



Genetics, evolution and conservation of Bromeliaceae

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Abstract

Bromeliaceae is a morphologically distinctive and ecologically diverse family originating in the New World. Three centers of diversity, 58 genera, and about 3,140 bromeliad species are currently recognized. We compiled all of the studies related to the reproductive biology, genetic diversity, and population structure of the Bromeliaceae, and discuss the evolution and conservation of this family. Bromeliads are preferentially pollinated by vertebrates and show marked variation in breeding systems, from predominant inbreeding to predominant outcrossing, as well as constancy in chromosome number ($2n = 2x = 50$). Autogamous or mixed mating system bromeliads have a high inbreeding coefficient (F_{IS}), while outcrossing species show low F_{IS} . The degree of differentiation among populations (F_{ST}) of species ranges from 0.043 to 0.961, which can be influenced by pollen and seed dispersal effects, clonal growth, gene flow rates, and connectivity among populations. The evolutionary history of the Bromeliaceae is poorly known, although some studies have indicated that the family arose in the Guayana Shield roughly 100 Mya. We believe that genetic, cytogenetic, and reproductive data will be essential for diagnosing species status and for assisting conservation programs.

Keywords: bromeliads, cytogenetics, genetic diversity, population structure, reproductive biology.

Introduction

The Bromeliaceae is one of the morphologically and ecologically most diverse flowering plant families native to the tropics and subtropics of the New World (Givnish *et al.*, 2011). Its geographical distribution ranges from the states of Virginia, Texas, and California in the USA (latitude 37° N) to northern Patagonia in Argentina (latitude 44° S). The family is known for its recent adaptive radiation. Bromeliads have different habits, varying from terrestrial to epiphytical, and are found from sea level to altitudes above 4,000 m, in both desert and humid regions, as well as in soils subject to regular floods and in places with very low or high luminosity. They can thrive on scalding sands and rocks, and withstand temperatures near 0 °C (Benzing, 2000).

Traditionally, the family has been divided into three subfamilies, Bromelioideae (~650 spp.), Pitcairnioideae (~890 spp.), and Tillandsioideae (~1000 spp.), based on

Smith and Downs (1979); this classification is adopted in the present study. However, in a recent phylogeny based on eight plastid regions, with representatives from 46 of 58 genera, Givnish *et al.* (2011) confirmed the eight-subfamily classification advanced by Givnish *et al.* (2007). The new classification splits the paraphyletic Pitcairnioideae into six subfamilies and proposes that they are related to each other as follow: (Brocchinioideae, (Lindmanioideae, (Tillandsioideae, (Hechtioideae, (Navioideae, (Pitcairnioideae, (Puyoideae, Bromelioideae)))))).

Bromeliads are especially appreciated for their ornamental value, but some species have proven medicinal properties (*e.g.*, *Bromelia antiacantha*) or are cultivated as tropical fruits (*e.g.*, pineapple: *Ananas comosus*). Here, we review the main genetic and evolutionary topics concerning Bromeliaceae, from a conservation standpoint.

Pollination and Reproductive Biology

Among the plant families, Bromeliaceae is the one with the highest diversity of pollination modes (ornithophily, chiropterophily, entomophily, mixed/unspecific, and autogamy) throughout its geographic distribution (Kessler

and Krömer, 2000; Canela and Sazima, 2005; Wendt *et al.*, 2008; Schmid *et al.*, 2010). Bromeliads have evolved floral displays with a great diversity of colors, shapes, and scents, which are related to pollinator attraction, with nectar being the usual reward (Benzing, 2000). The presence of Bromeliaceae in the New World has provided an important resource base, largely absent in the Old World, for small, hovering vertebrate pollinators (Fleming and Muchhala, 2008). A recent study (Krömer *et al.*, 2008) strongly supports the hypothesis that the composition of nectar sugars in Bromeliaceae is correlated with the pollinator syndrome (lepidopterophilous, trochilophilous, or chiropterophilous). Although the majority of bromeliads are pollinated by vertebrates, mainly hummingbirds and bats, bees are among the most frequent visitors to some short-corolla species with ornithophilous features. Nevertheless, few studies have identified insects as effective pollinators of these bromeliads (Kamke *et al.*, 2011).

Simultaneously with the divergence of bromeliad subfamilies (see “Evolution” below), the first split of modern hummingbird lineages appears to have occurred in the Andes about 13 Mya, with several other Andean lineages diverging during the Pliocene and Pleistocene (Givnish *et al.*, 2011). This might have contributed to the rapid expansion of the range of bromeliads and pollinators throughout the Neotropics. However, plant-pollinator interactions, seed dispersal, and the mechanisms promoting or constraining species diversification via these interactions are complex and poorly studied in the Neotropics (Antonelli and Sanmartín, 2011).

