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Origem híbrida e padrões de fluxo gênico entre três espécies simpátricas de *Dyckia* (Bromeliaceae) endêmicas do Rio Grande do Sul: implicações evolutivas e conservacionistas

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Sumário

Resumo

Bromeliaceae é uma das mais diversas famílias de plantas, distribuída quase que exclusivamente nos Neotrópicos, com somente uma espécie no oeste da África. A família sofreu uma radiação adaptativa recente, ocorrendo em diferentes habitats. *Dyckia* é o segundo maior gênero da subfamília Pitcairnioideae, abrigando aproximadamente 145 espécies. *Dyckia hebdingii*, *D. choristaminea* e *D. julianae* são espécies saxícolas e endêmicas de afloramentos rochosos do sul do Brasil, podendo ocorrer em simpatria. Quando espécies relacionadas e com isolamento reprodutivo incompleto ocorrem em simpatria, pode ocorrer hibridação e, a longo prazo, especiação. Uma vez que 25% das espécies de plantas são conhecidas por cruzarem com outras espécies e este número aumenta em grupos de radiação adaptativa recente, como em Bromeliaceae, o presente trabalho tem como objetivo geral investigar a ocorrência de hibridação entre essas três espécies do gênero *Dyckia* e a possível origem híbrida de *D. julianae*. O DNA genômico total foi extraído de duas populações simpátricas e três alopátricas e, usando sete marcadores microssatélite nucleares e seis plastidiais, foram acessados os padrões de diversidade genética e estruturação populacional das três espécies. Além disso, foram realizados cruzamentos artificiais entre as espécies para testar a compatibilidade e fertilidade das mesmas. Os principais resultados obtidos com nuSSR revelaram uma heterozigosidade esperada alta, com 0,444 para *D. hebdingii*, 0,649 para *D. choristaminea* e 0,708 para *D. julianae*/híbridos, com déficit de heterozigotos, quando comparado com a heterozigosidade observada (0,281, 0,456 e 0,585, respectivamente). A riqueza alélica média foi de 4,6 para *D. hebdingii*, 7,7 para *D. choristaminea* e 6,7 para *D. julianae*/hibrido. O coeficiente de endogamia (F_{IS}) foi alto para *D. hebdingii, D. choristaminea* e *D. julianae*/híbridos (0,274, 0,276 e 0,134, respectivamente). Os seis cpSSR

proporcionaram a identificação de 12 haplótipos e a diversidade haplotípica (*h*) variou de 0,0 (um haplótipo) a 0,736 (cinco haplótipos). Apenas seis cruzamentos produziram frutos e sementes, nenhum deles entre *D. hebdingii* e *D. choristaminea*. Os dados moleculares e os cruzamentos artificiais sugerem que *D. hebdingii* e *D. choristaminea* são geneticamente diferentes e não cruzam entre si atualmente. *Dyckia julianae* tem tanto morfologia quanto perfil molecular intermediário e parece ser capaz de cruzar com as outras duas espécies, indicando uma possível origem híbrida entre *D. hebdingii* e *D. choristaminea*.

Abstract

Bromeliaceae is one of the most diversity families of plants, distributed almost exclusively in Neotropics, with one species in west of Africa. Bromeliaceae have recent adaptive radiation, occoring in different habitats. *Dyckia* is the second major genus of subfamily Pitcairnioideae, comprising approximately 145 species. *Dyckia hebdingii*, *D. choristaminea* and *D. julianae* are saxicolous species, endemics to rock outcrops Southern of Brazil and may occur in sympatry. Speciation can occur by different mechanisms, but when different species with incomplete reproductive isolation meet each other again, may occur hybrid speciation, since 25% of plant species are known to cross with other species and this number increases in rapid radiations groups, as Bromeliaceae, the present study has the general objective to investigate the occurrence of hybridization in these three species and the hybrid origin of *D. julianae*. We extracted total genomic DNA from two sympatric and three allopatric populations and using seven nuclear and six plastid microsatellite *loci*, we accessed patterns of genetic diversity and population structure. Furthermore, we performed manual crosses between species to test compatibility and fertility of

the three species. The major results obtained with nuSSR, indicate high expected heterozygosities, with 0.444 for *D. hebdingii,* 0.649 for *D. choristaminea* and 0.708 for *D. julianae* and hybrids, with a deficit of heterozygotes, when compared with observed heterozygosities (0.281, 0.456 and 0.585, respectively). Allelic richness showed a mean of 4.6 for *D. hebdingii*, 7.7 for *D. choristaminea* and 6.7 *D. julianae*/ hybrids. Inbreeding coefficient (F_{IS}) was high for *D. hebdingii*, *D. choristaminea*, *D. julianae*/hybrid (0.274, 0.276 and 0.134, respectively). Six cpSSR identified 12 haplotypes and haplotype diversity (*h*) ranged from 0.0 (one haplotypes) to 0.736 (five haplotypes). Only six crosses generated fruits and seeds and none of them were between *D. hebdingii* and *D. choristaminea*. Molecular data and artificial crosses together suggest that *D. hebdingii* and *D. choristaminea* are genetically different and do not cross each other currently. However, *D. julianae* appears to be able to cross with both species. *Dyckia julianae* has intermediate morphology, intermediate molecular profile and seems to be able to cross with the other two species, indicating a possible hybrid origin between *D. hebdingii* and *D. choristaminea*.

Capítulo 1

Introdução Geral

1. Introdução Geral

1.1Bromeliaceae

Bromeliaceae é uma das famílias com maior riqueza e diversidade da Mata Atlântica. A família compreende cerca de 3.000 espécies que tradicionalmente estão divididas nas subfamílias Pitcairnioideae, Bromelioideae e Tillandsioideae (Smith e Downs, 1974; 1977; 1979). A monofilia bem definida dessa família de monocotiledôneas tem sido sustentado por diversos trabalhos filogenéticos e morfológicos (Chase *et al*., 1995 *in* Barfuss *et al*., 2005). Entretanto, Givnish *et al*. (2007) sugeriram que a subfamília Pitcairnioideae é parafilética e portanto a dividiram em outras seis subfamílias, resultando em uma família constituída de oito linhagens monofiléticas: Bromelioideae, Puyoideae, Pitcairnioideae, Navioideae, Hechtioideae, Tillandsioideae, Lindmanioideae e Brochinnioideae. Evidências sugerem que as bromélias surgiram no escudo das Guianas há 100 milhões de anos, durante o Cretáceo, e as subfamílias parecem ter começado a divergir cerca de 19 milhões de anos atrás (Givinish *et al*., 2011). As bromélias tem hábitos variados, podendo ser saxícolas, terrestres ou epífitas (Benzing, 2000), características que conferem à família um grande potencial para radiação adaptativa (Luther, 2012). Além disso, são encontradas desde o nível do mar até altitudes de 4.000 metros, em desertos ou regiões úmidas, com muita ou pouca luminosidade (Benzing, 2000). A família é considerada uma das mais distintas morfologicamente, apresentando grande diversidade

ecológica e em numero de espécies dentre as plantas com flores de regiões tropicais e subtropicais do continente americano (Givnish *et al*., 2011).

Uma das principais características do grupo é a disposição diferenciada de suas folhas, formando tanques centrais onde água e nutrientes são armazenados. Esses tanques são considerados caráter típico de Bromeliaceae (Crayn *et al*., 2004), entretanto o gênero em estudo *Dyckia* se caracteriza por não possuir tais estruturas (Leme *et al*., 2012). Bromeliaceae possui espécies de importância econômica, como o abacaxi (*Ananas comosus*), algumas espécies apresentam valor no paisagismo por serem ornamentais (*Vriesea gigantea*), outras por serem fonte de fibras ou por aplicação na medicina popular (*Bromelia pinguin*). Sua importância ecológica principal é a fonte de água e abrigo oferecida pelos tanques, assim como seus frutos e néctar (Benzing, 2000). Sua polinização é realizada principalmente por beija-flores e morcegos (Fischer e Araujo, 1995; Paggi *et al*., 2007), entretanto existem registros de abelhas como visitantes frequentes em bromélias de corola curta (Kamke *et al*., 2011).

Estão descritos centros de diversidade para bromélias em quatro regiões: América Central, Andes, Escudo das Guianas e o Escudo brasileiro, que abrange a Serra do Mar e faixas costeiras (Givnish *et al*., 2011). Uma grande fração das epífitas vasculares das Florestas Neotropicais é constituída por bromélias. Essas plantas apresentam grande diversidade nas elevações médias e em áreas de alta pluviosidade e umidade (Gentry e Dodson, 1987; Givnish *et al*., 2014). No Brasil são encontradas cerca de 50% das espécies de bromélias conhecidas, representando um grande contingente e tornando este País o mais importante centro de diversidade desta família (Leme e Marigo, 1993).

