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Diversidade genética de *Vriesea reitzii* (Leme & Costa): relevâncias no contexto de paisagem e comparação genética com espécies relacionadas

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Sumário

Resumo

A família Bromeliaceae representa um clássico exemplo de radiação adaptativa nos neotrópicos, sendo notável sua imensa versatilidade ecológica e variedade de formas. A Mata Atlântica abriga várias espécies de bromélias, algumas delas com alta semelhança morfológica, como é o caso de *Vriesea altodaserrae*, *Vriesea philippocoburgii* e *Vriesea reitzii*. Entretanto, tais espécies, diferem quanto à sua distribuição altitutinal e latitudinal, apesar de haver áreas de sobreposição, formando dessa maneira, gradientes. Com o objetivo de aprofundar os conhecimentos sobre a evolução da família Bromeliaceae, duas abordagens foram realizadas no presente trabalho: (a) análise da dinâmica populacional de seis populações de *V. reitzii* em fragmentos de Floresta Ombrófila Mista (b) análise da diferenciação populacional e fluxo gênico nas três espécies ao longo de um gradiente altitudinal, utilizando marcadores genéticos nucleares e análise Bayesiana. Essas abordagens nunca foram realizadas para uma espécie de Bromeliaceae nesta ecorregião da Mata Atlântica. Quanto à dinâmica populacional de populações de *V. reitzii* ao longo de toda sua distribuição geográfica, foi possível notar índices relativamente altos de diversidade genética (H_E=0,536), no contexto comparativo com outras espécies de Bromeliaceae e baixa a moderada entre suas populações (F_{ST}= 0,123), apesar da distância entre as mesmas e do isolamento natural imposto pela altitude. Considerando as três espécies estudadas, os perfis genéticos revelaram diferenças entre as mesmas, corroborando dados morfológicos e ecológicos de unidades taxonômicas distintas. Os resultados indicaram menor estruturação genética entre *V. altodaserrae* e *V. philippocoburgii* e um relativo isolamento de *V. reitzii* em relação às outras duas espécies. Os resultados, em conjunto, agregam informações importantes para o conhecimento da dinâmica evolutiva da Floresta Ombrófila Mista, bem como da família Bromeliaceae. Além disso, os dados aqui gerados podem ser levados em consideração para estratégias de conservação e manejo das espécies estudadas.

Abstract

The Bromeliaceae family is a classic example of adaptive radiation in the Neotropics, presenting high versatility and numerous ecological morphs. The Atlantic Forest harbors several species of bromeliads, some of them highly similar morphologically as *Vriesea altodaserrae*, *Vriesea philippocoburgii* and *Vriesea reitzii.* These species differ in its altitudinal and latitudinal distribution, although there are areas of overlaps, forming gradients. Aiming to contribute to the knowledge on the evolution of the family, two approaches were studied here: (a) population dynamics were analyzed in six populations of *V. reitzii* in Araucaria forest fragments and (b) population differentiation and gene flow was analyzed in three species along an altitudinal gradient using nuclear genetic markers and Bayesian analysis. These approaches have never been done in bromeliads species from this Atlantic Forest ecoregion. Considering the population's dynamics of *V. reitzii* throughout its geographical distribution, it was possible to note high levels of genetic diversity (H_E=0.536) in comparison to other bromeliads species and low to moderate differentiation among their populations (F_{ST} = 0.123), despite the distance between collect points and the natural isolation imposed by altitude. Considering the three species studied, the genetic profiles revealed differences between them, corroborating morphological and ecological data of distinct taxonomic units. The results indicated low genetic structure between *V. altodaserrae* and *V. philippocoburgii* and a relative isolation of *V. reitzii* from the other two species. Those results, together, can be considered important information to the knowledge of the evolutionary dynamics of the Araucaria Forest, as well as to Bromeliaceae family. Moreover, the data generated here may be considered for conservation strategies and management of the species.

Capítulo I

Introdução Geral

1. *Introdução*

1.1 Família Bromeliaceae

A família Bromeliaceae é um exemplo clássico de radiação adaptativa entre as plantas vasculares (Crayn *et al.*, 2004). Com 58 gêneros e aproximadamente 3.248 espécies (Luther, 2010), representa a maior família dentre as Angiospermas de distribuição exclusivamente Neotropical, ocorrendo em uma grande variedade de hábitats, desde rochas graníticas, dunas costeiras, florestas tropicais e florestas úmidas de altitude (Smith e Downs, 1974; Balke *et al.*, 2008). O único exemplo de espécie ocorrente fora das Américas é *Pitcairnia feliciana* (A. Chev.) Harms & Mildbraed, encontrada no continente africano, o que parece ter sido um evento recente de dispersão por longa distância (Benzing, 2000; Givnish *et al.*, 2011). No tocante a distribuição geográfica da família, tem-se como limite norte de ocorrência os estados da Virgínia, Texas e Califórnia, nos Sul dos Estados Unidos (Latitude 37°N) e como limite sul o norte da Patagônia, na Argentina (Latitude 44°S) (Smith, 1934; Leme e Marigo, 1993). Aproximadamente 50% das espécies de bromélias conhecidas são encontradas no Brasil (Louzada *et al.*, 2010).

Considerada basal dentro da ordem Poales (Bremer, 2002), Bromeliaceae tem sido tradicionalmente subdividida em três subfamílias: Bromelioideae (~650 sp), Pitcairnioideae (~890 sp) e Tillandsioideae (~1100 sp) (Smith e Downs, 1974, 1977 e 1979). Entretanto, uma nova classificação proposta inicialmente por Givnish *et al.* (2007) e confirmada posteriormente por Givnish *et al.* (2011), baseada dados moleculares com a sobreposição de em dados de morfologia de flores, frutos e sementes, propõe a circunscrição da subfamília Pitcairnoideae em novas seis subfamílias, com o relacionamento geral das subfamílias como segue: (Brocchinioideae, (Lindmanioideae, (Tillandsioideae, (Hechtiooideae, (Navioideae, (Pitcarnioideae, (Puyoideae, Bromelioideae))))))).

Sobre a evolução da família com base em dados de oito sequencias plastidiais, Givnish *et al.*, (2011) concluíram que as bromélias surgiram no Escudo das Guianas há 100 milhões de anos atrás (M.a.), reforçando a hipótese de Smith (1934), e que as linhagens existentes começaram a se divergir entre si por volta de 19 M.a. Os próprios autores salientam que há cerca de 15,4 M.a. o grupo se espalhou pela América e provavelmente chegou à África há cerca de 9,3 M.a. (Figura 1).

Figura 1: Distribuição geográfica da família Bromeliaceae, ocorrendo desde os estados da Virginia, Texas e Califórnia nos Estados Unidos até o norte da Patagônia na Argentina. Distribuição disjunta de *Pitcairnia feliciana*, ocorrendo na África. Eventos evolutivos citados na figura: (a) Origem da família no Escudo das Guianas; (b) Migração para outras partes da América e (c) Migração para África (adaptada de Steven, 2008).

Sabe-se que a grande radiação adaptativa do grupo se deve a algumas adaptações fisiológicas durante o curso evolutivo das mesmas, como Metabolismo Ácido das Crassuláceas (CAM), formação de rosetas e folhas com tricomas absorventes (Benzing, 2000; Crayn *et al.*, 2004).

Não surpreendentemente, os tanques aquáticos das bromélias abrigam uma diversa fauna aquática de mais de 400 espécies, incluindo insetos e anfíbios, muitos dos quais são estritamente dependentes desses habitats durante parte ou todo ciclo de vida (Kitching, 2000; Greeney, 2001; Ribera *et al.*, 2008). Desta forma, representantes da família apresentam importante papel nos ecossistemas em que ocorrem.

1.2 Estudos genéticos em espécies de plantas de altitude

Estudos recentes discutem que espécies que ocorrem em elevadas altitudes podem ter seu ciclo de vida diminuído, já que as baixas temperaturas podem influenciar no seu ciclo de crescimento, com consequências nos níveis e estruturação da diversidade genética dessas espécies (Thiel-Egenter *et al*., 2009). Outra potencial consequência de elevadas altitudes na distribuição espacial de indivíduos, e consequentemente na diversidade genética, é a diminuição no tamanho populacional, comparado com as populações de menores altitudes. Populações menores em geral têm aumento das taxas de endocruzamento, o que pode trazer sérias consequências às populações comprometidas, tais como fixação de alelos deletérios e depressão endogâmica (Ohsawa e Ide, 2008; Herrera e Basaga, 2010).

Estudos baseados em gradientes altitudinais têm apontado para várias linhas de raciocínio, como por exemplo: 1) Populações de altitude média tem maior diversidade genética que populações de baixas e altas altitudes (Isik e Kara, 1997; Ohsawa *et al*., 2007); 2) Populações de altas altitudes tem menos diversidade genética do que populações de altitude intermediária (Quiroga e Premoli, 2006); 3) Populações de altitude mais baixas tem menor diversidade genética que populações de altitudes mais altas (Gämperle e Schneller, 2002); 4) A variação genética intrapopulacional não é influenciada pela altitude (Truong *et al*., 2007).

1.3 Estudos genéticos em Bromeliaceae

Uma série de estudos genético-populacionais tem utilizado espécies de bromélias como modelo, em face de sua ampla radiação nos Neotrópicos, diversidade em sistemas de cruzamento, versatilidade ecológica, dentre outras inúmeras características. Entretanto, existem poucos trabalhos sobre genética de populações publicados com Bromeliaceae até o momento, somente para cerca de 20 espécies (Zanella *et al*., 2012).