Bromeliads possess specialized floral features such as herkogamy and dichogamy, which prevent spontaneous self-fertilization and facilitate animal-mediated outcrossing (Benzing, 2000; Martinelli G, 1994, PhD Thesis, University of St. Andrews). Floral morphology, hand-pollination experiments, and population genetics studies have shown that selfing and mixed are the most common mating systems in a large part of the family (Bush and Guilbeau, 2009; Matallana *et al.*, 2010; Table 1), although self-incompatibility systems can be found in all of the subfamilies (Pitcairnioideae: Vosgueritchian and Buzato, 2006; Bromelioideae: Canela and Sazima, 2003, 2005; Schmid *et al.*, 2010; Kamke *et al.*, 2011; Tillandsioideae: Hietz *et al.*, 2006; Ramírez-Morillo *et al.*, 2009). The Tillandsioideae subfamily has a particularly high frequency of selfing and mixed systems in various genera, including *Alcantarea*, *Guzmania*, *Racinea*, *Tillandsia*, *Vriesea*, and *Werauhia* (Benzing, 2000; Lasso and Ackerman, 2004; Paggi *et al.*, 2007, 2012; Matallana *et al.*, 2010; Martinelli G, 1994, PhD Thesis, University of St. Andrews; Table 1). Clonality is another reproductive strategy present in the family (Murawski and Hamrick, 1990; Izquierdo and Pinero, 2000; Sarthou *et al.*, 2001; Sampaio *et al.*, 2002; Sgorbati *et al.*, 2004; Cascante-Marín *et al.*, 2006; Barbará *et al.*, 2009), with important ecological and evolutionary consequences

(Gonzales *et al.*, 2008) such as recruitment and population maintenance (Villegas, 2001).

We studied the mating systems of two bromeliad species. *Vriesea gigantea* presented a high natural production of flowers, fruits, and seeds, with high rates of viable seeds, with an average germination rate of 94% (Paggi *et al.*, 2007, 2010). Furthermore, the species showed regular chromosome segregation and high pollen viability (84-98%, Palma-Silva *et al.*, 2008), which indicated that the populations analyzed were fertile. Manual hand-pollination indicated that *V. gigantea* is self-compatible (Paggi *et al.*, 2007) and showed low to moderate levels of inbreeding depression ($\delta = 0.02$ to 0.39 ; Sampaio *et al.*, 2012). In a study with *Vriesea friburgensis* we highlighted that it is pollinated by hummingbirds and produces high flower, fruit, and seeds together with high seed and pollen viability. We concluded that the wild populations studied were fertile. Self-sterility was observed from spontaneous selfing and manual self-pollination treatments, which may be a consequence of late-acting self-incompatibility. We proposed that this self-sterile species depends on pollinator services to maintain its population fitness and viability through cross-pollination (Paggi *et al.*, 2012).

Diversity and Genetic Structure

The genetic diversity of only a few species of Bromeliaceae has been studied. We compiled data from all diversity and genetic structure studies published before June 2011 (Table 1). Of the 58 genera and about 3,140 bromeliad species (Givnish *et al.*, 2011), only 20 species of the following nine genera have been previously evaluated: *Aechmea*, *Alcantarea*, *Bromelia*, *Dyckia*, *Encholirium*, *Pitcairnia*, *Puya*, *Tillandsia*, and *Vriesea*. Most of the studied species are endemic to the Atlantic rainforest in southeastern Brazil.

The use of co-dominant markers has been the preferred method for studying bromeliad population genetics, with nuclear microsatellite markers being the most frequently used molecular markers (nine species), followed by allozymes (eight species). Dominant markers such as amplified fragment length polymorphisms have been used in only one study of one species, and random amplified polymorphic DNA was applied in another study of three species (Table 1). A comparison of genetic diversity parameters among such studies is difficult, as the highly polymorphic SSRs usually show higher observed and expected heterozygosity (H_O and H_E , respectively) compared with other markers. For example, populations of *Pitcairnia geyskesii* have been evaluated using allozymes (Sarthou *et al.*, 2001) and SSRs (Boisselier-Dubayle *et al.*, 2010). With allozymes, H_O and H_E were 0.188 and 0.246, respectively; with SSRs, H_O and H_E were 0.293 and 0.324, respectively.

We found low inbreeding coefficient indices (F_{IS}) in almost all species with outcrossing mating systems. The ex-

Table 1 - Bromeliads studied: Mating system, genetic diversity and population structure descriptors, molecular markers used and geographical distribution.