1.2 O Gênero *Dyckia*

O gênero *Dyckia* Schult. & Schult.f. pertence à subfamília Pitcairnioideae e é caracterizado por suas folhas coriáceas ou suculentas distribuídas em forma de rosetas sem tanques de captação de água, frequentemente dotadas de espinhos marginais, porte variado, sementes aladas ou raramente nuas (Smith e Downs, 1974). Apresentam exclusivamente inflorescência racemosa lateral, simples ou ramificada, com o amarelo, vermelho e laranja sendo as cores predominantes, embora existam registros de flores de cor vinho-acastanhado e esverdeados. As espécies de *Dyckia* exibem propagação vegetativa por meio de rizomas subterrâneos curtos ou longos (Smith e Downs, 1974; Givnish *et al*., 2007). Esse gênero é o segundo maior dentro de Pitcairnioideae, agrupando cerca de 145 espécies (Givnish *et al*., 2007; Luther, 2012), tendo o Cerrado na região de Minas Gerais como seu centro de diversidade (Versieux e Wendt, 2007; Krapp *et al*., 2014). O gênero *Dyckia* é encontrado no Brasil, Argentina, Uruguai, Paraguai e Bolívia. Cerca de 83% das espécies conhecidas são encontradas no Brasil, com ocorrências desde o nível do mar até 1000 metros de altitude, geralmente em ambientes expostos ao sol nos biomas de Restinga, Floresta Atlântica, Caatinga, Campos Rupestres e Cerrado (Forzza *et al*., 2016). Os espécimes deste gênero podem ser encontrados como indivíduos únicos, formados por dispersão de sementes ou densos aglomerados, formados por propagação clonal. As espécies de *Dyckia* se caracterizam por serem xeromórficas, rupícolas, de ambientes úmidos ou, algumas poucas, são adaptadas a ambientes periodicamente inundados (Smith e Downs, 1974).

Dyckia hebdingii L.B. Smith, *D. choristaminea* Mez e *D. julianae* Strehl são espécies que podem ocorrer em simpatria, são saxícolas e endêmicas de afloramentos rochosos do Rio Grande

do Sul. *Dyckia hebdingii* é uma espécie com inflorescência ramificada e pode atingir 1 m de altura, as sementes possuem uma asa estreita apical com cerca de 3 mm de comprimento (Smith e Downs, 1974). *Dyckia choristaminea* possui inflorescência simples com altura entre 15-25 cm (Smith e Downs, 1974), sementes predominantemente na cor castanho, aladas e com cerca de 2,5 mm de comprimento (Strehl e Beheregaray, 2006). *Dyckia julianae* exibe inflorescência simples ou às vezes ramificada, altura variando de 20 a 40 cm, flores sésseis e igualmente distribuídas (Strehl, 2004). O "Livro Vermelho da Flora do Brasil" (Martinelli e Moraes, 2013) classifica essas espécies como "Espécies de Interesse para Pesquisa e Conservação" por suas distribuições restritas e deficiência de dados. Além disso, o livro "Campos Sulinos – Conservação e Uso Sustentável da Biodiversidade" (Instituto do Meio Ambiente e dos Recursos Naturais Renováveis, 2009) classificou *D. julianae* e *D. hebdingii* na categoria "vulnerável" e *D. choristaminea* na categoria "em perigo". Também, em 2014 a Fundação Zoobotânica do Rio Grande do Sul (FZB/RS) tornou pública a Lista da Flora Ameaçada do Rio Grande do Sul, na qual *D. hebdingii* e *D. choristaminea* foram classificadas como "Em Perigo" e *D. julianae* como "Criticamente em Perigo". Atualmente, existem alguns trabalhos para *D. hebdingii* e *D. choristaminea* a respeito da anatomia, morfologia (Sajo *et al*., 2004; Sajo *et al*., 2005; Strehl e Beheregaray, 2006) e filogenia (Krapp *et al*., 2014). Entretanto, parece não haver trabalhos para *D. julianae* com exceção de sua descrição (Strehl, 2004). A partir da observação de que *D. julianae* apresenta características morfológicas intermediarias entre *D. hebdingii* e *D. choristaminea* surgiu o questionamento se não seria *D. julianae* um híbrido entre as outras duas espécies e se elas exibem fluxo gênico contemporâneo.

1.3 *Barreiras Reprodutivas*

Grupos de espécies que sofreram radiação adaptativa recente são altamente informativos para o estudo do isolamento reprodutivo e na avaliação da deriva e da seleção na manutenção da coesão das espécies. Uma hipótese recente a respeito de radiações adaptativas refere-se ao papel do fluxo gênico interespecífico, sugerindo que a hibridação e introgressão em populações isoladas poderia ser o subsídio para a radiação adaptativa, por fornecer variação genética necessária para a especiação (Benzing, 2000; Barrier *et al*., 2001; Seehausen, 2004).

Plantas com flores exibem alto grau de diferenciação com impressionantes exemplos de radiação adaptativa e especiação (Rieseberg e Willis, 2007). Um dos processos considerado como força criativa na evolução é a hibridação (Arnold, 1997), a qual parece ter importante papel na especiação e manutenção da diversidade, principalmente em plantas (Soltis e Soltis, 2009). Assumindo-se o conceito de hibridação de Barton e Hewitt (1985), no qual hibridação é definida como a reprodução entre membros de populações geneticamente diferentes, então esse processo deve ocorrer em grande parte das especiações, com exceção de casos onde ocorre especiação alopátrica ou instantânea (Abbott *et al*., 2013). Especiação híbrida implica que a hibridação seja o principal fator no surgimento de uma nova espécie (Mallet, 2007), porém eventos de hibridação podem manter o nível de ploidia (especiação híbrida homoploide) ou alterar o nível de ploidia, através de alopoliploidia (especiação pela hibridação seguida de duplicação do genoma), sendo esse último sistema mais comum (Soltis e Soltis, 2009). A ampla ocorrência de alopoliploides em plantas indica a importância da hibridação na evolução e diversificação desse grupo (Seehausen, 2004). Portanto, a hibridação parece ser uma característica comum no processo de especiação das plantas (Soltis e Soltis, 2009).

Diversas barreiras reprodutivas isolam a maioria das espécies de plantas, sendo que qualquer redução da taxa de migrantes entre duas populações distintas aumentará a divergência entre elas e, passado algum tempo, poderá levar ao isolamento reprodutivo completo (Rieseberg e Willis, 2007). Nas plantas, as barreiras ao fluxo gênico são divididas em pré e pós-polinização. As barreiras pré-polinização são também exclusivamente pré-zigóticas e envolvem fatores ecológicos (como a distância excessiva entre plantas de linhagens diferentes, impedindo o fluxo gênico) e reprodutivos (diferenças em tempo de floração e morfologia floral que afeta diretamente a preferência de polinizadores). Por sua vez o isolamento pós-polinização pode ser pré ou pós-zigótico, incluindo baixas taxas de germinação, crescimento lento do tubo polínico e incapacidade do pólen penetrar o óvulo (Tiffin *et al*., 2001).

Compreender como atua o fluxo gênico intra e interespecífico na manutenção da integridade e coesão das espécies é um tema de grande interesse. A ideia de barreiras porosas, semipermeáveis ao fluxo gênico, está ganhando ampla aceitação no lugar do conceito de "isolamento reprodutivo de todo o genoma" (Wu, 2001). Para o isolamento reprodutivo entre duas espécies ser completo é necessário uma interação entre diferentes *loci* que influenciam o isolamento (*barrier loci*). *Loci* que não atuam no isolamento reprodutivo ou que não estão ligados a um *locus* que resulte em algum grau de isolamento poderá sofrer introgressão entre espécies que hibridizam (Abbott *et al.*, 2013). Portanto, muitas espécies simpátricas são mantidas por barreiras reprodutivas fortes, porém permeáveis (Palma-Silva *et al*., 2011).

Capítulo 2

Objetivos

1. Objetivos

1.1 Geral

A família Bromeliaceae é um modelo interessante para o estudo das barreiras reprodutivas, por ser uma família que sofreu radiação adaptativa recente e apresentar uma grande variação morfológica ainda pouco compreendida. Sendo assim, a presente dissertação tem como objetivo geral contribuir com informações importantes para a melhor compreensão de mecanismos de especiação, isolamento reprodutivo e coesão de espécies. Essas informações também serão fundamentais para traçar estratégias para conservação das espécies em estudo.

1.2 Objetivos Específicos

- Investigar sobre possível origem híbrida de *Dyckia julianae*;
- Determinar se *D. hebdingii* e *D. choristaminea* cruzam entre si e produzem híbridos;
- Estimar a diversidade e estruturação genética de populações de *Dyckia hebdingii, D.choristaminea* e *D. julianae*/híbridos;

- Avaliar os padrões de fluxo gênico nuclear, comparando com o fluxo gênico plastidial obtido entre as três espécies;
- Avaliar aspectos evolutivos do sistema reprodutivo e das barreiras reprodutivas pré e pós-zigóticas que estão atuando para a manutenção das espécies;
- Determinar as implicações dos padrões de fluxo gênico para a conservação e resiliência dessas espécies.