Inicialmente, Soltis *et al*., (1987) estudaram a variação genética de duas espécies epífitas com diferentes sistemas de cruzamento: *Tillandsia ionantha* Planch. e *T. recurvata* (Gaudich.) Baker. Posteriormente, em 1990, Murawski e Hamrick analisaram a variação genética de isoenzimas em uma espécie de crescimento clonal, *Aechmea magdalenae* (André) André ex Baker. Sarthou *et al.* (2001), utilizaram abordagem molecular em uma espécie saxícola, *Pitcairnia geyskesii* L.B. Sm. Já Izquierdo e Piñero (2000), González-Astorga *et al.* (2004) e Cavallari *et al.*, (2006) focaram seus trabalhos em espécies com alto grau de endemismo.

No tocante a dinâmica populacional de bromélias de altitude, merecem destaque os trabalhos de Sgorbatiti *et al.*, (2004) e Barbará *et al.*, (2007). Ambos os trabalhos encontraram populações altamente estruturadas (*Puya raimondii* Harms, G_{ST} = 0,962; *Alcantarea geniculata* (Wawra) J.R. Grant, F_{ST}=0,111; *Alcantarea imperialis* (Carriere) Harms, F_{ST} = 0,434).

Nos últimos anos, alguns trabalhos direcionados para bromélias endêmicas da Mata Atlântica foram desenvolvidos (Barbará *et al.*, 2007; Palma-Silva *et al.*, 2009; Palma-Silva *et al.*, 2011; Zanella *et al.*, 2011). Entretanto focaram, até o momento, em espécies de Mata Atlântica *stricto sensu*. Apesar da grande representatividade da família nos mais diversos ambientes dos Neotrópicos, algumas paisagens ainda não foram alvo de estudos como é o caso da Floresta Ombrófila Mista do sul do Brasil, dentre outras ecorregiões que o continente americano abriga.

1.4 *Vriesea reitzii* e sua semelhança com congenéricas

Pertencendo à subfamília Tillandsioideae, o gênero *Vriesea* Lindl. possui 266 espécies (Luther, 2010), e representa o segundo gênero mais representativo em número de espécies dentro da presente subfamília. O gênero possui dois centros de diversidade: o leste do Brasil, onde ocorrem cerca de 84% das espécies, e mais ao norte, na América do Sul, América Central e Caribe (Costa *et al.*, 2009).

Vriesea reitzii Leme & Costa ocorre somente no Brasil, nos estados do Paraná, Rio Grande do Sul e Santa Catarina, em altitudes que variam de 750 a 1.200 m, em fragmentos de Floresta Ombrófila Mista (Rech-Filho *et al.*, 2005; Alves *et al.*, 2006). Devido a sua semelhança morfológica com *Vriesea philippocoburgii* Wawra, *V. reitzii* foi, durante muito tempo, negligenciada como espécie. Entretanto, algumas autapomorfias morfológicas e ecológicas levaram recentemente ao reconhecimento de duas unidades taxonômicas distintas (Leme e Costa, 1991).

As cisternas de *V. reitzii* foram reconhecidas por Favretto *et al.*, (2011) como de grande importância ecológica para fauna de invertebrados de uma zona de Floresta Ombrófila Mista, com a criação de microhábitats para as espécies locais. Recentemente também foi reconhecida a importância da espécie como fonte alimentar e energética para os beija-flores que são mais dependentes de fontes de néctar, dentre eles o beija-flor de papo branco (*Leucochloris albicollis*) (Favretto *et al.*, 2010).

Além da semelhança morfológica com *V.philippocobrugii*, *V. reitzii* possui outra congenérica, *Vriesea altodaserrae* L.B. Sm., cuja semelhança morfológica também é notável (Figura 2). Em termos de distribuição geográfica, *V; altodaserrae* L.B. Sm. ocorre de Santa Catarina até Rio de Janeiro (Forza et al., 2010), *V. philippocoburgii* ocorre dos estados do Rio Grande do Sul ao Rio de Janeiro (Smith e Downs, 1977) e *V. reitzii* está restrita aos estados do Sul do Brasil (Leme e Costa, 1991).

Nesse contexto, têm-se três espécies de *Vriesea,* preferencialmente epífitas, com brácteas amarelas/ vermelhas e polinizadas por beija-flores (Kaehler *et al.*, 2005; Machado e Semir, 2006; Favretto *et al.*, 2011). Tais espécies podem ocorrer em total alopatria (*V. reitzii* e *V. philippocoburgii*) ou simpatria (*V. altodaserrae* e *V. philippocoburgii* entre 400-800m de altitude; *V. altodaserrae* e *V. reitzii* acima de 800m de altitude), o que torna as mesmas um interessante modelo para estudos genéticos e de especiação.

Figura 2: Semelhança em parte vegetativa e reprodutiva de três espécies do gênero *Vriesea*.(A) *V. philippocoburgii*, (B) *V. altodaserrae* e (C) *V. reitzii*.

Capítulo 2

Objetivos

2. Objetivo Geral

Examinar os padrões de diversidade genética de populações de *Vriesea reitzii* em toda a sua área de distribuição geográfica. Os dados obtidos contribuirão para elucidar aspectos evolutivos da espécie e da família Bromeliaceae como um todo, além de contribuir para a compreensão da história evolutiva da Floresta Ombrófila Mista, região à qual a espécie está associada.

2.1 Objetivos específicos

1. Descrever os índices de diversidade genética de *V. reitzii* (altitude) comparativamente com outras espécies de bromélias que ocorrem na Mata Atlântica *stricto sensu* (Capítulo 3);

2. Verificar se há estruturação genética alta entre as populações de *V. reitzii*, em função do isolamento geográfico (altitude) (Capítulo 3);

3. Determinar se há uma tendência altitudinal e/ou latitudinal na distribuição da diversidade genética das populações de *V. reitzii* (Capítulo 3);

4. Verificar se as espécies *V. reitzii, V. phillipocoburgii* e *V. altodasserae* apresentam perfis moleculares diferentes corroborando dados morfológicos de delimitação das espécies (Capítulo 4).

Capítulo 3

High altitudes and increased genetic variability on *Vriesea reitzii* (Bromeliaceae), in mountain region of Southern Brazil

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Abstract

It is well known that mountains may impose major barriers to gene flow and consequently cause genetic isolation due mainly by geographical separation and ecological diversification caused by altitudinal gradients.

Under a scenario of high level of endemism, is also expected that endemic plant species restricted to one particular association forestry harbors less genetic diversity than that highly distributed. However, these effects may vary in different landscapes and species. The Atlantic Forest in the southern Brazilian highlands, harbor the Mixed Ombrophilous Forest (FOM), or simply Araucaria forest, a distinct ecoregion with high levels of endemism. *Vriesea reitzii* is a bromeliad species restricted to a few isolated fragments of FOM in southern Brazil, being its geographic distribution restricted to higher elevations (750-1200m) and occurring in disjoint populations. Using seven microsatellite markers and Bayesian cluster analyses we examined genetic diversity levels in six populations of this epiphyte bromeliad. High levels of genetic variability (H_E=0.536) were detected when compared with levels of bromeliad's species with a broader geographic distribution. In addition, the genetic differentiation among populations was low to moderate, with pairwise F_{ST} values ranging from 0.034 to 0.212 with an average of 0.123. The genetic variation was higher within populations, with little variation between populations. Moreover, Bayesian analyses did not capture a significant population subdivision. Our results also indicate a tendency of increasing the genetic variability towards higher altitudes. A mean N_em value of 0.36 suggested a limited gene flow among the assayed populations, which leads us to hypothesize other causes to apparent connection. Our results also indicated a high potential for Araucaria forest as venues for molecular ecology studies of altitude species.

Key words: Mixed Ombrophilous Forest; southern Brazil; bromeliad; high altitudes; genetic diversity; microsatellites.

Introduction

Ecogeographically subdivided, the Brazilian Atlantic forest (AF) harbor in its southernmost limit the Mixed Ombrophilous Forest (MOF), or Araucaria Forest, one of the most threatened Neotropical ecoregions. This region encompasses 12.93% of AF domain, and is distributed almost continuously across the southern plateau, between 24° and 30° S latitude in altitudes that vary between 500 to 1400 m above sea level, with disjoint occupation in sites of elevated areas as the Serra do Mar and Mantiqueira Mountains in southeastern Brazil (Klein 1960; Galindo-Leal and Câmara 2003).

From an evolutionary perspective, it's known that, in this region, before the last glacial maximum, the vegetation was dominated by grasslands and shrubs, with forests restricted to deep valley refuges (Ledru 1993). Accumulated evidences suggest that about 3000 years before present the Araucaria forests expanded from the gallery forests along the rivers, indicating a shift to a somewhat wetter climate (Behling et al. 2004). Under natural conditions on the highlands, Araucaria forest apparently continues to expand today (e.g. Klein 1960, 1975; Rambo 1956a, b).

In this landscape context, a bromeliad endemic to this region draws attention by its degree of endemism. *Vriesea reitzii,* unlike many bromeliad species that occurs in Atlantic Rainforest *strictu senso*, is spread over southern Brazilian highlands, associated with FOM. It occurs in altitudes ranging from 750 to 1200 m, in Brazilian states of Santa Catarina, Paraná and Rio Grande do Sul, between 25° and 29° S latitude. For many decades this species was neglected as distinct taxonomy unity, due to its morphological similarity with its congener *V. philippocoburgii*. However, in 1991, morphological and ecological features led to the recognition of *V. reitzii* as a new taxonomic unit (Leme and Costa 1991; Baensch and Baensch 1994).