Species	Mating system	Marker	H_O mean/all	H_F mean/all	F_{IS} mean	F_{ST} mean	Geographical distribution	Reference
<i>Aechmea magdalenae</i>	ND	Allozyme	0.099/-	0.084/-	-	0.356 ^a	Mexico to Ecuador	Murawski and Hamrich, 1990
<i>Aechmea tuitensis</i>	ND	Allozyme	0.061/-	0.12/-	0.631	0.196	Endemic to Mexico	Izquierdo and Piñero, 2000
<i>Alcantarea geniculata</i>	Out	SSR	0.356/0.357	0.380/0.429	0.094	0.111	Rio de Janeiro, Brazil	Barbará <i>et al.</i> , 2007
<i>Alcantarea glaziouana</i>	Out	SSR	0.259/0.299	0.334/0.472	0.156	0.217	Rio de Janeiro, Brazil	Barbará <i>et al.</i> , 2009
<i>Alcantarea imperialis</i>	Out	SSR	0.357/0.362	0.398/0.615	0.099	0.434	Rio de Janeiro, Brazil	Barbará <i>et al.</i> , 2007
<i>Alcantarea Regina</i>	Out	SSR	0.479/0.484	0.458/0.523	-0.051	0.195	Rio de Janeiro, Brazil	Barbará <i>et al.</i> , 2009
<i>Bromelia antiacantha</i>	Out	SSR	0.326/-	0.559/-	0.431	0.224	southeastern Brazil	Zanella <i>et al.</i> , 2011
<i>Dyckia ibiramensis</i>	Mix	Allozyme	0.055/0.064	0.098/0.219	0.436	0.674 ^b	Endemic to southern Brazil	Hmeljevski <i>et al.</i> , 2010
<i>Encholirium biflorum</i>	ND	RAPD	-	-	-	0.160 ^c	Cadeia do Espinhaço, Brazil	Cavallari <i>et al.</i> , 2006
<i>Encholirium pedicellatum</i>	ND	RAPD	-	-	-	0.084 ^c	Cadeia do Espinhaço, Brazil	Cavallari <i>et al.</i> , 2006
<i>Encholirium subsecundatum</i>	ND	RAPD	-	-	-	0.012 ^c	Cadeia do Espinhaço, Brazil	Cavallari <i>et al.</i> , 2006
<i>Pitcairnia albiflos</i>	Out	SSR	0.383/-	0.429/-	0.109	0.336	Rio de Janeiro, Brazil	Palma-Silva <i>et al.</i> , 2011
<i>Pitcairnia geyskesii</i>	ND	SSR	0.293/-	0.325/-	0.125	0.156	French Guyana and Suriname	Boisselier-Dubayle <i>et al.</i> , 2010
<i>Pitcairnia geyskesii</i>	ND	Allozyme	0.185/0.188	0.183/0.246	-0.037	0.266	French Guyana and Suriname	Sarthou <i>et al.</i> , 2001
<i>Pitcairnia staminea</i>	Aut	SSR	0.347/-	0.452/-	0.240	0.336	Rio de Janeiro, Brazil	Palma-Silva <i>et al.</i> , 2011
<i>Puya raimondii</i>	Aut	AFLP	-	-	-	0.961 ^a	Peru	Sgorbati <i>et al.</i> , 2004
<i>Tillandsia achyrotachys</i> ^d	ND	Allozyme	0.127/-	0.210/-	0.433	0.391	Mexico	González-Astorga <i>et al.</i> , 2004
<i>Tillandsia ionantha</i>	ND	Allozyme	0.064/-	0.069	0.056	0.043	Central Mexico to Nicaragua	Soltis <i>et al.</i> , 1987
<i>Tillandsia recurvata</i>	ND	Allozyme	0/-	0.01/-	1.000	0.906	USA to Argentina	Soltis <i>et al.</i> , 1987
<i>Vriesea friburgensis</i>	Mix	Allozyme	-0.234	-0.226	-0.035	-	Rio Grande do Sul to Pernambuco, Brazil	Alves <i>et al.</i> , 2004
<i>Vriesea gigantea</i>	Mix	SSR	0.431/-	0.579/-	0.273	0.211	Brazil (south and southeast)	Palma-Silva <i>et al.</i> , 2009

ND = Not determined; Out = Outcrossing; Mix = Mixed; Aut = Autogamous; AFLP = Amplified Fragment Length Polymorphism; RAPD = Random Amplified Polymorphic DNA; SSR = Microsatellite.

^a G_{ST} (Nei, 1973, 1977).

^b G_{ST} (Hedrick, 2005).

^c θ_{ST} (Excoffier *et al.*, 1992).