Capítulo 3

Hybrid origin and patterns of gene flow in three species of *Dyckia* (Bromeliaceae) endemics from Southern Brazil

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HYBRID ORIGIN AND PATTERNS OF GENE FLOW IN THREE SPECIES OF *DYCKIA* (BROMELIACEAE) ENDEMICS FROM SOUTHERN BRAZIL

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ABSTRACT

Dyckia hebdingii, *D. choristaminea* and *D. julianae* are bromeliads endemic to rock outcrops of Southern Brazil that can occur in sympatry. *Dyckia hebdingii* and *D. choristaminea* has distinct morphology, but *D. julianae* exhibits an intermediate floral and vegetative morphology between the other two species. Here we used seven nuclear and six plastid microsatellite *loci* to access the patterns of genetic diversity and population structure aiming to elucidate the boundaries of these three species and the patterns of interspecific gene flow among them. Furthermore, we performed manual crosses between species to test compatibility and fertility of the three species. The major results indicate high expected heterozygosities when compared with observed heterozygosities. Inbreeding coefficient (F_{IS}) was high for all species. Six cpSSR identified 12 haplotypes and haplotype diversity (*h*) ranged from 0.0 to 0.736. Crosses between *D. julianae* and the other two species generated fruits and seeds as well as interspecific crosses of *D. julianae* and *D. hebdingii*. None cross between *D. hebdingii* and *D. choristaminea* generated fruits or seeds. Our data suggest a hybrid origin of *D. julianae* from *D. hebdingii* and *D. choristaminea*. However these two species are genetically different and have low migration rate between them. Therefore, *D. hebdingii* and *D. choristaminea* do not cross each other currently, probably due to strong reproductive barriers.

INTRODUCTION

Hybridization is an important element to understand the processes that lead to speciation, since several speciation events in plants involve hybridization. According to Mallet (2007), 25%

of plant species are known to cross with at least one species and this number increases in groups that suffered rapid radiation. Plants exhibit different mechanisms of speciation and modes of reproductive isolation (Soltis and Soltis, 2009). Complete speciation process can take many generations, except for hybrid speciation of fully isolated polyploidy, which may arise in one or two generations (Rieseberg and Willis, 2007). When different species meet again and the reproductive isolation is incomplete may occurs a special evolutionary case – hybrid speciation (Schmickl and Koch, 2011). Hybridization seems to be common in plants and may play an important role in great diversity and speciation, becoming an object of study of great interest among evolutionists.

Reproductive isolation plays a major role in speciation, and the knowledge of the reproductive barriers mechanisms between closely related species is an important factor to better understand these processes (Bedinger *et al*., 2011). There are different reproductive barriers in plants, including prepollination (ecogeographic, mechanical, temporal and pollinator isolation), postpollination prezygotic isolation (preference for co-specific pollen) and postpollination postzigotic isolation (hybrid inviability or sterility; Rieseberg and Willis, 2007). For the most part of plants, prezigotic barriers is an important contribution to reproductive isolation comparing to postzigotic barriers, since prezigotic barriers are individually stronger and act earlier over life history of an organism (Lowry *et al*., 2008). The determination of reproductive barriers strength, particularly in rapidly diverging lineages, can provide insights on speciation process of these species (Runquist *et al*., 2014). The presence and classification of reproductive barriers are essential for understanding the persistence of species, especially when there are sympatric populations.

Bromeliaceae is one of the most diverse families native to the tropics and subtropics of New World (Martinelli *et al.*, 2008; Givnish *et al*., 2011; Zanella *et al*., 2012), comprising approximately 3.000 species and 58 genera (Benzing, 2000; Luther, 2012; Givnish *et al*., 2014). This group has distribution almost exclusively Neotropical, with one species in west of Africa (Porembski and Barthlott, 1999). Bromeliaceae had a recent adaptive radiation (Benzing, 2000; Barfuss *et al*., 2005), with geographical distribution ranging from the states of Virginia, Texas, and California in the USA (longitude 37° W) to northern Patagonia in Argentina (latitude 44° S). Bromeliads may dwell in various habitats and the occurrence of several congeneric species in sympatry can be considered common (Benzing, 2000; Wendt *et al*., 2001, 2002, 2008; Givnish *et al*., 2014). However, a few reports on natural hybridization in Bromeliaceae suggest the existence of strong isolation barriers among species (Luther, 1985; Schulte *et al*., 2010; Palma-Silva *et al*., 2011; Zanella *et al.*, 2016 *in press*). Despite this, some studies revealed weak prezygotic isolation among bromeliads species. Wendt *et al*., (2008) studied 42 sympatric bromeliad species and highlighted the importance of postzigotic barriers in bromeliads.

Dyckia Schult. & Schult.f. is the second major genus of Pitcairnioideae (Givnish *et al*., 2007), comprising approximately 147 species (Luther *et al*., 2012). About 83% of *Dyckia* species occur in Brazil, being this genus the most diverse in Rio Grande do Sul state, with 28 species registered of which 17 possibly endemics (Sthrel, 2004). *Dyckia hebdingii*, *D. choristaminea* and *D. julianae* are related and endemic species from Southern of Rio Grande do Sul state growing in sympatry. *Dyckia hebdingii* and *D. choristaminea* has distinct morphology, but *D. julianae* exhibits an intermediate morphology between the other two species. According to literature and our field trips there is no allopatric populations of *D. julianae*, apparently this species occurs only in sympatry with the other two species (Strehl, 2004). Furthermore, we recognize

morphologically different individuals, regarding to the three described species and we named these individuals as hybrids.

Molecular markers are powerful tools to investigate natural hybridization, since they are able to detect low levels of introgression. Nuclear and plastid markers have been used successfully in detection of interspecific gene flow (Barton and Hewitt, 1985). Plastid genome do not undergone recombination and is inherited from one of the parental (in Bromeliaceae inheritance seems to be maternal; Wagner *et al*., 2015), offering valuable information on introgression direction (Rieseberg and Brunsfeld, 1992).

Here, we aim to elucidate the boundaries of these three species and the patterns of interspecific gene flow among them. Also, we investigated the intraspecific variability and the genetic structure of *D. hebdingii*, *D. choristaminea*, *D. julianae* and putative hybris, in sympatric and allopatric populations. Specifically, we seek to answer the following questions: a) does *Dyckia julianae* has hybrid origin from *D. hebdingii* and *D. choristaminea* (since *Dyckia julianae* morphology is intermediate)? b) does *Dyckia hebdingii*, *D. choristaminea* and *D. julianae* cross each other? These crosses produce viable hybrids? c) what patterns of gene flow between these species and how this can affect the resilience of species (conservation)? The discussion of these questions will contribute to better understand aspects as speciation, reproductive isolation and cohesion of these species, which is fundamental for planning conservation strategies.

MATERIALS AND METHODS

Plant Species and Populations Sampling

In this study we sampled populations of three species of *Dyckia* (Bromeliaceae): *Dyckia hebdingii, D. choristaminea* and *D. julianae*. These species are saxicolous and endemics to rock outcrops from Southern Brazil (east of Rio Grande do Sul federative state) and may occur in sympatry (Fig. S1). *Dyckia hebdingii* shows a branched inflorescence with 1 meter high, yellow petals and many flowers (Smith and Downs, 1974). In contrast, *D. choristaminea* shows a single inflorescence with 15-25 cm high, yellow petals and a few flowers (Smith and Downs, 1974). As well as *D. julianae* shows single or branched inflorescence with 20-40 cm high, yellow petals and many flowers equally distributed (Strehl, 2004; Fig. S2).

We collected young leaf tissue from 13 to 125 flowering or fruiting individuals per population from two sympatric sites (Cabaña de los Brettes and Serrinha, both in Barra do Ribeiro/RS), two allopatric sites for *D. hebdingii* (Horto in Mariana Pimentel/RS and Faxinal in Arroio dos Ratos/RS) and one allopatric site for *D. choristaminea* (Morro do Osso in Porto Alegre/RS; Fig. S1), totalizing 255 individuals sampled (Table 1). Individuals were classified by morphology and collected with a minimum distance of five meters from each other. None allopatric population was found for *D. julianae*. For each plant, fresh leaves were collected and stored in silica gel. Total genomic DNA was extracted following the protocol described by Doyle and Doyle (1990).

Nuclear and Plastid Microsatellite Markers and Genotyping Assays

We used a total of seven nuclear microsatellite markers (nuSSR) to access the patterns of genetic diversity and admixture in the sympatric and allopatric populations studied. These markers were isolated from different bromeliad species: *Ananas comosus* (Acom_109.6, Acom_12.12, Acom_82.8, Acom_101.1; Wöhrmann and Weising, 2011), *Dyckia distachya* (Dd03, Dd19; Zanella *et al*. 2012) and *Fosterella rusbyi* (ngFos22; Wöhrmann *et al.*, 2012). In

addition, a total of six plastid microsatellites markers were amplified (cpSSR) to study the haplotype variation among populations. The cpSSR markers used in this study were isolated from *Dyckia marnier-lapostollei* (DSSR-L01, DSSR-L04, DSSR-N01, DSSR-N07, DSSR-N16, DSSR-N11; Krapp *et al*., 2012). The polymerase chain reaction (PCR) and genotyping were performed in a Veriti 96-well terrmal cycler (Applied Biosystems), according to Zanella *et al.* (2012) for nuclear and according to Krapp *et al.* (2012) for cpSSR. Nuclear and plastid SSR were resolved on an ABI 3500 DNA analyser sequencer (Applied Biosystems) compared with GS500 LIZ molecular size standard (Applied Biosystems) using GENEMARKER demo version 1.97 software (SoftGenetics).