One topic of interest to molecular ecologists is the dynamics of interacting between the organisms used as model with their environment and the potential genetic consequences of this interaction. The massif works on Bromeliaceae family, until now, comprised species from coastal regions, or AF *stricto sensu* (Barbará et al. 2007; Palma-Silva et al. 2009; Zanella et al. 2011). Studies on FOM species could help in understanding the family evolutionary history as well the biome history as a whole. Also, studies on population structure of narrow range montane taxa are rare and usually appoint to low amounts of genetic diversity, which may be a direct consequence of the species' biological and ecological traits (Hamrick and Godt 1996), or of particular historical events (Hewitt 1996). Here we addressed the following questions:

1. Concerning its restricted geographic distribution, this species shows low levels of genetic diversity?
2. Does natural fragmentation imposed by interspersed isolated high-mountain habitats provide high genetic structure among *Vriesea reitzii* populations?
3. Is there any decrease/increase in levels of genetic diversity towards higher latitudes or altitudes?
4. Do the genetic diversity patterns agree with the bromeliads from Atlantic Forest *stricto sensu*?

Materials and methods

Sampling and DNA extraction

Six populations of *Vriesea reitzii* were sampled covering its entire geographical distribution. Fresh leaves from 187 individuals (approximately 30 per population) were collected and fast dried in silica gel. The altitudes of the sampled populations ranged between 778 m and 1031 m above sea level (Table S1 and Figure 1). Total genomic DNA was extracted as described by Doyle and Doyle (1990).

Molecular markers and genotyping assays

All samples were genotyped for seven nuclear microsatellite loci described for different bromeliads species: e6B, and e19, isolated from

Tillandsia fasciculata (Boneh et al. 2003); PaA10, isolated from *Pitcairnia labiflos* (Paggi et al. 2008), and VgB10, VgC01, VgF02 and VgF05 isolated from *Vriesea gigantea* (Palma-Silva et al. 2007). Amplification reactions were carried out in a TC-412 Thermal Cycler (Techne, Burlington, New Jersey, USA) in 10- µL reactions using 10 ng of DNA template, 1 × Taq buffer, 2 mM $MgCl_2$, 0.2 mM dNTPs, 10 pmol forward (labelled with one of four fluorescent tags: VIC, NED, 6FAM or PET) and reverse primers, and 0.5 U Taq DNA polymerase (GoTaq, Promega), making use of a standard touchdown cycling program described by Palma-Silva et al. (2007). The microsatellite alleles were resolved on an *ABI 3100* DNA Analyzer Sequencer (Applied Biosystems, Foster City, CA, USA) and sized against the *GS500 LIZ* molecular size standard (Applied Biosystems, Foster City, CA, USA) using GENEMARKER Demo version 1.97 (*SoftGenetics*, State College, PA, USA).

Data Analyses

Levels of genetic diversity of each population and for all variable loci was characterized using the number of alleles (A), allelic richness (R_S), expected (H_E) and observed (H_O) heterozygosities, and the inbreeding coefficient F_{IS} (Weir and Cockerham 1984), using the programs FSTAT 2.9.3.2 (Goudet 1995) and Microsatellite Analyser Software 4.05 (Dieringer and Schlötterer 2003). To examine departures from the Hardy–Weinberg equilibrium (HWE) were used exact tests in Genepop4.0 (Raymond and Rousset 1995). The data also were tested for genotyping errors resulting from stuttering, short allele dominance and null alleles using a Monte Carlo simulation of expected allele-size differences implemented in Micro-Checker2.2.3 (van Oosterhoutet et al. 2004).

To test genetic differentiation among all sampling localities, analysis of molecular variance (AMOVA) was performed in ARLEQUIN (Excoffier et al. 2005). In addition, we estimated genetic distances among sampling localities by computing population pairwise F_{ST} (Weir and Cockerham 1984), using FSTAT 2.9.3.2 (Goudet 1995). The hypothesis that populations are differentiated because of isolation-by-distance (Wright 1965) was tested by calculating the correlation between geographic and genetic distance matrices with a standardized Mantel test (Sokal and Rohlf 1995) using NTSYS (Rohlf 2005).

We also used a Bayesian assignment approach to investigate the number of genetic clusters represented in these data with STRUCTURE 2.3.4 (Pritchard et al. 2000) aiming to assign individuals to genetic clusters (K) and to estimate admixture proportions (Q) for each individual. The proportion of membership for each cluster was calculated without the consideration of sampling localities. The analyses were carried out under the admixture model assuming independent allele frequencies and using a burn-in period of 50 000, run lengths of 300 000 and 10 iterations per K ranging from 1 to 8 to confirm stabilization of the summary statistics (Pritchard et al. 2000). We used the admixture model and independent allele frequencies. To determine the most likely number of clusters (K), we used the method proposed by Evanno et al. (2005), which is based on an *ad hoc* measure of ΔK that evaluates the second-order rate of change of the likelihood function with respect to K.

The effective number of migrants (N_em) between pairs of populations was estimated following a coalescent theory and maximum-likelihood-based approach using MIGRATE 2.0.6 (Beerli and Felsenstein, 1999) as described by Barbará et al. (2007). Computations were carried out in both the infinite allele model (IAM) and Stepwise Mutation Model SMM, and mutation rates (μ) among loci were estimated from the data.

The existence of latitudinal, longitudinal and altitudinal gradients in relation to genetic diversity (i.e. allelic richness, variance in allele size and gene diversity) was tested using both linear regression and Spearman's rank correlation.

We also used the program BOTTLENECK to determine if a mode shift in the distribution of allele frequencies, which is characteristic of recent severe bottlenecks, had occurred (Luikart et al. 1998). We ran BOTTLENECK for 10 000 iterations. Piry et al. (1999) suggested that the

Wilcoxon signed-rank test is the most appropriate and powerful for analysis of <20 loci.

Results

All seven microsatellite loci were polymorphic in *Vriesea reitzii*, with an average of 9.29 alleles, ranging from four to 20 alleles per locus (Table S2). Four loci departed significantly from Hardy-Weinberg equilibrium within populations at the 0.05 level, probably due to presence of null alleles, (Table S2 and data not shown). Relatively high levels of genetic diversity were observed in all populations for all diversity genetic parameters (Table 1). The allelic richness (R_S) in all populations ranged from 3.589 to 5.531. The observed and expected heterozygosities per population ranged from 0.360 to 0.498 and from 0.452 to 0.629, respectively (Table 1). The inbreeding coefficients were low to high and ranged from 0.080 to 0.301. Five of six populations displayed significant departures from HWE showing heterozygote deficits, which may be the result of a Wahlund effect or inbreeding. Using bottleneck software, we did not find support for a recent bottleneck with the TPM, IAM and SMM models, in any population.

The six populations of *V. reitzii* showed moderate levels of genetic differentiation among populations (F_{ST} = 0.118). The pairwise F_{ST} values also suggest low to moderate structure between population's pairs, and the geographical proximity seems not to be the main factor that determine low F_{ST} (e.g., Cambará do Sul and Lages F_{ST} = 0.109 and 241 km apart; Campo Alegre and Lages, F_{ST} = 0.034 and 314 km apart; see Figure1 and Table 2). The Mantel test revealed that geographical distances were not significantly correlated with genetic differentiation as estimated by r = -0.07205 (*P* = 0.3829), suggesting the absence of isolation-by-distance (Figure S3).

The hierarchical multi-locus AMOVA indicated that the largest percentage of variation (91.96%) was attributed to the among-individuals-within-sites component and only small portion of the genetic variance (8.04%) was attributed to the among populations component (Table S3).

Bayesian analysis confirmed that a model of $K = 2$ groups was able to best capture the variation in the data of *V. reitzii* (Figure S1), and the admixture proportions (*Q*) for each individual plant are shown in Figure 2. The mean number of migrants per generation in *V. reitzii* was 0.36 (Table 2), which is < 1 migrant per generation. One migrant per generation has traditionally been regarded as the minimum required for maintaining species cohesion.

The distribution of genetic diversity (allelic richness and expected heterozygosity) was not correlated with latitude and longitude (Figure S2). On the other hand, a continued increase in genetic diversity must to be linked to higher altitudes using allelic richness (strong r=0.950 and $P = 0.004$) as parameter (Figure 3).

Discussion

Our study focused on genetic diversity patterns of one bromeliad species endemic from mountains of southern Brazil, a biogeographically distinct ecoregion from Atlantic rainforest. Although the species' geographic range could be suggested as a good predictor of genetic diversity of natural populations, where endemic species attain lower genetic diversity levels than their widespread congeners, new examples of narrow endemics with high genetic diversity has been regularly added to the literature (Torres-Díaz et al. 2007, Ægisdóttir et al. 2009, Eliades et al 2011). High genetic diversity in narrowly endemic species can be associated with factors other than the species' geographical distribution, such as recent origin from widespread congeners, hybridization, maintenance of genetic diversity within refugial populations and ecological traits (Torres-Díaz et al. 2007).

Among the factors influencing levels of genetic diversity, species traits and population history have been regarded as major determinants (Ellstrand and Elam. 1993; Hamrick and Godt. 1996). Altitudinal habitats have long been hypothesized to influence species' genetic diversity (Thiel-Egenter et al. 2009). For example, on the one hand, high elevations restrict plant's life cycle and the pollinator activity decreases, due to low temperatures (Arroyo et al. 1982; Ellenberg, 1988; Körner, 1999). On the other hand, the longer the stigmatic receptivity, more efficient will be pollination, which can compensate the low pollinator visits in high altitudes (Bingham and Orthner 1998).