^d*Tillandsia achyrotachys* var *achyrotachys*.

ceptions were *B. antiacantha* ($F_{IS} = 0.431$), possibly due to the Wahlund effect and/or null alleles, and *Alcantarea glaziouana* ($F_{IS} = 0.156$), owing to biparental inbreeding. *Pitcairnia staminea*, which is autogamous, had a high inbreeding coefficient ($F_{IS} = 0.240$; Table 1). *V. gigantea* and *Dyckia ibiramensis*, which have a mixed mating system, also showed high inbreeding coefficients ($F_{IS} = 0.273$ and 0.436 , respectively; Table 1). The degree of differentiation among populations (F_{ST}) of species evaluated ranged from 0.043 to 0.961. These differences in plant population structure can be influenced by pollen and seed dispersal effects, clonal growth (Gliddon *et al.*, 1987), gene flow rates, and connectivity among populations. Compared with species from continuous forest habitats, species restricted to inselberg habitats (Barbará *et al.*, 2007, 2009; Palma-Silva *et al.*, 2011; Table 1) showed more highly structured populations, with extremely high population differentiation and isolation based on the distance among inselbergs. Thus, rock outcrops could be highly useful venues for studies regarding the molecular ecology and genetics of continental radiations.

Cytogenetics

Few cytogenetic studies of Bromeliaceae are available. Chromosome numbers have been determined for nearly 12% of the known species (Cotias-de-Oliveira *et al.*, 2004), most of which are horticulturally important as ornamentals or fruit producers. Owing to the scarcity of cytogenetic data, the chromosomal evolution of the family has not been completely elucidated. The major hindrances to cytogenetic studies are probably the very small size and poor staining ability of the chromosomes, together with a marked cytoplasmic content (Sharma and Ghosh, 1971; Brown and Gilmartin, 1986).

Billings (1904) was the first to determine the chromosome number of a bromeliad, using *Tillandsia usneoides*, after which several studies were carried out. The first reports revealed a great variety of diploid numbers ($2n = 16, 34, 36, 46, 48, 50, 52, 54, 56, 64, 96, \text{ and } 100$) and basic numbers ($x = 5, 8, 9, 16, 17, \text{ and } 25$; Brown and Gilmartin, 1986; Bellintani *et al.*, 2005). In contrast, most of the 72 bromeliad species studied by Marchant (1967) showed a basic number of $x = 25$ (except *Cryptanthus*: $x = 17$). Since then, studies in several different species have generally found the basic chromosome number to be a multiple of $x = 25$, corroborating Marchant's finding (Brown and Gilmartin, 1989; Cotias-de-Oliveira *et al.*, 2000, 2004; Palma-Silva *et al.*, 2004; Gitaí *et al.*, 2005; Ceita *et al.*, 2008; Louzada *et al.*, 2010). Polyploidy of this base number ($2n = 4x = 100$ and $2n = 6x = 150$) has been observed in all subfamilies, but with low frequency (Brown and Gilmartin, 1989; Gitaí *et al.*, 2005; Louzada *et al.*, 2010).

Brown and Gilmartin (1989) have proposed a model to explain the evolution of the chromosome base number. In their model, two paleodiploids ($x = 8$ and $x = 9$) hybrid-

ized, resulting in a paleotetraploid lineage ($x = 17$), which in turn hybridized with the $x = 8$ paleodiploid, and the poliploidization stabilized at the hexaploid level of $x = 25$. Electrophoretic data (Soltis *et al.*, 1987) suggest that a "diploidization" of the dibasic paleohexaploid occurred. The dibasic model could explain the origin of the distinctive chromosome number in *Cryptanthus*, which may represent a paleotetraploid with $2n = 34$. One alternative hypothesis is that *Cryptanthus* evolved from $x = 25$ via aneuploidy (Brown and Gilmartin, 1989). Flow cytometric results obtained by Ramírez-Morillo and Brown (2001) indicated that the *Cryptanthus* chromosome number originated by descending aneuploidy.

Bromeliaceae chromosomes are usually exceedingly small (0.21–2.72 μm), although the size varies widely among species. According to Gitaí *et al.* (2005), larger chromosomes are usually found at lower ploidy levels, with diploids exhibiting a higher contrast between maximal and minimal chromosome sizes compared with polyploids. Chromosome banding and triple staining with CMA₃/Actinomycin/DAPI has revealed that bromeliads have relatively little heterochromatin, with only one or two CMA⁺/DAPI terminal bands corresponding to nucleolus organizing regions. B chromosomes have been reported in three Bromelioideae species (Cotias-de-Oliveira *et al.*, 2000, 2004; Bellintani *et al.*, 2005).

Evolution

Recently, Givnish *et al.* (2011) reinforced the *i.e.* of Smith (1934) that bromeliads arose in the Guayana Shield roughly 100 Mya during the Cretaceous Period, with the extant subfamilies beginning to diverge only about 19 Mya. Givnish *et al.* (2011) also suggested that about 15.4 Mya, bromeliads began to spread from that hyper-humid, extremely infertile center to other parts of tropical and subtropical America, and probably arrived in tropical Africa about 9.3 Mya, in a recent long-distance dispersal event. During the evolution of this family, events such as climatic oscillations throughout the Pleistocene have resulted in the dispersion of some clades, including Bromelioideae (Givnish *et al.*, 2011). As of the current time, *V. gigantea* has survived glaciation periods in two fragmented refugia in southeastern Brazil (Palma-Silva *et al.*, 2009).