Data Analysis

We performed admixture analysis using a Bayesian clustering approach to assign individuals to genetic clusters and to identify the hybrids. The analysis was implemented in the software STRUCTURE 2.3.4 (Pritchard *et al*., 2000), using nuclear data. Allopatric populations of *D. hebdingii* and *D. choristaminea* were used as reference profiles, because these individuals were considered pure. STRUCTURE analysis was performed under the admixture model, assuming correlated allele frequencies and using a burn-in period of 50,000, run length of 300,000 and 10 replicates per K, ranging from one to ten with all populations in the data set (sympatric and allopatric). We used the method of Evanno *et al*. (2005) to determine the most likely number of clusters (K) in our data, implemented in STRUCTURE HARVESTER (Earl and vonHoldt, 2012). Also, we performed analysis for each sympatric population separately, using allopatric populations as pure individuals for each species. The K=2 model was used with 10 replicates per K, because we presumed that *D. hebdingii* and *D. choristaminea* contribute to

the gene pool of the sample. In the STRUCTURE we assumed a threshold of $q \geq 0.90$ to classify pure individuals of *D. hebdingii*, $q \leq 0.10$ to classify pure individuals of *D. choristaminea* and $0.10 < q < 0.90$ to classify hybrids (Vähä and Primmer, 2006). All subsequent analyses with hybrids were performed with individuals identified by the STRUCTURE approach.

Genetic Diversity

Genetic diversity was characterized in *D. hebdingii*, *D. choristaminea* and *D. julianae*/hybrids, in both sympatric and allopatric populations, using nuclear microsatellites markers. We estimated the number of alleles, allelic richness, observed and expected heterozygosities and inbreeding coefficient (F_{IS}; Weir and Cockerham, 1984) calculated for each *locus* using the software FSTAT (Goudet, 1995) and ARLEQUIN 3.11 (Excoffier *et al*., 2005). Departures from HWE for each population were tested using exact tests in the software GENEPOP 4.2 (Raymond and Rousset, 1995). We assessed nuclear genetic differentiation using estimates of F_{ST} (Weir and Cockerham, 1984) and G'_{ST} (Hedrick, 2005) calculated in the software FSTAT, considering only pure individuals. We carried out the analysis of molecular variance (AMOVA), implemented in ARLEQUIN, in order to partition the genetic diversity within and among pure individuals of *D. hebdingii* and *D. choristaminea*. The significance of the model was tested using 10,000 permutations. Pairwise effective migration rates (N_em) were estimated among *D. hebdingii*, *D. choristaminea* and *D. julianae*/hybrids in the sympatric sites, following a coalescent theory and maximum-likelyhood based approach using MIGRATE 3.0.3 software (Beerli and Felsenstein, 1999). The computations were carried out under both the infinite allele model (Kimura and Crow, 1964) and the stepwise mutation model (Kimura and

Ohta, 1978). Also, pairwise F_{ST} from nuclear and plastid microsatellites was computed by ARLEQUIN.

Plastid microsatellite *loci* were used to identify and characterize plastid DNA haplotypes. Diversity was accessed for all populations, using number of haplotypes observed in each population, haplotype diversity (*h*) and the allelic richness (*AR*), implemented in software Contrib 1.4 (Petit *et al*., 1998). A haplotype network was built based on cpSSR using the software NETWORK v. 4.5.1.0 and applying median-joining option (Bandelt *et al*., 1999). Single haplotypes were excluded to simplify the network.

Compatibility and Fertility Estimate

In addition to the molecular data, intra and interspecific artificial crosses were performed to test genome compatibility and fertility of *D. hebdingii*, *D. choristaminea, D. julianae* and putative hybrids by testing seed viability. Manual crossing experiments were conducted at the green house of the Botanical Department (UFRGS, Rio Grande do Sul, Brazil). Artificial crosses were performed following Cafasso *et al*. (2005) and Pinheiro *et al*. (2010) with a few modifications. The treatments used were: (a) intraspecific cross-pollination control (*D. hebdignii* and *D. choristaminea*); (b) interspecific cross-pollination between *D. hebdingii* and *D. choristaminea*; (c) intraspefic between possible hybrids (*D. julianae* x *D. julianae*); (d) interspefic between *D. julianae* with each parental species (*D. hebdingii* and *D. choristaminea*). In total it was used 34 flowers from 14 individuals. All crosses were conducted in both directions (each plant provided and received pollen). After the formation and maturation, the fruit was collected and stored for subsequent seed count and viability test; germination was performed in MS medium (Murashige and Skoog, 1962) plus vitamin B5 (Gamborg *et al*., 1968), 3% of

saccharose and 0.25% of Phytagel with temperature controlled (25ºC). Seeds were checked daily and the criterion for determining the germination was the emergence of the radicle.

RESULTS

Genetic Composition of Sympatric Population

Using the admixture analysis by STRUCTURE for all data set, we obtained a K = 2 as the most likely number of genetic clusters (Fig. 1), indicating individuals with intermediate molecular profile. Bayesian STRUCTURE results for each sympatric population, considering two genetic groups, showed that individuals of *D. julianae* and putative hybrids exhibit, mostly, intermediate molecular profile (Fig. 2), for this reason, subsequent analysis of genetic diversity were performed clustering *D. julianae* and individuals morphologically identified as hybrids. Bayesian STRUCTURE results for each sympatric population identified a total of 49 individuals with intermediate profile, of which 25 was identified morphologically as *D. julianae*, 15 as hybrids, six as *D. hebdingii* and three as *D. choristaminea* in Cabaña de los Brettes and Serrinha populations (Fig. 3). Therefore, 30.2% of individuals from sympatric populations showed genetic mixture between both clusters (STRUCTURE threshold of $0.10 < q < 0.90$). Allopatric populations, used as a reference for each parental species, were composed of purebreds (Fig. 3). STRUCTURE Bayesian analysis identified 42 hybrids in Cabaña de los Brettes and seven in Serrinha populations (Fig. 3). Only 10 samples from 35 collected as *D. julianae* during field expeditions, did not show an intermediate molecular profile (six had *D. hebdingii* profile and four presented *D. choristaminea* genetic constitution, according to STRUCTURE). As well as only two samples from 17 morphologically classified as hybrid did not show an intermediate

molecular profile (classified as *D. hebdingii* and *D. choristaminea*). Also, six samples of *D. hebdingii* and three of *D. choristaminea* showed intermediate profile. For subsequent analyzes the samples were classified according to the result of the STRUCTURE.

Nuclear and Plastid Microsatellite Diversity

The seven nuSSR markers used were polymorphic for *D. hebdingii*, *D. choristaminea* and *D. julianae*/hybrids with total number of alleles ranging from six to 16 per *locus* (data not shown). *Dyckia julianae* and hybrids exhibited one *locus* (Acom_12.12) monomorphic. *Dyckia hebdingii* showed a mean of six alleles (ranging from four to nine) and *D. choristaminea* exhibited a mean of nine alleles per *locus* (ranging from five to 15). *Dyckia julianae* and hybrids showed a mean of seven alleles (ranging from one to 11). Allelic richness showed a mean of 4.6 alleles per *locus* for *D. hebdingii* and 7.7 for *D. choristaminea*. For *D. julianae* and hybrids a mean of 6.7 to allelic richness was found. The observed and expected heterozygosities were 0.281 and 0.444 for *D. hebdingii*, 0.456 and 0.649 for *D. choristaminea* and 0.585 and 0.708 for *D. julianae* and hybrids, respectively. Inbreeding coefficient (F_{IS}) was high and departed significantly from HWE for almost all *loci* ($P < 0.05$) in the three species and hybrids analyzed. Also, almost all *loci* showed at least one private allele, except the *locus* Dd19, totalizing 19 unique alleles (data not shown). *Dyckia hebdingii*, *D. choristaminea* and *D. julianae*/hybrids exhibited four, 15 and none private allele, respectively. *Dyckia hebdingii* and *D. julianae*/hybrids shared six alleles absent in *D. choristaminea* (data not shown). As well as *D. choristaminea* shared 12 alleles with *D. julianae*/hybrids absent in *D. hebdingii* (data not shown). *Dyckia julianae* and hybrids showed, on average, intermediate genetic diversity index, comparing to other two species (Table 2).

Dyckia choristaminea showed higher allelic richness levels compared to *D. hebdingii*, considering all populations. The largest allelic richness for both species was found in Cabaña de los Brettes population. Allelic richness was similar between Cabaña de los Brettes and Serrinha populations, but Cabaña de los Brettes presented higher number of alleles than Serrinha. Inbreeding coefficient (F_{IS}) was high and displayed significant departures from HWE in Cabaña de los Brettes population, but in Serrinha none F_{IS} presented significant departures from HWE. In general, genetic diversity indexes were lower in allopatric populations than sympatric ones and only Faxinal population did not show significant departures from HWE (Table 3).

High levels of nuclear genetic differentiation between *D. hebdingii* and *D. choristaminea* was found (F_{ST} = 0.253 and G'_{ST} = 0.244; data not shown). The AMOVA results showed that 15.8% of differentiation were among species, with F_{CT} = 0.158 (Table 4). Maximum-likelihood based estimates of effective migration rates (N_em) for sympatric populations (Cabaña de los Brettes and Serrinha) were low between *D. hebdingii* and *D. choristaminea*, but high between *D. julianae* and the other to species (Fig. 4).