Here we used microsatellite loci to evaluate the genetic diversity and structure of populations from all geographical distribution of *V. reitzii* in southern Brazilian highlands. We found that genetic diversity within populations was similar or higher (mean H_E=0.536) than that reported for bromeliads from the same genus which occur in the Atlantic forest *stricto sensu* (*Vriesea gigantea*, mean H_E = 0.579, Palma-Silva et al 2009; *V. friburgensis*, mean H_E = 0.226, Alves et al. 2004. Reviewed in Zanella et al. 2012). Also, the values of H_E found in *V. reitzii,* which has disjunct populations and restricted geographic distribution, are comparable to that reported in bromeliad's species with broad geographic distribution (e.g. *V. gigantea*, Palma-Silva et al. 2009; *Bromelia antiacantha*, Zanella et al. 2011). This relatively ample genetic diversity in *V. reitzii* could be interpreted as ancestral diversity, according to the argument that maintaining high genetic diversity in a narrow endemic species is due to its origin from a widespread congener species (Eliades et al. 2011). Also, the breeding system can be related with genetic diversity levels; outcrossing species can be responsible for high genetic diversity in populations. The breeding system of *V. reitzii* has not been studied yet, however, it is assumed that it is pollinated by hummingbirds (Favretto et al. 2010), as its congener species *V. phillipocoburgii* and *V. altodaserrae* (Machado and Semir. 2006), and therefore would be an outcross species.

Another factor that could be responsible for high genetic diversity in mountain species would be the populations' foundation by multiple genetically diverse individuals. The most of this initial diversity could be maintained in the stable mountainous conditions throughout the Quaternary glacial/interglacial cycles via a combination of relatively large population sizes and recurrent interpopulation gene flow, as encountered in the terrestrial orchid *Oreorchis patens* from Korean mountain ranges (Chung et al. 2012). *Vriesea reitzii* is a species highly associated with araucaria forest. The Late Quaternary vegetation and the climate history of

Southern Brazilian highlands are poorly studied, however, Behling et al. (2004), using paleoenviromental data, found a marked expansion of Araucaria forests on the Southern highlands, replacing grasslands vegetation about 1500 to 1000 years ago. This expansion probably started by migration from the gallery forests along the rivers and could be related with *V. reitzii* evolutionary history as well its population's foundation and high genetic diversity.

Mountainous environments are expected to present landscape heterogeneity and the mountain ridges may impose major barriers to gene flow among populations that inhabit different peaks of the same chain (Zhan et al. 2009, Frei et al. 2010). However, in contrast to this expectation, some studies reported that populations in fragmented landscapes are genetically not much differentiated (Jacquemyn et al. 2004; He et al. 2010). Here, we found low to moderate population subdivision, with pairwise F_{ST} ranging from 0.034 to 0.208. Similar levels of differentiation have been found in other study with bromeliads from inselbergs, *Alcantarea geniculata* (F_{ST}= 0.111; Barbará et al. 2007) in southeastern Brazil. In contrast, other altitude bromeliads, *Alcantarea imperialis* and *Puya raimondii*, showed high differentiation among their populations (F_{ST}= 0.434, G_{ST}=0.961, respectively).These results suggest that levels of gene flow between populations within mountainous regions appear to be highly variable (Byars et al. 2009).

The majority of the total genetic diversity found on *V. reitzii* resides within populations, which are normally found in outcrossing species. We can also observe that populations with higher rates of genetic diversity are genetically closer as observed in pairwise F_{ST} (Table 2). In this case populations separated by greater geographical distances weren't showed completely different and isolated, as evidenced by Mantel's test.

Maximum-likelihood-based estimates of migration rates were extremely low (N_em =0.362), since one migrant per generation has traditionally been regarded as the minimum required for maintaining species cohesion. Other studies on migration rate in other bromeliads have found also low values (e.g *A. imperialis* and *A. geniculata*, Barbara et al. 2007).

No evidence of migration, with the possibility of cohesion among populations, was found and bottleneck has no occurred. These results suggest that migration was caused by more ancient events. In some cases, intraspecific genetic diversity is not correlated to such ecological and biological traits. It seems to have been modeled mainly by past historical events such as the Pleistocene glaciations (e.g. Hewitt 1996). Chung et al 2012, studying orchids from *Oreorchis* genus, suggest that historical gene flow among neighboring populations has been high along the main mountainous ranges in the Korean Peninsula. García-Fernández et al. (2012) studying altitudinal gradients of *Silene ciliate*, proposed that the high genetic similarity among populations from different mountains may be due historical population movements related to ice contraction and expansion during glaciations in the Quaternary period. In this way, the factors involved in species evolutionary history might have significantly contributed to genetically homogenizing *V. reitzii* populations. In this sense, it would be interesting a study focusing on phylogeographic patterns of this species.

In a comparative altitude landscape context, similar levels of genetic diversity were found in other studies using various molecular markers on plants from distinct mountainous regions (e.g. *Elymus alaskanus* on mountains of Canada, Greenland, and the U.S.A- Sun and Salomon, 2003; *Athyrium distentifolium* above 600m in the Highlands of Scotland- Woodhead et al., 2005; *Picea abies* wich occurs above 450 meters in Scandinavian mountains- Heuertz et al., 2006; *Alcantarea imperialis* and *Alcantarea geniculata* on Neotropical inselbergs- Barbará et al. 2007; *Quercus crispula* from 850 to 1750m around the Chichibu Mountains, central Japan- Ohsawa et al. 2007; *Athyrium filix-femina* in different elevations 460-1800m in the northern Swiss Alps- Schneller and Liebst 2007, *Poa hiemata* in Australian alpine zone above 1500 m- Byars et al. 2009).

As this study was focused on a species restricted to narrow geographical area, we did not expect to find substantial differences in genetic diversity (or in other genetic variables) among populations (Table 1). However, contrary to our expectations, high values of genetic diversity and little structure among populations were found.

Concerning the latitudinal and altitudinal gradients, we found a tendency to increase the genetic variability towards higher altitudes. Similar results were obtained to *Rhodiola sachalinensis* (Yan et al. 1999). In contrast, no correlation was obtained with latitudinal gradient as observed on *V. gigantea,* a bromeliad from Atlantic forest *stricto sensu* (s.s.) (Palma-Silva et al. 2009). This led us to conclude about different patterns of organization of genetic variability among Atlantic forest *s.s* and and *lato sensu (l.s).*

Conclusions

Taken together, our results reject one possible hypothesis of low genetic diversity and high population isolation, resulting landscape and biological features of species analyzed. This was an unexpected fact, due to the complex topography of this region and the distance between collection points. Probably historical factors and adaptability can have great influence on obtained results. Despite the relative degree of endemism, *V. reitzii,* the diversity indexes were comparable to those of other bromeliad's species with broader distribution. Nevertheless, further fragmentation and isolation of habitats may threaten this rare plant in the future. Isolation of a small population can reduce gene flow, resulting in decreased genetic variation due to drift and/or inbreeding (Lande 1980; Caughley 1994).

We also found higher indices of genetic diversity in higher altitudes. The basic idea of increasing genetic variation towards higher altitudes, led us to think that in one moment in the past, higher altitudes opens up better ecological opportunities for this species. This is an interesting that what leads spectacular examples of adaptive radiation, such as the bromeliads in Neotropical region. These findings also suggest that the altitudinal gradient can be a major driver of population genetic diversity in this bromeliad species. This tendency could be explained also with the premise that the Araucaria Forest is still expanding towards the major to lower altitudes, and the lowest indices of genetic diversity would be explained by founder effect, due to the expansion.

Likewise, due to lack of studies focused on endemic species of Southern Brazilian highlands we suggest this landscape as venues for studies of the molecular ecology and genetics of continental radiations. Deeper future analysis should show other interesting example of plants with unexpected features in plant genetics, physiology, and ecology.

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Table 1: Summary of genetic variation for seven microsatellite loci in six populations of *Vriesea reitzii* with sample size (N), allelic richness (R_S), expected (H_E) and observed (H_O) heterozygosities as well as inbreeding coefficient (F_{IS}). Departures from Hardy–Weinberg equilibrium are indicated by asterisks (* $P < 0.001$) (SFRS = São Francisco de Paula; CSRS = Cambará do Sul; LGSC = Lages; PDSC = Papanduva; CASC = Campo Alegre; SMPR = São Mateus do Sul)

Population	**N**	R_S	H_o	H_e	F_{IS}
SMPR	31	3.589	0.411	0.452	0.080
CASC	31	5.531	0.438	0.629	0.301*
PDSC	30	3.846	0.360	0.504	0.278*

LGSC	32	5.419	0.498	0.541	0.046*
CSRS	32	4.955	0.493	0.571	0.128*
SFRS	31	5.176	0.423	0.519	0,157*

Table 2: Estimation of effective number of migrants (N_em) for populations of *Vriesea reitzii* (above diagonal), and genetic divergence (F_{ST}) (below diagonal) between pairs of *Vriesea reitzii* populations. (SFRS = São Francisco de Paula; CSRS = Cambará do Sul LGSC=Lages; PDSC= Papanduva; CASC = Campo Alegre; SMPR = São Mateus do Sul) (* $P < 0.001$).

Population	SMPR	CASC	PDSC	LGSC	CSRS	SFRS
SMPR	-	0.49	0.25	0.33	0.36	0.28
CASC	0.108*	-	0.40	0.43	0.45	0.27
PDSC	0.187*	0.106*	-	0.41	0.49	0.35
LGSC	0.104*	0.034	0.212*	-	0.29	0.26
CSRS	0.120*	0.040	0.054	0.109*	-	0.37
SFRS	0.200*	0.118*	0.118*	0.208*	0.059	-

Table Supplementary 1: Populations of *Vriesea reitzii* sampled, with their geographical parameters.