The "bromeliad revolution" probably occurred after the uplift of the northern Andes and shift of the Amazon to its present course (Givnish *et al.*, 2007). Some morphological and physiological adaptations, including crassulacean acid metabolism (CAM) photosynthesis and the formation of rosettes and leaf absorptive scales, might have been crucial to the adaptive radiation of bromeliads (Benzing, 2000; Crayn *et al.*, 2004).

An ecological peculiarity of Bromeliaceae, compared with other families of the order Poales, is their epiphytic habit (Linder and Rudall, 2005). Based on plastid loci,

Crayn *et al.* (2004) proposed that the epiphytic habit of bromeliads evolved a minimum of three times, most likely in response to geological and climatic changes in the late Tertiary.

The more than 3,000 bromeliad species that currently occupy the Neotropical region have evolved to fill numerous niches, with an incredible diversity of adaptations. Some aspects of the complex evolutionary history of this family are still unclear, indicating the need for further molecular studies, in combination with paleontological data, to explain the evolutionary gaps in the wide diversity of bromeliad forms and adaptations.

Conservation

Bromeliads are widely distributed in the Neotropics, with three centers of diversity: the Brazilian Atlantic rainforest; the Andean slopes of Peru, Colombia, and Ecuador; and Mexico and adjacent Central America (Zizka *et al.*, 2009). Many species are presently distributed in endangered biomes, are endemic, or have a relict distribution, threatening the survival of many members of this family. For example, the Brazilian Atlantic rainforest is a diverse biome with multiple extremely endangered vegetation types occupying only 7.91% of the extent of their original distribution (Fundação SOS Mata Atlântica and Instituto Nacional de Pesquisas Espaciais, 2009; Carnaval and Moritz, 2008). As the Atlantic rainforest contains at least 803 bromeliad species, 653 of which are endemic and 40% of which are endangered, the preservation of the Atlantic rainforest is vital for the conservation of Bromeliaceae (Martinelli *et al.*, 2008).

Few studies of Bromeliaceae connect genetic data and conservation planning. All of the works cited in the above section “Diversity and genetic structure” contain data that could be used in making conservation decisions. Considerations of the clonal and sexual reproduction, demography, genetic structure within and among populations, gene flow, and mating systems of Bromeliaceae are of primary importance in developing successful conservation strategies (Bizoux and Mahy, 2007).

Our group has studied mainly Brazilian bromeliads, and our field records show a significant reduction in the current distribution of species, compared with the first records in the literature. We believe that genetic, cytogenetic, and reproductive data will be essential for diagnosing species status and for assisting conservation programs and will help to elucidate aspects of evolution and environmental and climate change for Bromeliaceae and the Brazilian Atlantic rainforest.

References

Alves GM, Filho AR, Puchalski A, Reis MS, Nodari RO and Guerra MP (2004) Allozymic markers and genetic characterization of a natural population of *Vriesea friburgensis* var.