Concerning the six cpSSR used, we identified 12 haplotypes. Haplotype diversity (*h*) ranged from 0.0 to 0.736 and *D. hebdingii* in Cabaña de los Brettes population exhibited the highest haplotype diversity (0.433; Table 3). The plastidial allelic richness (*AR*) ranged from 0.0 (one haplotype) to 11 (five haplotypes; Table 3). The H1 haplotype was the most frequent (144 individuals) and was observed in three species and hybrids (Fig. 5). *Dyckia hebdingii* and *D. choristaminea* shared 5 haplotypes (Fig. 5). *Dyckia hebdingii* exhibited four unique haplotypes (H6, H7, H9, H11) and *D. choristaminea* presented only one private haplotype (H10; Fig. 5). In contrast, *D. julianae*/hybrids showed no unique haplotypes, exhibiting five of the 12 haplotypes observed (Fig. 5). None haplotype were exclusively shared between *D. julianae* and *D.*

choristaminea. *Dyckia choristaminea* exhibited the haplotypes 1 and 4 in all populations (Fig. 5). The H6 single haplotype was excluded from network.

DISCUSSION

Hybrid Origin of *D. julianae*

Our STRUCTURE results showed individuals with intermediated profile (Fig. 3), of which 48% had morphology of *D. julianae*. Also, we were unable to find allopatric populations of *D. julianae* in our field expeditions, fact that may contribute to confirm our hypothesis of hybrid origin of *D. julianae*. Besides, only eight individuals collected as *D. julianae* do not showed intermediate profiles, suggesting that *D. julianae* may have originated from hybridization between *D. hebdingii* and *D. choristaminea*. *Dyckia hebdingii* and *D. choristaminea* are highly related species, with a close genetic relationship between them (Krapp *et al*., 2014) and, although low, effective contemporary migration rates were obtained in our study (Fig. 4). On the other hand, F_{ST} statistics and AMOVA results (Table 4) showed high genetic structure, suggesting that these two species are genetically different and hybridization between them may not be common currently. Our results from controlled crosses experiments corroborated this suggestion, since *D. hebdingii* and *D. choristaminea* are able to cross with hybrids and form seeds, but do not form seeds when they cross each other (Table S1). In this sense, we proposed a hybrid origin for *D. julianae* in sufficient time for existing relatively strong reproductive barriers between the parental species, but still permeable between the parents and the hybrid species. In general, hybrids have more variation than parental species and may be the trigger to ecological speciation in sympatric populations (Seehausen, 2004) and our data show

highest genetic diversity (H_E) in *D. julianae*/hybrids than in the two other species (Table 2). Also, we observed that *D. julianae*/hybrids exhibited high effective migration rates with *D. hebdingii* and *D. choristaminea*, indicating gene flow among them (Fig. 4).

Migration patterns for the two sympatric populations diverged (Fig. 4). In Cabaña de los Brettes we can notice gene flow among *D.julianae*/hybrids and the other two species in similar proportions, indicating hybridization in both directions, but in Serrinha population *D. julianae*/hybrids exhibited higher effective migration rates with *D. choristaminea* than *D. hebdingii*, maybe as a result of asymmetric gene flow (Fig. 4). These divergent results may be due to the minor number of individuals collected in Serrinha (Table 1), which can caused a bias in migration rate estimative. Indeed, hybrid speciation require, *a priori*, isolation of hybrid gene pool from parental species (Schmickl and Koch, 2011) and evolution of complete reproductive isolation between species may take millions of generations (Abbott *et al*., 2013). Several factors may be involved in reproductive isolation between species and even different flower size can be a structural barrier to gene flow (Field *et al*., 2010). Despite this, a few cases of hybrid speciation are known (Arnold, 1993; Abbott and Lowe, 2004; Schmickl and Koch, 2011), in part because of the difficulty to prove that hybridization led to speciation (Mallet, 2007). Accordingly, hybridization may lead to generation of new populations of mixed ancestry that preserve distinct characteristics from both parental populations (Abbott *et al*., 2013).

In this study we found a few inconsistencies between morphology and molecular patterns for the purpose of species delimitation. As commented above, in both sympatric populations we collected 10 individuals of *D. julianae* and two hybrids which were categorized as *D. hebdingii* or *D. choristaminea* by genetic data. As well as six samples collected as *D. hebdingii* and three as *D. choristaminea* that presented intermediate molecular profiles. These results highlight that it

can be difficult to classify these species using only morphological traits, in part because *D. julianae* presents intermediate morphological characteristics between the other two species, resulting in misclassification. Zanella *et al*. (2016, *in press*), studying hybridization patterns between two *Vriesea* (Bromeliaceae) species also pointed out the difficulty in classifying hybrids based on only one approach, since hybrids may not always present an intermediate morphology.

Dyckia julianae appears to be able to cross with both species, since interspecific artificial crosses produced seeds and estimated migration rates were high (Fig. 4; Table S1). The individuals classified as hybrids by morphology can be an outcome of these interspecific crosses. Interspecific artificial crosses involving *D. julianae* produced fruits with aborted seeds, which may indicate incomplete post-zigotic barrier (Fig. S3; Table S1). In order to corroborate the hypothesis of hybrid origin of *D. julianae*, cytogenetic approaches (meiotic and mitotic analysis) are necessary and will be important aiming recognize whether this species has undergone to homo or polyploid speciation.

Interspecific Gene Flow Between *D. hebdingii* and *D. choristaminea*

In this study, STRUCTURE analysis showed individuals with intermediate profiles between *D. hebdingii* and *D. choristaminea* (Fig. 2) which were considered as hybrids. Moreover, MIGRATE results exhibited an extremely low N_em between *D. hebdingii* and *D. choristaminea*, indicating that hybridization between these species may be rare (Fig. 4). Meanwhile, effective migration rates between *D. choristaminea* and *D. hebdingii* with hybrids were high (Fig. 4), supporting the idea that hybrids might be in speciation process (Abbott *et al*., 2013). Another evidence that gene flow between *D. hebdingii* and *D. choristaminea* may be low is that these two species has different blooming periods (Smith and Downs, 1974 ; Strehl, 2004).

In our field trips we observed that *D. choristaminea* blooms in October/November and *D. hebdingii* in Dezember/January, with a short period of overlapping that probably difficult interspecific gene flow. Also, in our artificial crosses experiments, *D. hebdingii* and *D. choristaminea* were not able to generate fruits or seeds when crossed (Table S1). Likewise, none intraspecific cross with *D. hebdingii* generated fruits or seeds and further studies are needed to elucidate this result.

Three haplotypes (H1, H2 and H3) were shared between *D. hebdingii*, hybrids and *D. choristaminea*. Two other haplotypes (H4 and H8) were shared only among *D. hebdingii* and *D. choristaminea* (Fig. 5), and this sharing can be explained by many process, as recent introgressive hybridization, ancestral polymorphism and homoplasy (Palma-Silva *et al*., 2011, Zanella *et al*., 2016 *in press*). Unlike our nuclear data, the cpSSR exhibited low genetic differentiation between species (F_{ST} = 0.07; Table 5), with five shared haplotypes between species. *Dyckia julianae*/hybrids shared five haplotypes with the other two species, but do not shared any haplotypes exclusively with *D. choristaminea*, suggesting asymmetric hybridization towards *D. choristaminea* (*D. julianae* as obligatory pollen donor; Fig. 5). Nuclear data showed low genetic admixture (Table 4), despite plastid data indicated a long history of gene exchange between *D. hebdingii* and *D. choristaminea* (Table 5; Fig. 5), suggesting that pre and post-zigotic barriers contribute to reproductive isolation between these species (Widmer *et al*., 2009; Palma-Silva *et al*., 2011).

The results obtained here indicated that *D. hebdingii* and *D. choristaminea* are genetically different and migration rates between them are low. None artificial crosses among these two species generated seeds or fruits, confirming a possible zygotic barrier.

Patterns of Gene Flow and Conservation

The analysis of our data indicated that *D. hebdingii* and *D. choristaminea* did not presented a significant gene flow, as suggested by extremely low effective migration rates (Fig. 4), high F_{ST} (Table 4) and just a few shared alleles between them (four alleles; data not shown). However, the gene flow among *D. julianae*/hybrids and the other two species seems to have meaningfulness. According to our molecular data, *D. julianae*/hybrids exhibit considerable effective migration rate (Fig. 4), low F_{ST} with *D. choristaminea* (F_{ST} = 0.09) and intermediate F_{ST} with *D. hebdingii* in Cabaña de los Brettes and high F_{ST} with both two species in Serrinha population (Table 5), furthermore many alleles shared with *D. choristaminea* (12 alleles; data not shown).

These patterns of gene flow suggest that *D. hebdingii*, *D. choristaminea* and *D. julianae* are well established species. Meanwhile, if *D. julianae* cross with *D. hendingii* and *D. choristaminea* producing hybrids, particularly asymmetric hybridization, homogenization of species should be taken into account. Supposing that *D. choristaminea* receives pollen of *D. julianae*, but do not serve as pollen donator, *D. choristaminea* may lose some features over generations because asymmetric quota of *D. julianae* genomes towards *D. choristaminea*. Hybridization and introgression between species are ongoing and regular, contributing to the adaptive radiation and diversification (Mallet, 2005). However, it is important understand these implications because hybridization between native and introduced species should be taken into account when considering conservation strategies (Allendorf *et al*., 2001).

The indication of pre-zygotic barriers (yet to be established) between *D. hebdingii* and *D. choristaminea* may have allowed these species persisted in sympatry. The low levels admixture of nuclear alleles (Table 4) suggests that there is no greater threat of genetic assimilation.