State	**Sampling site**	**Population Code**	**Coordinates**	**Altitude (m)**
RS	São Francisco de Paula	SMPR	S29 26.699 W50 36.743	927
RS	Cambará do Sul	CSRS	S29 08.508 W50 05.217	977
SC	Lages	LGSC	S27 47.450 W50 21.239	1031
SC	Papanduva	PDSC	S26 30.126 W50 14.005	790
SC	Campo Alegre	CASC	S26 10.094 W49 13.635	977
PR	São Mateus do Sul	SMPR	S25 52.980 W50 18.779	778

Table Supplementary 2: Characterization of seven microsatellite markers in altitude bromeliad *Vriesea reitzii*, with the number of alleles (A), expected (H_E) and observed (H_O) heterozygosities, within-population inbreeding coefficient (FI_S), and fixation index (F_{ST}). Departures from Hardy–Weinberg equilibrium are indicated by asterisks (* $P < 0.001$).

Locus	**A**	H_o	H_e	F_{IS}	F_{ST}
e6B	11	0.334	0.421	0.209*	0.042
e19	3	0.340	0.347	0.011	0.023
PaA10	4	0.350	0.396	0.093	0.191

VgB10	13	0.598	0.769	0.221*	0.156
VgC01	10	0.529	0.596	0.110*	0.064
VgF02	20	0.651	0.844	0.217*	0.063
VgF05	4	0.258	0.379	0.294	0.322
Mean	9.29	0.437	0.536	0.105	0.123

Table Supplementary 3: Analysis of molecular variance (AMOVA) of *Vriesea reitzii* based on seven microsatellite markers.

Source of Variation	**d.f.**	**Sum of squares**	**Variance components**	**Percentage of variation**
Among populations	5	27.07	0.07	8.04
Within populations	368	309.09	0.83	91.96
Total	373	336.17	0.91	
Fixation Index	F_{ST}: 0.08			

Fig 1: The six *Vriesea reitzii* populations sampled for this study (colorful dots). Map of southern Brazil highlighting marked altitude differences. For population abbreviations see Table S1.

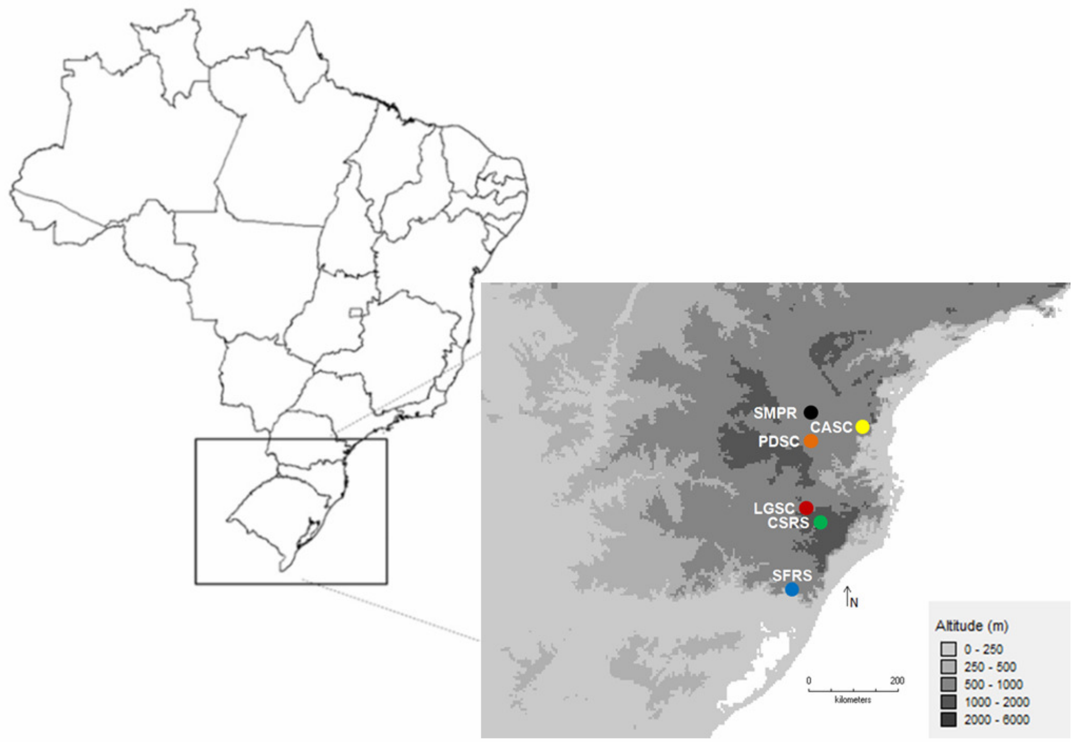


Figure 2: Bayesian admixture proportions (*Q*) of individual plants of *Vriesea reitzii* for a *K*=2 genetic clusters. Each plant individual is represented by a vertical line, and cluster assignments are indicated by color. (SFRS = São Francisco de Paula; CSRS = Cambará do Sul LGSC = Lages; PDSC= Papanduva; CASC = Campo Alegre; SMPR = São Mateus do Sul).

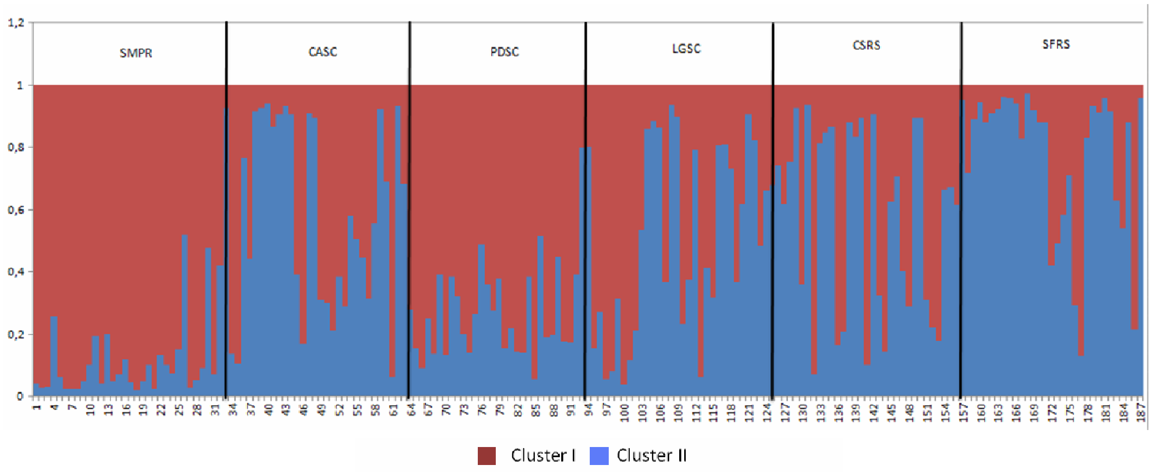
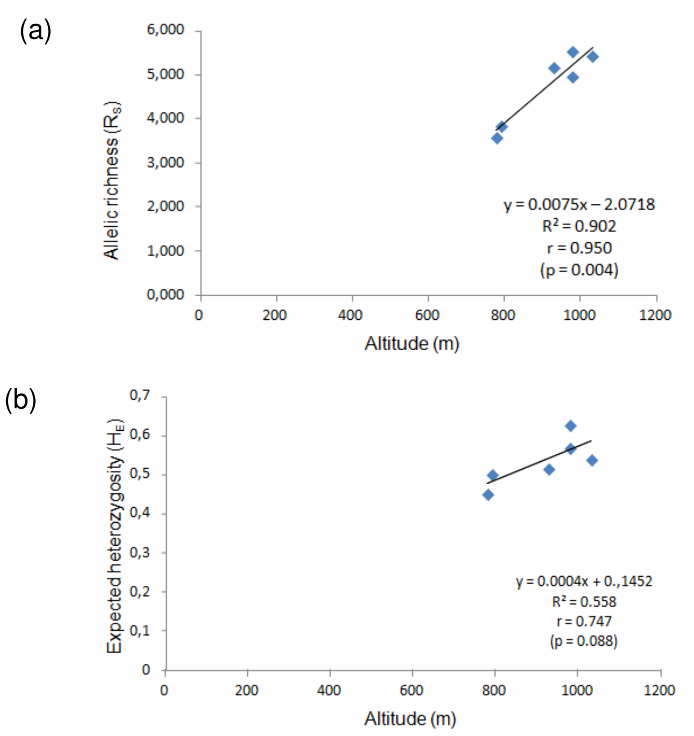
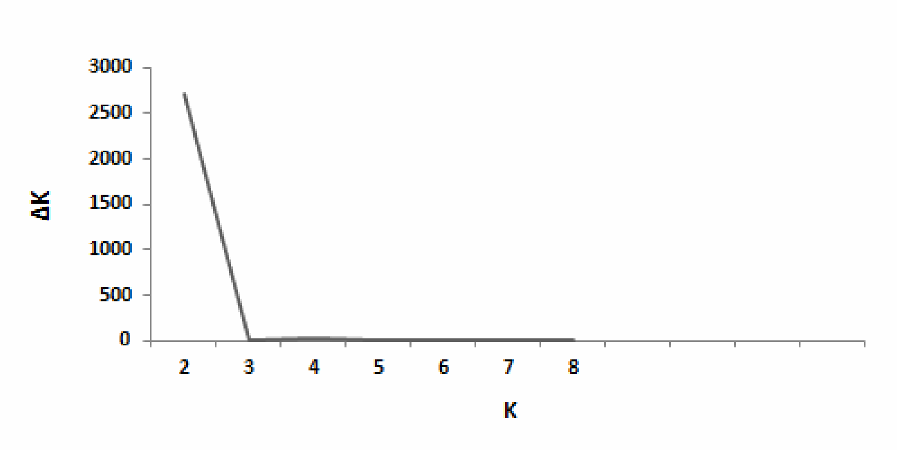