- paludosa*, a bromeliad from the Atlantic Forest. *Plant Genet Resour* 2:23-28.
- Antonelli A and Sanmartín I (2011) Why are there so many plant species in the Neotropics? *Taxon* 60:403-414.
- Barbará T, Martinelli G, Fay MF, Mayo SJ and Lexer C (2007) Population differentiation and species cohesion in two closely related plants adapted to neotropical high-altitude ‘inselbergs’, *Alcantarea imperialis* and *Alcantarea geniculata* (Bromeliaceae). *Mol Ecol* 16:1981-1992.
- Barbará T, Martinelli G, Palma-Silva C, Fay MF, Mayo SJ and Lexer C (2009) Genetic relationships and variation in reproductive strategies in four closely related bromeliads adapted to neotropical ‘inselbergs’: *Alcantarea glaziouana*, *A. regina*, *A. geniculata* and *A. imperialis* (Bromeliaceae). *Ann Bot* 103:65-77.
- Bellintani MC, Assis JGA and Cotias-de-Oliveira ALP (2005) Chromosomal evolution of Bromeliaceae. *Cytologia* 70:129-133.
- Benzing DH (2000) Bromeliaceae: Profile of an Adaptive Radiation. Cambridge University Press, Cambridge, 690 pp.
- Billings FH (1904) A study of *Tillandsia usneoides*. *Bot Gaz* 38:99-121.
- Bizoux JP and Mahy G (2007) Within-population genetic structure and clonal diversity of a threatened endemic metallophyte, *Viola calaminaria* (Violaceae). *Am J Bot* 94:887-895.
- Boisselier-Dubayle MC, Leblois R, Samadi S, Lambourdière J and Sarthou C (2010) Genetic structure of the xerophilous bromeliad *Pitcairnia geyskesii* on inselbergs in French Guiana - A test of the forest refuge hypothesis. *Ecography* 33:175-184.
- Brown GK and Gilmartin AJ (1986) Chromosomes of the Bromeliaceae. *Selbyana* 9:88-93.
- Brown GK and Gilmartin AJ (1989) Chromosome numbers in Bromeliaceae. *Am J Bot* 76:657-665.
- Bush SP and Guilbeau JE (2009) Early autonomous selfing in the hummingbird-pollinated epiphyte *Pitcairnia brittoniana* (Bromeliaceae). *J Torr Bot Soc* 136:313-321.
- Canela MBF and Sazima M (2003) *Aechmea pectinata*: A hummingbird-dependent bromeliad with inconspicuous flowers from the rainforest in south-eastern Brazil. *Ann Bot* 92:731-737.
- Canela MBF and Sazima M (2005) The pollination of *Bromelia antiacantha* (Bromeliaceae) in southeastern Brazil. *Plant Biol* 7:1-6.
- Carnaval AC and Moritz C (2008) Historical climate modelling predicts patterns of current biodiversity in the Brazilian Atlantic forest. *J Biogeogr* 35:1187-1201.
- Cascante-Marin A, De Jong M, Borg ED, Oostermeijer JGB, Wolf JHD and Den Nijs JCM (2006) Reproductive strategies and colonizing ability of two sympatric epiphytic bromeliads in a tropical premontane area. *Int J Plant Sci* 167:1187-1195.
- Cavallari MM, Forzza RC, Veasey EA, Zucchi MI and Oliveira GCX (2006) Genetic variation in three endangered species of *Encholirium* (Bromeliaceae) from Cadeia do Espinhaço, Brazil, detected using RAPD Markers. *Biodivers Conserv* 15:4357-4373.
- Ceita GO, Assis JGA, Guedes MLS and Oliveira ANPC (2008) Cytogenetics of Brazilian species of Bromeliaceae. *Bot J Linn Soc* 158:189-193.