However, *D. julianae* seems to be able to cross with the other two species, being necessary to confirm the extension of introgression (by classification of hybrids) to access the consequences in species resilience. Interspecific hybridization is usually deleterious, but several examples (Sperling, 1990; Freeland and Boag, 1999; Weill *et al*., 2000) suggest that hybridization and introgression can sometimes allow adaptive combinations (Mallet, 2005). To elucidate if *D. julianae* is already well established as a single entity or a hybrid is an important task, since hybrids are in most cases deemed unworthy of conservation by conservation policies. However, we should remember that invasions of genome (introgression) are sometimes natural and can contribute to adaptability and diversification (Mallet, 2005). Ultimately, our data indicate that *D. julianae* had hybrid origin from *D. hebdingii* and *D. choristaminea*, but other approaches may provide further answers about the origin and genetic flow between these three sympatric species.

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Table 1. *Dyckia* populations sampled, including population names, municipalities, sample sizes and geographic coordinates for each population. Dh (*D. hebdingii*) and Dc (*D. choristaminea*).

Populations	Localities	Sample Size	Coordinates	
			Latitude S	Longitude W
Allopatric				
Horto (Dh)	Mariana Pimentel/RS	40	30º21’	51º34’
M. do Osso (Dc)	Porto Alegre/RS	40	30º07’	51º14’
Faxinal (Dh)	Arroio dos Ratos/RS	13	30º11’	51º42’
Sympatric				
Cabaña de los Brettes	Barra do Ribeiro/RS	125	30º18’	51º30’
Serrinha	Barra do Ribeiro/RS	37	30º16’	51º28’
Overall		255		

Table 2. Genetic variability at seven microsatellite *loci* in *D. hebdingii*, *D. choristaminea* and *D. julianae*/hybrids, including *locus* name, number of alleles (*A*), allelic richness (*Rs*), observed (H_O) and expected (H_E) heterozygosities and inbreeding coefficient (F_{IS}) for each *locus*.

	Dyckia hebdingii					*D. julianae*/Hybrids					*Dyckia choristaminea*				
Locus	*A*	*Rs*	*Ho*	*He*	*Fis*	*A*	*Rs*	*Ho*	*He*	*Fis*	*A*	*Rs*	*Ho*	*He*	*Fis*
A109.6	6	5.0	0.152	0.256	0.408**	7	7.0	0.5	0.801	0.379**	7	6.0	0.618	0.652	0.053**
A12.12	4	2.2	0.031	0.031	-0.007	1	1.0	-	-	-	6	4.5	0.116	0.113	-0.031
Dd03	6	4.2	0.223	0.206	-0.08	8	7.9	0.581	0.596	0.026	10	9.9	0.458	0.855	0.466**
ngF22	6	5.0	0.347	0.691	0.499**	10	9.9	0.418	0.681	0.389**	15	12.9	0.7	0.833	0.162**
A82.8	7	5.4	0.409	0.616	0.337**	4	4.0	0.65	0.552	-0.18**	5	4.6	0.106	0.576	0.816**
Dd19	5	4.3	0.365	0.678	0.463**	6	6.0	0.568	0.802	0.294**	6	5.4	0.355	0.683	0.482**
A101.1	9	6.3	0.44	0.628	0.301**	11	10.8	0.795	0.817	0.027*	11	10.8	0.842	0.829	-0.015
Mean	6	4.6	0.281	0.444	0.274**	7	6.7	0.585	0.708	0.134**	9	7.7	0.456	0.649	0.276**

Imbreeding coefficient (F_{IS}) departed significantly from Hardy-Weinberg equilibrium (HWE) are indicated by asterisks (*P < 0.05; **P < 0.005).

Table 3. Genetic characterization of populations of *D. hebdingii*, *D. choristaminea*, *D. julianae*/hybrids, with seven nuclear and six plastid microsatellite markers, including number of alleles (*A*), allelic richness (*Rs*), observed (H_O) and expected (H_E) heterozygosities, imbreeding coefficient (F_{IS}), haplotype diversity (*h*) and allelic richness (*AR*).

	Nuclear Microsatellites					cpDNA		
Species	*A*	*Rs*	*Ho*	*He*	*Fis*	*h*	*AR*	Haplotypes
Allopatric								
Horto								
D. hebdingii	3	1.2	0.280	0.320	0.128**	0.000	0.000	H9
Morro do Osso								
D. choristaminea	5	3.1	0.507	0.560	0.096**	0.148	0.895	H1,H4,H10
Faxinal								
D. hebdingii	3	1.8	0.415	0.423	0.020	0.538	1.000	H8,H11
Sympatric								
Cabaña								
D. hebdingii	5	2.4	0.275	0.408	0.328**	0.433	2.665	H1,H2,H3,H4,H5,H6,H7
D. julianae/Hybrids	7	3.3	0.589	0.676	0.130**	0.383	1.870	H1,H2,H3,H5
D. choristaminea	7	3.6	0.404	0.595	0.325**	0.367	2.168	H1,H2,H3,H4,H8
Serrinha								
D. hebdingii	3	2.0	0.288	0.320	0.102	0.736	3.380	H1,H3,H4,H5,H12
D. julianae/Hybrids	4	3.2	0.571	0.670	0.158	0.733	11.000	H1,H5,H12
D. choristaminea	3	2.7	0.458	0.565	0.214	0.667	11.000	H1,H4

*Imbreeding coefficient (F_{IS}) departed significanty from Hardy-Weinberg equilibrium (HWE) at the $P < 0.05$ level.

Table 4. Analysis of molecular variance (AMOVA) for seven nuclear microsatellite *loci* at one hierarchical level (species), including only pure individuals of *D. hebdingii* and *D. choristaminea*.

		Nuclear Microsatellite	
Source variation	Variation(%)	*F-statistic*	*P*
Among species	15.78	F_{CT}: 0.158	0.027
Among populations within species	22.15	F_{ST}: 0.379	0.000
Within populations	62.08	F_{SC}: 0.262	0.000

Table 5. Genetic divergence (F_{ST}) of nuclear data (below diagonal) and plastid data (above diagonal) for two sympatric populations, including *Dyckia hebdingii* (Dh), *Dyckia julianae* (Dj)/hybrids and *Dyckia choristaminea* (Dc).

	Cabaña de los Brettes				Serrinha		
	Dh	Dj/hybrid	Dc		Dh	Dj/hybrid	Dc
Dh	-	0.759*	0.533*	Dh	-	-0.025	0.224
Dj/hybrid	0.131*	-	0.852*	Dj/hybrid	0.295*	-	0.034
Dc	0.319*	0.090*	-	Dc	0.449*	0.219*	-

*Fixation index (F_{ST}) departed significantly from Hardy-Weinberg equilibrium (HWE) at the $P < 0.05$ level.

Suplementary material Table S1. Artificial crosses treatments, including number of viable and aborted seeds for each treatment.

	Number of Seeds	
Artificial Crosses	Viable	Aborted
D. hebdingii x *D. hebdingii*	0	0
D. choristaminea x *D. choristaminea*	48	43
D. julianae x *D. julianae*	69	0
D. hebdingii x *D. choristaminea*	0	0
D. hebdingii x *D. julianae*	47	27
D. choristaminea x *D. julianae*	57	15

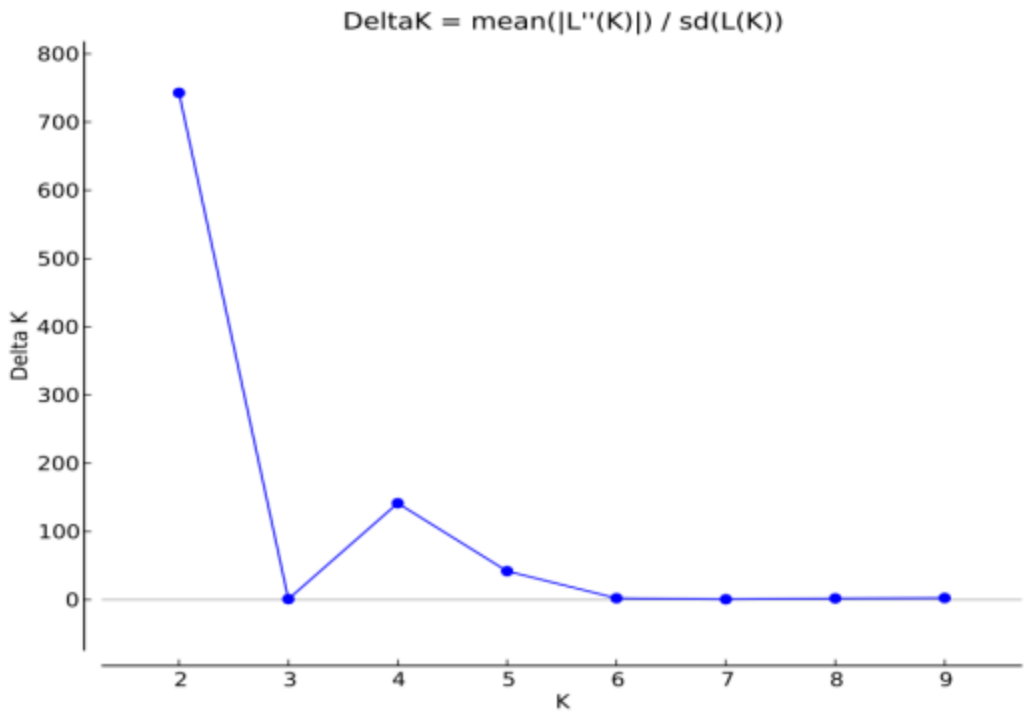


Figure 1. Mostly likely number of clusters (K) by STRUCTURE HARVESTER.