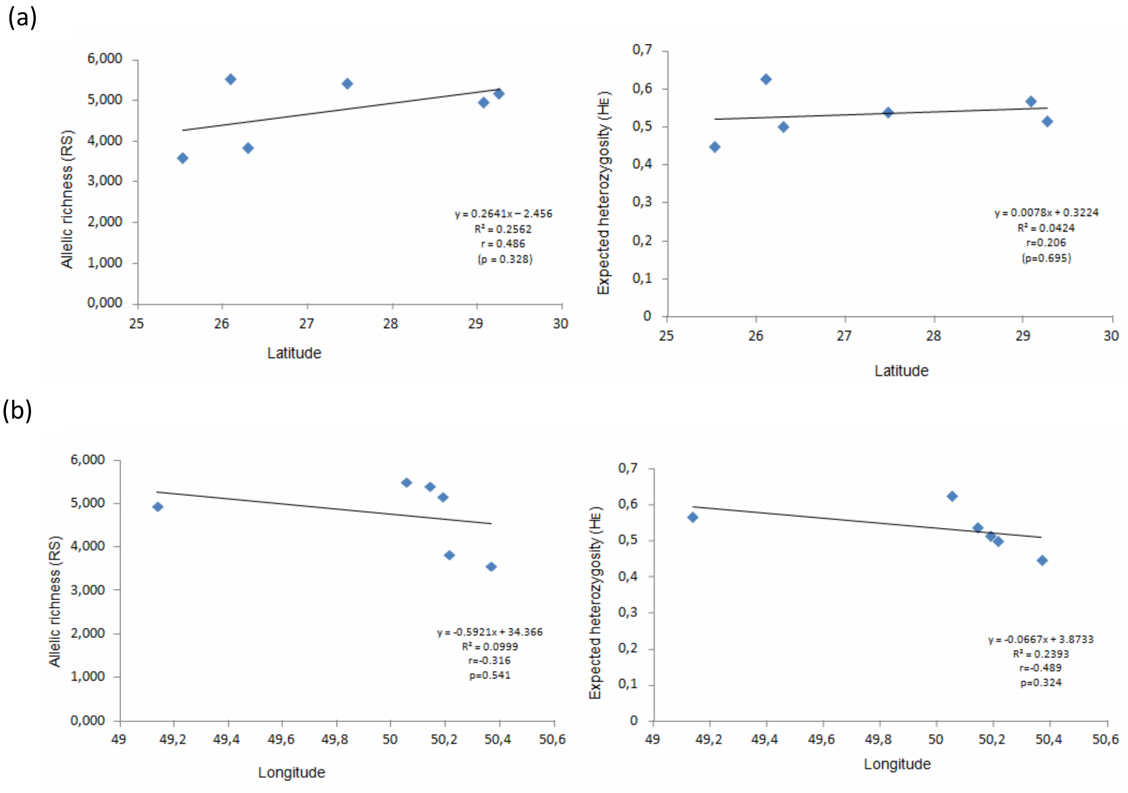
Figure 3: (a) Plot of allelic richness (R_S) vs. altitude (in meter) and (b) plot of expected heterozygosity (H_E) vs. altitude (in meter) for six populations of *Vriesea reitzii* (blue squares).



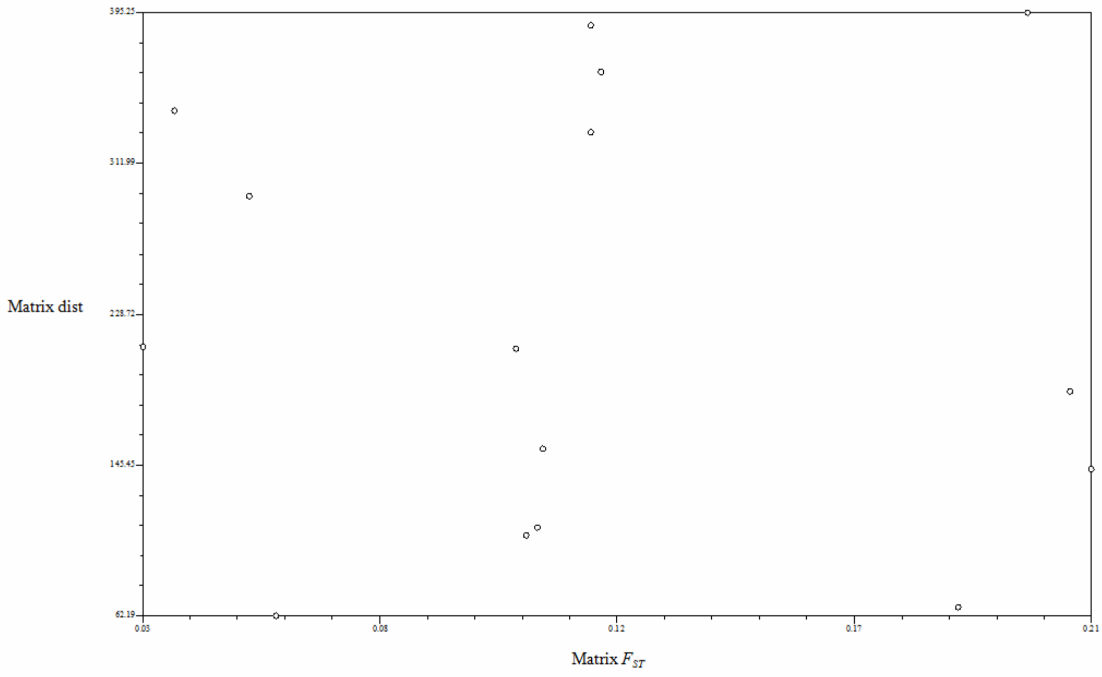
Supplementary Fig 1. Magnitude of ΔK from structure analysis of K (mean ±SD over 10 replicates), calculated by following the ΔK method proposed by Evanno et al. (2005), for *Vriesea reitzii* microsatellite data. The modal values of these distributions indicate the true K or the uppermost level of structure is three “genetic clusters”.



Supplementary Figure 2: (a) Plot of allelic richness (R_S) and expected heterozygosity (H_E) vs. latitude and (b) Plot of allelic richness (R_S) and expected heterozygosity (H_E) vs. longitude for six populations of *V. reitzii* (blue squares).



Supplementary Figure 3. Multilocus estimates of pairwise differentiation (F_{ST}) are plotted against linear geographical distances (in kilometers) according to Hutchison and Templeton (1999). There was not a significant positive linear relationship between pairwise F_{ST} and linear geographical distance (r = -0.07205, P = 0.3829)


395.25
311.99
228.72
145.45
62.19
Matrix dist
0.03
0.08
0.12
0.17
0.21
Matrix F_{ST}

Capítulo 4

Genetic structure of three congeners bromeliad's species along an elevational gradient in Southern Brazil

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Short Communication

Abstract

Bromeliaceae is an outstanding example of adaptive radiation in vascular plants, and show a wide morphological, physiological and ecological variability, factors that have attracted many evolutionary biologists. Environmental gradients caused by altitude, in long term, can promote genetic barriers due to isolation. These species can occur in sympatry or in total isolation by altitude. It’s known that these three species can share pollinators (hummingbirds), according to their floral characteristics and previous studies. We use nuclear microsatellites and Bayesian approach to study population differentiation and gene flow in three morphologically similar species of Bromeliaceae, *Vriesea altodaserrae*, *Vriesea philippocoburgii* and *Vriesea reitzii*. Samples were taken from three localities along an altitudinal gradient and analyzed with seven nuclear microsatellite loci. The molecular profile of the three species was distinct and we consider that they are different taxonomic units. The genetic structure between *V. altodaserrae* and *V. philippocoburgii* was low. In contrast, *V. reitzii* showed relative isolation from the other two species.

Key words:Brazilian Atlantic Rainforest; bromeliad; gene flow; genetic diversity; microsatellites; elevational gradient.

The Atlantic Forest Domain is constituted by many forest types and associated ecosystems, presenting high environmental heterogeneity

(Metzger, 2006). In this way, the Atlantic forest can be classified in two main forms: *stricto sensu* (s.s.) and *lato sensu* (l.s.). While the first (s.s) comprises only tropical rainforests of the Atlantic coast, the second (l.s.) includes seasonal forests (Deciduous and Semi-deciduous), Araucaria forests (Mixed Araucaria forest; MAF) and low coasts rainforests (Restingas) (Oliveira-Filho and Fontes, 2000).

Elevational gradients in species diversity are nearly as ubiquitous as latitudinal gradients, offering many characteristics that make them more suitable for uncovering the underlying cause(s) of spatial variation in diversity (Sanders and Rahbek, 2012). In this way, this environmental heterogeneity can be an excellent model system for ecological and evolutionary studies.

Vriesea altodaserrae L.B. Sm., *Vriesea philippocoburgii* Wawra and *Vriesea reitzii* Leme & Costa, are three species highly similar morphologically (Figure 1) which can occur sympatrically (*V. altodaserrae* and *V. philippocoburgii*, between 400-800m above sea level; *V. altodaserrae* and *V. reitzii* 800m above sea level) (Figure 2C). Regarding latitudinal distribution, it is known that *V. altodaserrae* occurs from Santa Catarina to São Rio de Janeiro states (Forza et al., 2010), *V. philippocoburgii* occurs from Rio Grande do Sul to Rio de Janeiro Brazilian states (Smith and Downs, 1977) and *V. reitzii* is restricted to the southern states of Brazil (Leme and Costa 1991) (Figure 2A). It might be argued the potential gene flow between *V. atodaserrae* and *V. reitzii*, since they occur in sympatry and share the same pollinator agent (hummingbird), *Leucochloris albicolis* (Vieillot) (Kaehler *et al.*, 2005; Machado and Semir, 2006; Favretto *et al.*, 2011).In the same way, it can also be hypothesized gene flow between *V. philippocburgii* and *V. reitzii* take into account the high degree morphological similarity, which led these two species to be considered a single taxa until 1991 (Leme and Costa, 1991).

