here, we investigated the potential of natural hybridization in four sympatric populations of *Vriesea carinata* and *Vriesea incurvata*. A total of 279 individuals of four sympatric populations were sampled, one allopatric population of *V. carinata* and one allopatric population of *V. incurvata* were also sampled, to be analyzed as reference populations. All samples were genotyped with 14 nuclear microsatellites and two cpDNA regions (matK gene and trnL-trnF intergenic spacer) were sequenced. Bayesian Structure results of all four sympatric populations analyzed presented hybrids (a total of 19; 9.1% of the total individuals sampled; $0.10 < q < 0.90$) between *V. carinata* and *V. incurvata*. Nevertheless, considering NewHybrids results, more hybrids were identified. Bayesian assignment analysis detected the presence of F2 and backcrosses towards *V. incurvata*. cpDNA network identified low haplotypic sharing, resulting probably from ancestral polymorphism or bidirectional introgression between these two species. Interestingly, populations at lower latitudes showed more hybrids than populations further south, this pattern could be influenced by ecological factors, since these species show sequential flowering

with a short period of overlap and pollinator sharing. A latitudinal gradient can be influenced by the seasons, temperature, rainfall and consequently affect the species' flowering period. The temporal difference in the flowering period of the two species may act as a strong prezygotic reproductive barrier, being the main force responsible for species cohesion. The presence of reproductive barriers has allowed these species to persist in sympatry for extended periods of time, ensuring the maintenance of species cohesion.

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Key words: *Vriesea*; Hybridization; Introgression.

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Morphology, collections and molecules – results and perspectives

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Since 1990, molecular approaches have provided a steadily increasing wealth of information on the systematics and phylogeny of Bromeliaceae. The monophyly of the family has never been doubted, but the intra-familial classification has recently undergone considerable changes. While the concept of eight subfamilies is widely accepted now, the genus delimitations are in many cases far from being settled. The *Aechmea* alliance in the subfamily Bromelioideae is one of the most intriguing examples for which phylogenetic analyses identified remarkable polyphyly. At the species level, we face similar problems. In the few cases where comprehensive molecular studies at this level are on hand, we have evidence for gene flow between species and genera. Additionally, the low genetic divergence in Bromeliaceae combined with high morphological plasticity and the as yet rudimentary knowledge of key morphological characters suggest that only a holistic approach will lead to a stable taxonomic solution. Another problem whose consequences are not to be overlooked is that species identification in the larger genera lacking recent revision (e.g. *Aechmea*, *Pitcairnia*, *Puya*, *Hechtia*) is highly problematic, meaning that misidentifications in molecular studies can be comparatively high. More genetic data and comprehensive sampling of groups will surely improve our knowledge and are highly important, e.g. as an essential contribution towards a new generic concept. However, they alone will most probably not be sufficient to establish a broadly accepted genus and species concept in the family. This can only be achieved when the considerable gaps in “classical” revisions are closed. Some morphological characters are obviously still underexploited (e.g. perianth, stigma, pollen, fruits). Progress in Bromeliaceae systematics could be enhanced considerably if a comprehensive, well documented and reliably determined reference collection could be established with officialised, but feasible access for the international scientific community. Examples are presented, where i) morphological approaches recently helped to identify groups that could be confirmed with molecular methods, ii) different topologies obtained from nuclear and plastid markers tell different stories and indicate special gene flow, and iii) genetic patterns of populations and related species cannot yet be interpreted satisfactorily.

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Key words: Phylogeny; Morphology; Collections.

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Heteroblasty in bromeliads

Zotz, Gerhard

More than a century ago, the term heteroblasty was coined by Carl Goebel to describe abrupt changes in form in the ontogeny of a range of vascular plants with diverse taxonomic affiliations and representing different life forms (e.g. herbs, vines, trees). Today, in 2015, we are still struggling with terminological issues, with unambiguous definitions of heteroblastic vs. homoblastic species, and are still far from an understanding of

the functional and evolutionary consequences of these sudden changes for any of the groups with heteroblastic members. Considering the diversity of ecologies it is probably futile to expect one single explanation. Hence, I will focus on heteroblasty in one particular group with a common phylogenetic background and a relative similarity in ecology: epiphytic bromeliads. Considering that water scarcity is arguably the most limiting abiotic factor in the epiphytic habitat, it seemed straightforward to interpret the observed ontogenetic shift from juveniles with many narrow leaves to later stages with broad leaves (tank-forming individuals) as an adaptation to intermittent water supply. This appeared particularly obvious because the early stages resemble so-called atmospheric bromeliad species, which are typically associated with drier habitats. Although early experimental studies provided some support for this notion, extensive work in the last years challenged this view. For example, it could be shown that most of the changes were actually continuous changes with plant size (ontogenetic drift). Moreover, heteroblastic species are as common in the understory of very wet forest as they are in more seasonal forest. Thus, the functional role of heteroblasty in bromeliads is still an open question – we are currently testing alternative hypotheses, e.g. the role of leaf form in the avoidance of self-shading in the understory. Reviewing the current state of knowledge demonstrates that heteroblastic changes are a fascinating phenomenon in many ways and an underutilized resource in the study of ecological and evolutionary processes.

Key words: Water Relations; Ecophysiology; Ontogeny.

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