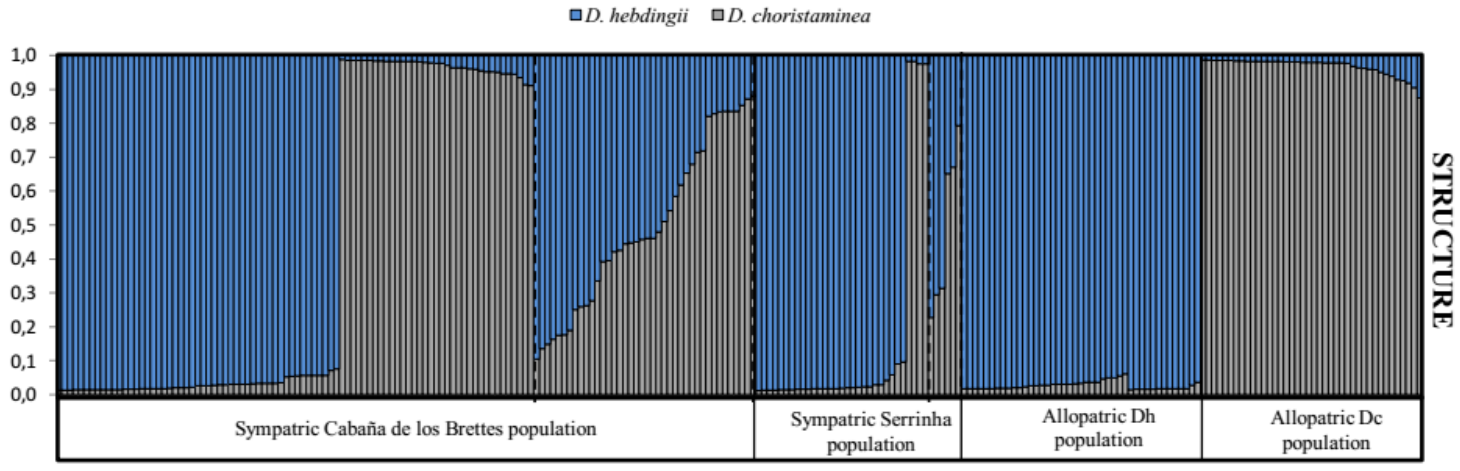


Figure 2. Posterior probabilities (*q*) for sympatric and allopatric populations analyzed together. The proportion of color in each bar represents an individual's assignment probability, which correspond to

D. hebdingii (blue), *D. choristaminea* (gray) and *D. julianae*/hybrids are delimited by dashed line.

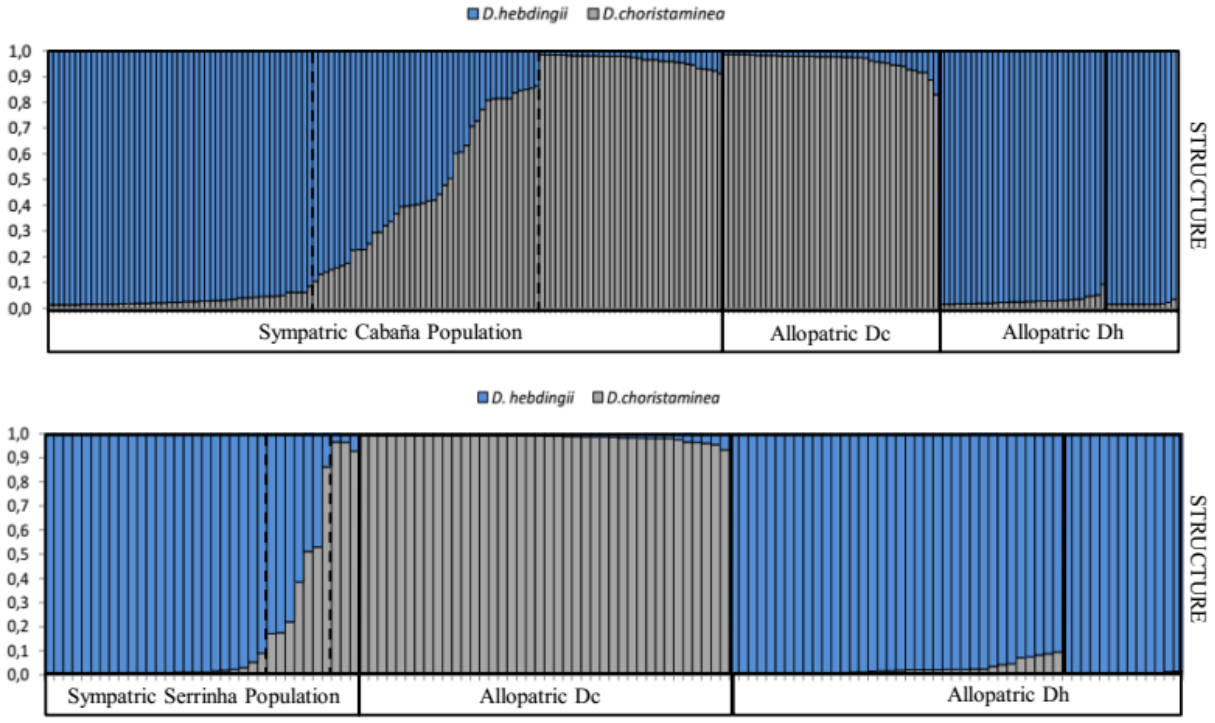


Figure 3. Posterior probabilities (*q*) for Serrinha and Cabaña de los Brettes sympatric populations analyzed with STRUCTURE. Sympatric and allopatric localities are delimited by solid lines. The proportion of color in each bar represents an individual's assignment probability, which correspond to *D. hebdingii* (blue), *D. choristaminea* (gray) and *D. julianae*/hybrids are delimited by dashed line.

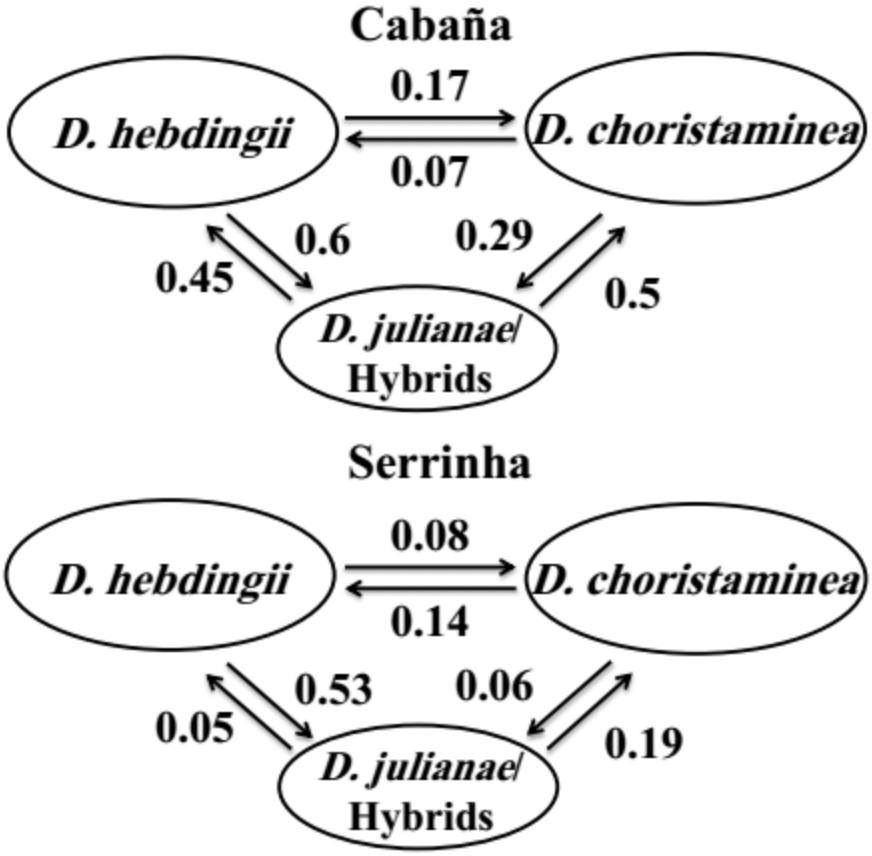


Figure 4. Bidirectional migration rates (effective number of migrants, N_em) among *D. hebdingii*, *D. choristaminea* and *D. julianae*/hybrids from two sympatric populations.