In this study we aimed to test if these three species have different molecular profiles, corroborating taxonomical delimitation using morphological data. In the same way we discuss the distribution of genetic diversity among and within species and the possibility of gene flow among species' pairs.

Due to the fragmentation of the environment selected for this study, the sampling was limited to populations located in the only one elevational gradient (110, 745, and 977 m) encountered in Atlantic Rain Forest, southern Brazil (Figure 2B) which consists of two major classes of vegetation according to the IBGE (1986): Dense Ombrophilous Forest (DOF) and Mixed Ombrophilous Forest (MOF).

A total of 127 individuals from all three species were used in our analysis (Table 1). DNA was extracted from fresh leaves as described by Doyle and Doyle (1990). A total of seven nuclear microsatellite loci previously characterized for other bromeliads species were selected for genetic analysis (Table S1). Amplification was performed using protocols as detailed in Palma-Silva *et al.* (2007). The microsatellite alleles were resolved on an *ABI 3100* DNA Analyzer Sequencer (Applied Biosystems, Foster City, CA, USA) and sized against the *GS500 LIZ* molecular size standard (Applied Biosystems, Foster City, CA, USA) using GENEMARKER Demo version 1.97 (*SoftGenetics*, State College, PA, USA).

Levels of genetic diversity within loci and populations were estimated by Microsatellite Analyser (Dieringer and Schlötterer, 2003) and FSTAT 1.2 (Goudet, 1995). Genetic differentiation among populations was characterized by estimating pairwise F_{ST} (Weir and Cockerham, 1984) using the Microsatellite Analyser software (Dieringer and Schlötterer, 2003).

To depict relationships among populations and species in a graphical way, a neighbour-joining (NJ) tree was constructed based on the chord distance of Cavalli-Sforza and Edwards (1967). Yet, to gain insights about the degree of similarity among the populations analyzed, Bayesian analysis was performed in Structure version 2.3.4 (Pritchard *et al.* 2000) and a method proposed by Evanno et al. (2005), which is based on an *ad hoc* measure of ΔK that evaluates the second-order rate of change of the likelihood function with respect to K, was used to infer the most probable value of K.

The seven microsatellite markers used were polymorphic in all populations and species and the number of alleles per locus varied from five to 21 (Table 3). Deviation from Hardy–Weinberg equilibrium was observed in four of five populations, caused by heterozygote deficit (Table 1). Patterns of genetic diversity were similar in the three species evaluated (Table 1), except to one population of *V. philippocoburgii* (745m), probably due to its small sample size. Hahn et al (2012), studying patterns of genetic variation across altitude in three plant species, did not found differences in genetic diversity and inbreeding estimates among populations. The authors suggested that the altitudinal gradient is not a major driver of population genetic diversity in the studied species. Here we can observe a tendency of increasing genetic diversity towards higher altitudes (using H_E and R_s as references, Table 1), but this estimate must be tested and replicated to have a more robust conclusion. It is known that the altitudinal gradients may influence the distribution of genetic variation within and among plant populations or species in a complex and variable ways (Ohsawa and Ide, 2008; Byars *et al.*, 2009; Hahn *et al.*, 2012).

Overall gene-flow estimates yielded a low to moderate values of F_{ST} between allopatric populations of the same species, and between species (Table 2). The moderate F_{ST} between different species could be a clue to the occurrence of gene flow between them, although the sharing of ancestral polymorphism cannot be ruled out. Moreover, the paired F_{ST} between species are tendentiously higher (even when they are in sympatry) when compared with F_{ST} between populations of the same species, even at different altitudes (Table 2). *Vriesea reitzii* showed higher F_{ST} values in relation to the two other species, indicating some degree of isolation. The Bayesian analysis indicated that the most likely number of groups in the total sample was two, being all *V. reitzii* samples isolated in one cluster and the two other species distributed between groups (Figure 2C). The genetic structure among populations from an altitudinal gradient can be extremely variable, according to the species evaluated and molecular marker used (neutral vs. non-neutral for example). Ohsawa and Ide (2008), in a revision on patterns of genetic variation in plant species along mountain gradients, found that almost all the studies of herbaceous species report some evidence of differentiation among populations. However, factors as overlap flowering fenology, extensive pollen flow, long distance seed dispersal, short population history and interspecific hybridization could contribute to limit differentiation among populations and species (Ohsawa and Ide, 2008; Hahn *et al.*, 2012; Acosta *et al.*, 2012)

Corroborating the Bayesian analysis, the NJ tree based on microsatellite genetic distances separated the five populations in two main groups with high bootstrap support (Figure 3). The two populations of *V. altodasserae* clustered together and *V. reitzii* formed a separate group. The two *V. philippocoburgii* populations did not clustered probably due to the small sample size of 475m population (Figure 3). The occurrence of hybrids between these three species is a plausible assumption, since they share pollinator (hummingbirds) and its morphology is compatible. The spatial isolation of *V. reitzii*, and *V. philippocoburgii* provided a certain genetic isolation between them, and a distinct flowering period could provided isolation in relation to the sympatric species as *V. altodaserrae* x *V. reitzii* and *V. altodaserrae* x *V. philippocoburgii* (see Figure 2C). Works on phenology and reproductive biology of these three species could help in understanding the reproductive isolation mechanism involved.

The three species studied here represent a good model for further studies on ecological adaptation, speciation and hybridization. Our preliminary results revealed that these species have different molecular profiles (mainly *Vriesea reitzii*) and could be considered as distinct taxonomic units. Also, through field expeditions, we could confirm that the species occur in different environments (altitudes), which can be a fertile ground for studies on ecological adaptation through adaptive traits and also for understanding the evolutionary histories of these species, which are probably linked. A complete phylogeographic study of these bromeliads will also contribute to understanding its evolutionary dynamics.

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Population	N	R_S	H_O	H_E	F_{IS}
V. retzii **(977m)**	31	6.00	0.438	0.629	0.328*
V.altodaserrae **(977m)**	30	5.29	0.331	0.530	0.417*
V. altodaserrae **(745m)**	32	5.14	0.421	0.594	0.274*
V. philippocoburgii**(745m)**	6	2.25	0.830	0.689	-0.200
V. philippocoburgii **(110m)**	28	4.57	0.391	0.506	0.176*

Table 1: Characterization of genetic diversity of *Vriesea altodaserrae*, *Vriesea philippocobrugii* and *Vriesea reitzii* populations with seven nuclear micro-satellite markers, allelic richness (R_S) allelic richness expected (H_E) and observed (H_O) heterozygosities and inbreeding coefficient (F_{IS}). Departures from Hardy–Weinberg equilibrium are indicated by asterisks (*P< 0.001)

Population	***V. retzii*** **(977m)**	***V.altodaserrae*** **(977m)**	***V. altodaserrae*** **(745m)**	***V. philippocoburgii*** **(745m)**
V.altodaserrae **(977m)**	0.223*			
V. altodaserrae **(745m)**	0.228*	0.024		
V. philippocoburgii **(745m)**	0.276*	0.111*	0.122*	
V. philippocoburgii**(110m)**	0.139*	0.104*	0.145*	0.110*

Table 2: Genetic divergence (F_{ST}) between pairs of *Vriesea altodaserrae*, *Vriesea philippocobrugii* and *Vriesea reitzii* populations. Significant values are indicated by asterisks (*P< 0.001).

Locus	A	H_o	H_e	F_{IS}	F_{ST}
e6B[a]	14	0.509	0.725	0.363*	0.126
e19[a]	7	0.210	0.395	0.447*	0.324
PaA10[b]	5	0.263	0.313	0.140	0.280
VgB10[c]	14	0.809	0.825	0.097	0.064
VgC01[c]	7	0.434	0.630	0.179	0.028
VgF02[c]	21	0.259	0.762	0.598*	0.178
VgF05[c]	5	0.276	0.413	0.313	0.337
Mean	10.429	0.394	0.580	0.289	0.191

[a] Microsatellite markers isolated by Boneh *et al.* (2003)

[b] Microsatellite markers isolated by Paggi *et al.* (2008)

[c] Microsatellite markers isolated by Palma-Silva *et al.*(2007)

Table 3: Characterization of microsatellite markers in populations of *Vriesea altodaserrae*, *Vriesea philippocobrugii* and *Vriesea reitzii.* A = number of alleles per locus; H_O= observed heterozygosity; H_E= expected heterozygosity; F_{IS}= inbreeding coefficient; F_{ST} = fixation index.

Figure 1: Three analyzed species (A) *Vriesea pilippocoburgii*, (B) *V. altodaserrae* (C) *V. reitzii*.