- Cotias-de-Oliveira ALP, Assis JGA, Bellintani MC, Andrade JC and Guedes MLS (2000) Chromosome numbers in Bromeliaceae. *Genet Mol Biol* 23:173-177.
- Cotias-de-Oliveira ALP, Assis JGA, Ceita G, Palmeira ACL and Guedes MLS (2004) Chromosome number for Bromeliaceae species occurring in Brazil. *Cytologia* 69:161-166.
- Crayn DM, Winter K and Smith JAC (2004) Multiple origins of crassulacean acid metabolism and the epiphytic habit in the Neotropical family Bromeliaceae. *Proc Natl Acad Sci USA* 101:3703-3708.
- Excoffier L, Smouse PE and Quattro JM (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes - application to human mitochondrial - DNA restriction data. *Genetics* 131:479-491.
- Fleming TH and Muchhala N (2008) Nectar-feeding bird and bat niches in two worlds: Pantropical comparisons of vertebrate pollination systems. *J Biogeogr* 35:764-780.
- Fundação SOS Mata Atlântica and Instituto Nacional de Pesquisas Espaciais (1993) Atlas dos Remanescentes Florestais da Mata Atlântica Período 2005-2008. Fundação SOS Mata Atlântica, São Paulo, 156 pp.
- Gitaí J, Horres R and Benko-Iseppon AM (2005) Chromosomal features and evolution of Bromeliaceae. *Plant Syst Evol* 253:65-80.
- Givnish TJ, Millam KC, Berry PE and Sytsma KJ (2007) Phylogeny, adaptive radiation, and historical biogeography of Bromeliaceae inferred from *Ndhf* sequence data. *Aliso* 23:3-26.
- Givnish, TJ Barfuss MHJ, Ee BV, Riina R, Schulte K, Horres R, Gonsiska PA, Jabaily RS, Crayn DM, Smith JAC, et al (2011) Phylogeny, adaptive radiation, and historical biogeography in Bromeliaceae: Insights from an eight-locus plastid phylogeny. *Am J Bot* 98:872-895.
- Gliddon C, Belhassen E and Gouyon pH (1987) Genetic neighborhoods in plants with diverse systems of mating and different patterns of growth. *Heredity* 59:29-32.
- Gonzales E, Hamrick JL and Smouse PE (2008) Comparison of clonal diversity in mountain and piedmont populations of *Trillium cuneatum* (Melanthiaceae-Trilliaceae) a forest understory species. *Am J Bot* 95:1254-1261.
- González-Astorga J, Cruz-Angon A, Flores-Palacios A and Vovides AP (2004) Diversity and genetic structure of the Mexican endemic epiphyte *Tillandsia achyrostachys* E. Morr. ex Baker var. *achyrostachys* (Bromeliaceae). *Ann Bot* 94:545-551.
- Hedrick PW (2005) A standardized genetic differentiation measure. *Evolution* 59:1633-1638.
- Hietz P, Winkler M, Cruz-Paredes L and Jiménez-Aguilar A (2006) Breeding systems, fruit set, and flowering phenology of epiphytic bromeliads and orchids in a Mexican humid mountain forest. *Selbyana* 27:156-164.
- Hmeljevski KV, Reis A, Montagna T and Reis MS (2011) Genetic diversity, genetic drift and mixed mating system in small subpopulations of *Dyckia ibiramensis*, a rare endemic bromeliad from southern Brazil. *Conserv Genet* 12:761-769.
- Izquierdo LY and Piñero D (2000) High genetic diversity in the only known population of *Aechmea tuitensis* (Bromeliaceae). *Aust J Bot* 48:645-650.
- Kamke R, Schmid S, Zillikens A, Lopes BC and Steiner J (2011) The importance of bees as pollinators in the short corolla bromeliad *Aechmea caudata* in southern Brazil. *Flora* 206:749-756.
- Kessler M and Krömer T (2000) Patterns and ecological correlates of pollination modes among bromeliad communities of Andean Forests in Bolivia. *Plant Biol* 2:659-669.
- Krömer T, Kessler M, Lohaus G and Schmidt-Leubuh AN (2008) Nectar sugar composition and concentration in relation to pollination syndromes in Bromeliaceae. *Plant Biol* 10:502-511.
- Lasso E and Ackerman JD (2004) The flexible breeding system of *Werauhia sintenisii*, a cloud forest bromeliad from Puerto Rico. *Biotropica* 36:414-417.
- Linder HP and Rudall PJ (2005) Evolutionary history of Poales. *Annu Rev Ecol Evol Syst* 36:107-24.
- Louzada RB, Palma-Silva C, Corrêa AM, Kaltchuk-Santos E and Wanderley MGL (2010) Chromosome number of *Orthophytum* species (Bromeliaceae). *Kew Bull* 65:53-58.
- Marchant CJ (1967) Chromosome evolution in Bromeliaceae. *Kew Bull* 21:161-170.
- Martinelli G, Vieira CM, Gonzalez M, Leitman P, Piratininga A, Costa AF and Forzza RC (2008) Bromeliaceae da Mata Atlântica Brasileira: Lista de espécies, distribuição e conservação. *Rodriguésia* 59:209-258.
- Matallana G, Godinho MAS, Guilherme FAG, Belisario M, Coser TS and Wendt T (2010) Breeding systems of Bromeliaceae species: Evolution of selfing in the context of sympatric occurrence. *Plant Syst Evol* 289:57-65.
- Murawski DA and Hamrick JL (1990) Local genetic and clonal structure in the tropical terrestrial bromeliad, *Aechmea magdalenae*. *Am J Bot* 77:1201-1208.
- Nei M (1973) Analysis of gene diversity in subdivided populations. *Proc Natl Acad Sci USA* 70:3321-3323.
- Nei M (1977) F-statistics and analysis of gene diversity in subdivided populations. *Ann Hum Genet* 41:225-233.
- Paggi GM, Palma-Silva C, Silveira LCT, Kaltchuk-Santos E, Bodanese-Zanettini MH and Bered F (2007) Fertility of *Vriesea gigantea* Gaud. (Bromeliaceae), in southern Brazil. *Am J Bot* 94:683-689.
- Paggi GM, Sampaio JAT, Bruxel M, Zanella CM, Goetze M, Büttow MV, Palma-Silva C and Bered F (2010) Seed dispersal and population structure in *Vriesea gigantea*, a bromeliad from the Brazilian Atlantic Rainforest. *Bot J Linn Soc* 164:317-325.
- Paggi GM, Silveira LCT, Zanella CM, Bruxel M, Bered F, Kaltchuck-Santos E and Palma-Silva C (2012) Reproductive system and fitness of *Vriesea friburgensis*, a self-sterile bromeliad species. *Plant Spec Biol* doi: 10.1111/j.1442-1984.2012.00374.x.
- Palma-Silva C, Santos DG, Kaltchuk-Santos E and Bodanese-Zanettini MH (2004) Chromosome numbers, meiotic behavior, and pollen viability of species of *Vriesea* and *Aechmea* genera (Bromeliaceae) native to Rio Grande do Sul, Brazil. *Am J Bot* 91:804-807.
- Palma-Silva C, Paggi GM, Felicetti RA, Ferraz RS, Kaltchuk-Santos E, Bered F and Bodanese-Zanettini MH (2008) Meiotic behavior and pollen viability of wild populations of the neotropical species *Vriesea gigantea* (Bromeliaceae). *Plant Spec Biol* 23:217-221.
- Palma-Silva C, Lexer C, Paggi GM, Barbará T, Bered F and Bodanese-Zanettini MH (2009) Range-wide patterns of nuclear and chloroplast DNA diversity in *Vriesea gigantea* (Bromeliaceae), a Neotropical forest species. *Heredity* 103:503-512.