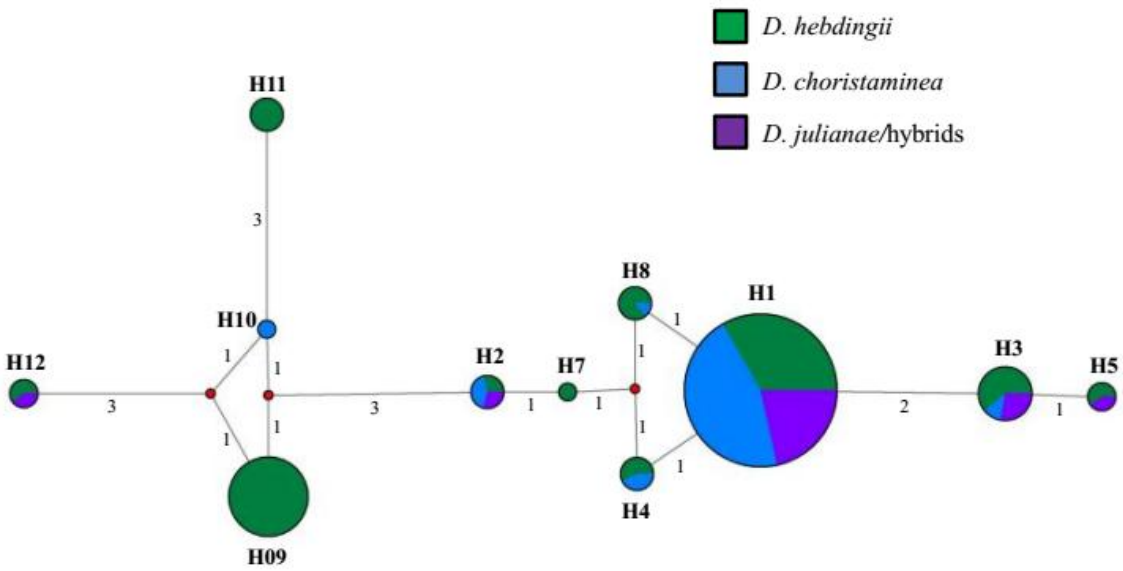
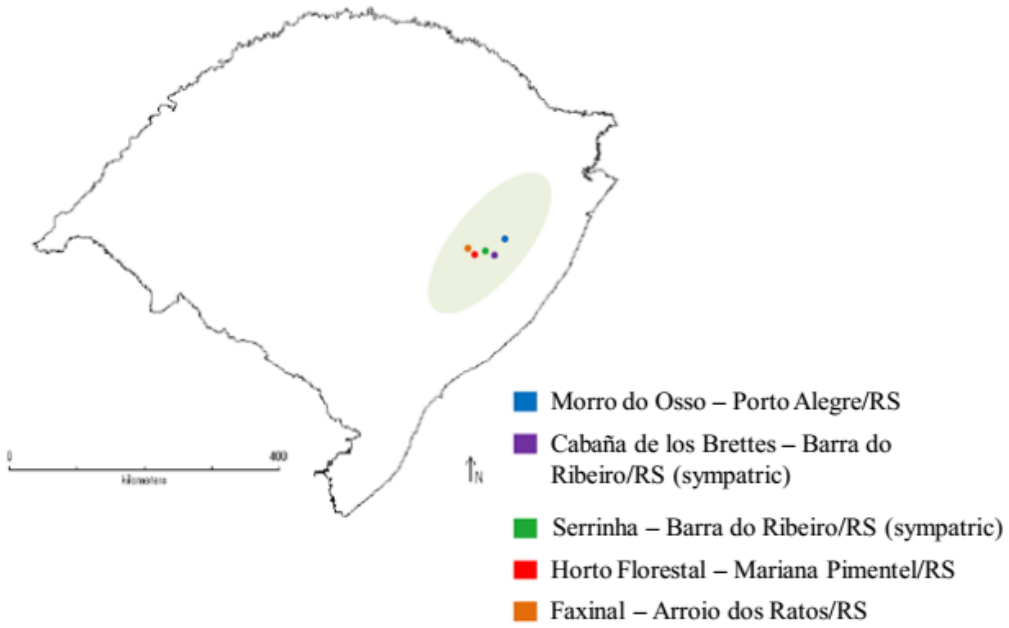
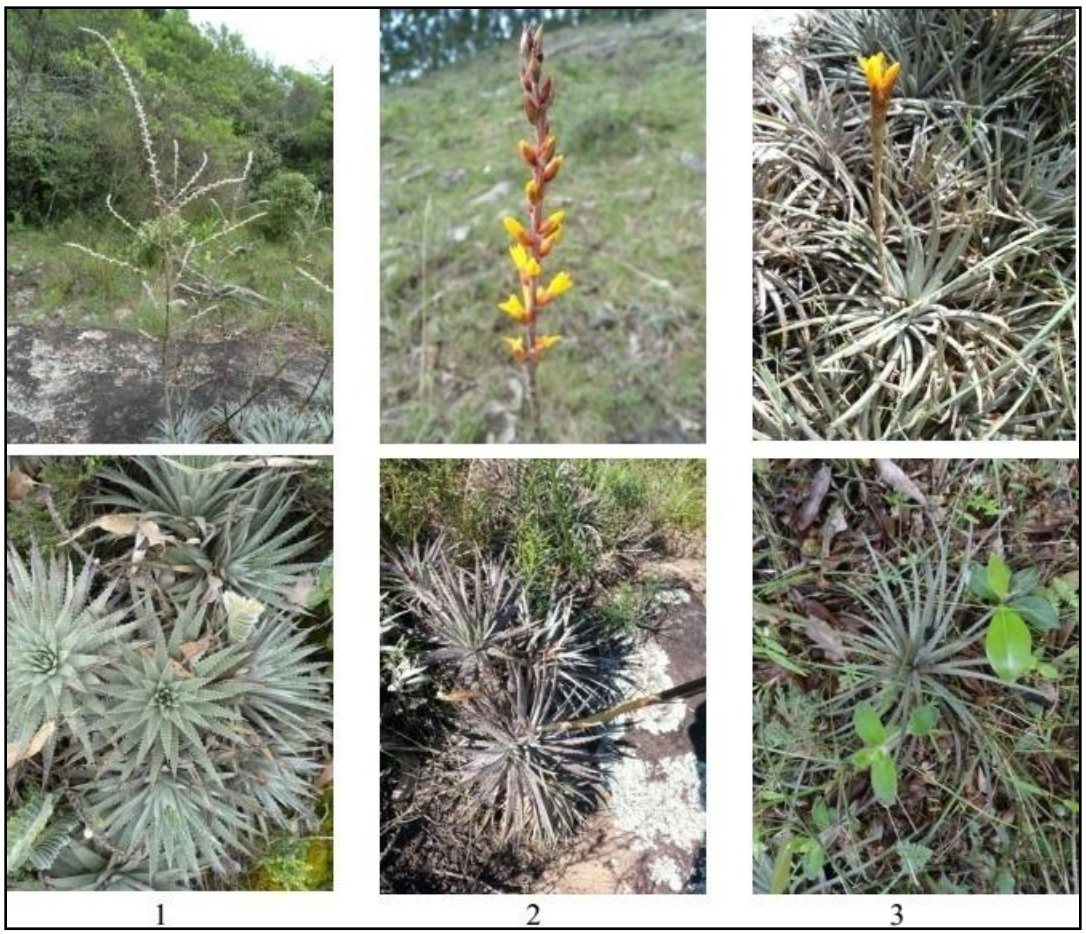


Figure 5. Median-joining network among plastid DNA haplotypes. The haplotype are indicated by circles, the size of each circle being proportional to the observed frequency of each haplotype. The colors indicate the individuals classified as *D. hendingii*, *D. choristaminea* and *D. julianae*/hybrids. Lines draw between haplotypes represent mutation events and the number identify how many mutations were observed.



Suplementary material Figure S1: Map of Rio Grande do Sul federative state, including approximate distribution in gray and localities of the sampled populations.



Suplementary material Figure S3: (1) Morphology of *D. hebdingii*; (2) Morphology of *D. julianae*; (3) Morphology of *D. choristaminea*.

Suplementary material Figure S3. Normal seed (left) and aborted seed (right).

Capítulo 4

Considerações Finais

1. Considerações Finais

A presente dissertação visa contribuir para o conhecimento da biologia e genética de três espécies da família Bromeliaceae, principalmente espécies ameaçadas de extinção do Rio Grande do Sul. Tais informações serão importantes para a melhor compreensão dos mecanismos de especiação, isolamento reprodutivo e coesão de espécies. Essas informações também serão fundamentais para traçar estratégias para conservação das espécies em estudo.

Dyckia hebdingii, *D. choristaminea* e *D. julianae* são espécies em risco de extinção e por esse motivo tornaram-se alvo deste estudo. Além disso, são encontradas em simpatria em algumas regiões e por este motivo são espécies de grande interesse no estudo da evolução das espécies.

Para tentar esclarecer a composição genética dessas três espécies e os padrões de fluxo gênico entre elas, foram realizadas análises de diversidade genética utilizando marcadores microssatélites nucleares e plastidiais. Os resultados sugerem uma possível origem híbrida de *D. julianae*, tendo surgido através do cruzamento entre *D. hebdingii* e *D. choristaminea*. Ainda, os dados nucleares mostraram que atualmente *D. hebdingii* e *D. choristaminea* apresentam alta estruturação genética. Já os dados plastidiais resultaram em 12 haplótipos com baixa diferenciação genética entre as espécies, compartilhando cinco haplótipos entre elas.

Os cruzamentos artificiais geraram poucos frutos e será necessária uma investigação mais profunda da biologia reprodutiva dessas espécies, utilizando um maior número de flores. Entretanto os resultados foram compatíveis com os dados moleculares, apoiando a hipótese de uma origem híbrida para *D. julianae* e alta diferenciação contemporânea entre *D. hebdingii* e *D. choristaminea*.

Capítulo 5

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1. Referências Bibliográficas

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