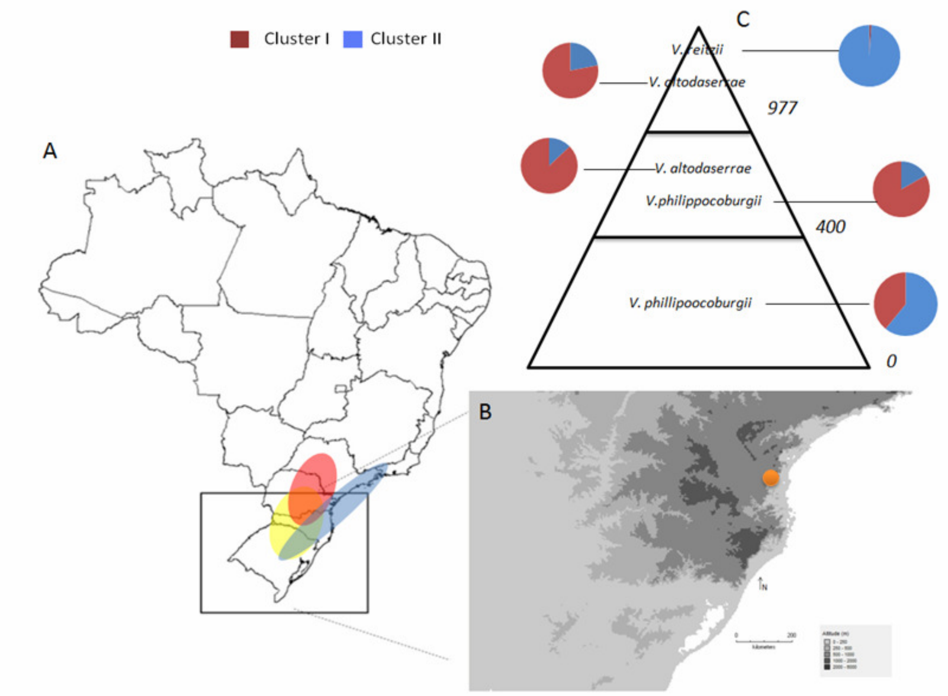

Figure 2: (A) Approximated distribution area of: *Vriesea atodaserrae* (red area), *Vriesea philippocoburgii* (blue area) and *Vriesea reitzii* (yellow area) (B) Location of collection sites in the cities of Joinville and Campo Alegre, Santa Catarina, Brazil (C) Distribution of sample sites along an altitudinal gradient and Bayesian admixture proportions of 5 populations of three species, for K=2 model.

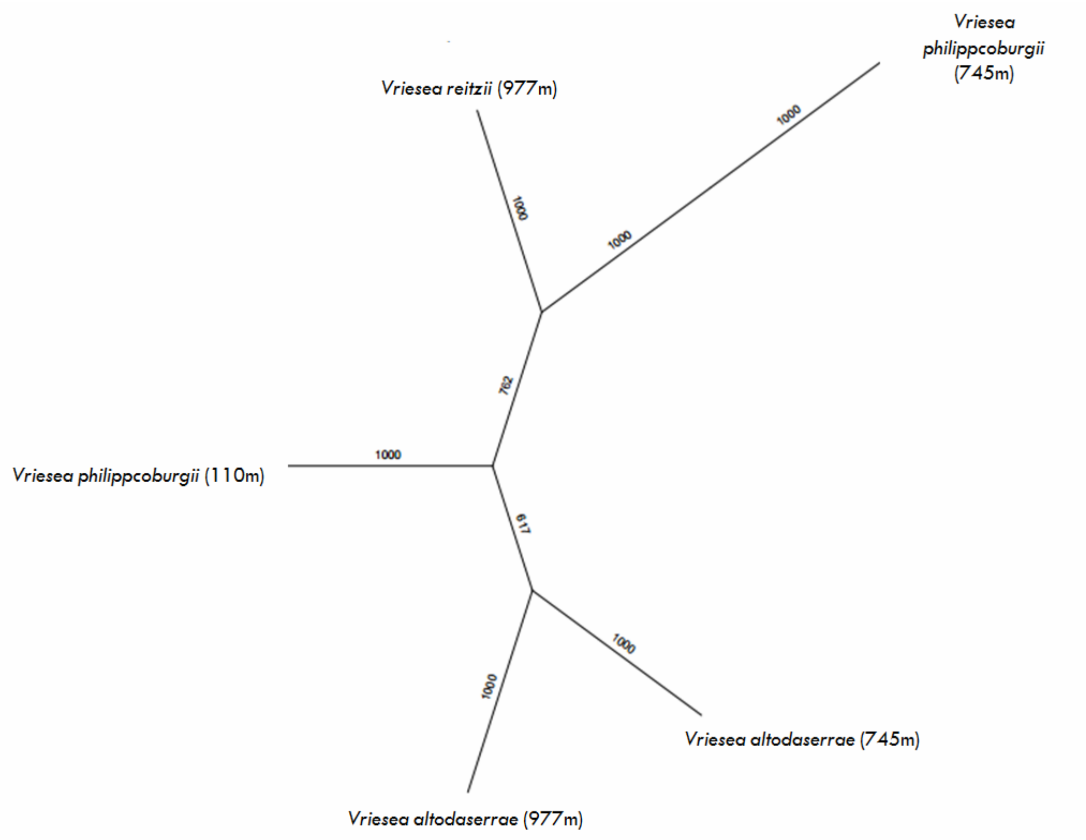

Figure 3: Unrooted neighbour-joining tree of populations based on Cavalli-Sforza & Edward's (1967) chord distance, including bootstrap support values in percent. A scale for genetic distance is provided at the bottom of the graph.

Capítulo 5

Discussão e considerações finais

5. *Discussão e considerações finais*

A Mata Atlântica é um bioma extremamente heterogêneo em sua composição, se estendendo de 4° a 32°S e cobrindo uma variedade de climas e formações vegetacionais, incluindo as tropicais e subtropicais. Além disso, compreende diferentes níveis altitudinais, do nível do mar a 2900m, com mudanças abruptas no tipo de solo e na temperatura média anual (Tabarelli *et al.*, 2005). Neste contexto encontra-se a Floresta ombrófila mista (FOM), ou floresta de araucárias, uma ecorregião da Mata Atlântica típica de áreas montanhosas do sul do Brasil. Esta formação ocorre de aproximadamente 500m a 1600m acima do nível do mar (WWW, 2001). A história da Mata Atlântica foi marcada por períodos de conexão com outras florestas sul-americanas, como a Amazônica e as Andinas, o que resultou em fluxo gênico entre espécies seguido de períodos de isolamento, levando a processos de especiação geográfica (Silva *et al.*, 2004). Embora todos esses processos ainda sejam muito pouco compreendidos, devido a esse isolamento da Mata Atlântica das outras florestas sul-americanas, muito provavelmente esse mecanismo esteja envolvido no grande grau de endemismo da floresta como um todo e também de suas ecorregiões, como é o caso da FOM (Myers *et al.*, 2000). Relativamente poucos trabalhos com plantas e animais têm sido publicados focando na evolução de espécies endêmicas da Mata Atlântica, e menos ainda considerando espécies da FOM (Dantas *et al.*, 2011; Turchetto-Zolet *et al.*, 2012). Sendo assim, qualquer contribuição abordando aspectos genéticos e evolutivos de espécies endêmicas dessa região é extremamente relevante no sentido de colaborar na elucidação da história evolutiva do bioma.

O presente trabalho foi focado em *Vriesea reitzii*, uma espécie endêmica da FOM, utilizando abordagens genéticas, e tem como objetivo principal contribuir para os estudos da família Bromeliaceae e da Mata Atlântica. O trabalho está dividido em dois artigos; o primeiro (Capítulo 3), além de contemplar a espécie *V. reitzii,* trata de duas outras espécies muito similares morfologicamente (*V. altodaserrae* e *V. philippocoburgii*) e que podem ocorrer em simpatria em algumas situações ou altitudes. O artigo está escrito sob forma de nota científica e, além de demonstrar que as espécies possuem perfis genéticos distintos, podendo ser realmente consideradas unidades taxonômicas, demonstra que esse grupo de espécies pode ser usado de forma mais detalhada como modelo de estudos de fluxo gênico e especiação. O segundo artigo (Capítulo 4) trata da descrição da diversidade genética e estrutura de populações de *V. reitzii* e demonstrou que essa espécie possui índices de diversidade genética semelhantes aos encontrados em outras congenéricas, tais como *V. gigantea* que possui distribuição mais ampla e é endêmica de Mata Atlântica *stricto sensu* (Palma-Silva *et al.*, 2009). A comparação entre espécies de altitude e suas congenéricas que ocorrem na região costeira do bioma certamente ajudará a elucidar a história evolutiva da família e do gênero. Segundo Amaro *et al.*, 2012, dados preliminares sugerem diferentes respostas às mudanças climáticas do Pleistoceno considerando espécies de montanha em comparação com as espécies costeiras do Brasil. Rodrigues *et al.*, 2009 sugerem que os taxa hoje restritos a altas altitudes possivelmente mostrarão padrão oposto àqueles observados nas formas que ocorrem nas regiões costeiras. Além disso, a porção sul da Mata Atlântica, conhecida pela sua topografia complexa, apresenta assinatura de expansão recente, refletindo colonização do Norte para o Sul do bioma (Carnaval *et al.*, 2009). No presente estudo foi evidenciado certo grau de influência da altitude nos índices de variabilidade genética de *V. reitzii*. Entretanto não foi possível observar influência da latitude como ocorre em *V. gigantea* e outras espécies com distribuição restrita à Mata Atlântica. Ao encontro desses resultados, Dantas *et al.*, 2011 discute que a biota da Mata Atlântica é provavelmente o resultado de uma história evolutiva bastante complexa. Nesse sentido, trabalhos que visem gerar dados genéticos de espécies endêmicas de regiões diferenciadas e pouco estudadas como a FOM são de extrema importância. Para uma melhor descrição deste cenário evolutivo, dados filogeográficos das espécies aqui estudadas serão de grande valia.

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Capítulo 6

Referências Bibliográficas

6. *Referências Bibliográficas*

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