- Palma-Silva C, Wendt T, Pinheiro F, Barbará T, Fay MF, Cozzolino S and Lexer C (2011) Sympatric bromeliad species (*Pitcairnia* spp.) facilitate tests of mechanisms involved in species cohesion and reproductive isolation in Neotropical inselbergs. *Mol Ecol* 20:3185-3201.
- Ramírez-Morillo IM and Brown GK (2001) The origin of the low chromosome number in *Cryptanthus* (Bromeliaceae). *Syst Bot* 26:722-726.
- Ramírez-Morillo IM, May FC, Carnevali G and Pat FM (2009) It takes two to tango: Self incompatibility in the bromeliad *Tillandsia streptophylla* (Bromeliaceae) in Mexico. *Rev Biol Trop* 57:761-770.
- Sampaio MC, Perissé LE, de Oliveira GA and Rios RI (2002) The contrasting clonal architecture of two bromeliads from sandy coastal plains in Brazil. *Flora* 197:443-451.
- Sampaio JAT, Paggi GM, Zanella CM, Bruxel M, Palma-Silva C, Goetze M, Büttow MV and Bered F (2012) Inbreeding depression in *Vriesea gigantea*, a perennial bromeliad from southern Brazil. *Bot J Lin Soc* 169:312-319.
- Sarthou C, Samadi S and Boisselier-Dubayle MC (2001) Genetic structure of the saxicole *Pitcairnia geyskesii* (Bromeliaceae) on inselbergs in French Guiana. *Am J Bot* 88:861-868.
- Sgorbati S, Labra M, Grugni E, Barcaccia G, Galasso G, Boni U, Mucciarelli M, Citterio S, Benavides Iramátegui A, Venero Gonzales L, *et al.* (2004). A survey of genetic diversity and reproductive biology of *Puya raimondii* (Bromeliaceae), the endangered queen of the Andes. *Plant Biol* 6:222-230.
- Schmid S, Schmid VS Zillikens A, Harter-Marques B and Steiner J (2010) Bimodal pollination system of the bromeliad *Aechmea nudicaulis* involving hummingbirds and bees. *Plant Biol* 13:41-50.
- Sharma AK and Ghosh I (1971) Cytotaxonomy of the family Bromeliaceae. *Cytologia* 36:237-247.
- Smith LB (1934) Geographical evidence on the lines of evolution in Bromeliaceae. *Bot Jahrb Syst, Pflanzengesch Pflanzengeogr* 66:446-468.
- Smith LB and Downs RJ (1979) Bromelioideae (Bromeliaceae). In: *Flora Neotropica Monograph 14*. Hafner Press, New York, pp 1658-1660.
- Soltis DE, Gilmartin AJ, Rieseberg L and Gardner S (1987) Genetic variation in the epiphytes *Tillandsia ionatha* and *T. recurvata* (Bromeliaceae). *Am J Bot* 74:531-537.
- Villegas AC (2001) Spatial and temporal variability in clonal reproduction of *Aechmea magdalenae*, a tropical understory herb. *Biotropica* 33:48-59.
- Vosgueritichian SB and Buzato S (2006) Reprodução sexuada de *Dyckia tuberosa* (Vell.) Beer (Bromeliaceae, Pitcairnioideae) e interação planta-animal. *Rev Bras Bot* 29:433-442.
- Wendt T, Coser TS, Matallana G and Guilherme FAG (2008) An apparent lack of prezygotic reproductive isolation among 42 sympatric species of Bromeliaceae in southeastern Brazil. *Plant Syst Evol* 275:31-41.
- Zanella CM, Bruxel M, Paggi GM, Goetze M, Büttow MV, Cidade FW and Bered F (2011) Genetic structure and phenotypic variation in wild populations of the medicinal tetraploid species *Bromelia antiacantha* (Bromeliaceae). *Am J Bot* 98:1511-1519.
- Zizka G, Schimidt M, Schulte K, Novoa P, Pinto R and König K (2009) Chilean Bromeliaceae: Diversity, distribution and evaluation of conservation status. *Biodivers Conserv* 18:2449-2471.

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