Universidade Federal do Rio Grande do Sul

Especiação híbrida: citogenética, filogenia e reprodução de três espécies de *Dyckia* (Bromeliaceae)

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Tese submetida ao Programa de Pós-Graduação em Genética e Biologia Molecular da UFRGS como requisito parcial para a obtenção do grau de **Doutor em Genética e Biologia Molecular.**

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Sumário

Resumo

Hibridação é um elemento importante para entender os processos que levam a especiação, principalmente em plantas, uma vez que híbridos naturais são comuns para este grupo. *Dyckia hebdingii*, *D. julianae* e *D. choristaminea* são espécies ameaçadas de extinção, endêmicas dos Campos Sulinos, que ocorrem em simpatria. Considerando que *D. julianae* tem morfologia intermediária entre as outras duas espécies e que estudos prévios, utilizando marcadores microssatélites, também revelaram um perfil molecular intermediário, a especiação híbrida é proposta para a origem dessa espécie. Esta tese contempla três diferentes abordagens utilizadas para discutir a possível origem híbrida de *D. julianae* a partir do cruzamento de *D. hebdingii* e *D. choristaminae*. Primeiramente analisamos estas espécies com uma abordagem citogenética, investigando os números cromossômicos, sua regularidade meiótica, a morfologia e viabilidade do dos grãos de pólen e o tamanho do genoma. Os resultados mostraram que as três espécies são diploides 2n = 50, e com tamanho de genoma bastante similar (2C = ~1,71 pg); todas apresentam alta regularidade meiótica e alta viabilidade polínica. A partir exclusivamente desses dados a hipótese de hibridação ainda não poderia ser confirmada nem refutada, porém indicando um possível híbrido homoploide. Complementarmente, análises moleculares utilizando sequenciamento de regiões plastidiais e nucleares foram realizadas para inferir a herança biparental (nuDNA) e materna (cpDNA) de *D. julianae*. Os resultados indicaram que *D. choristaminea* provavelmente contribuiu maternalmente na origem de *D. julianae*, sendo *D. hebdingii* o provável progenitor paterno. Além disso, os padrões de relacionamento tanto na filogenia nuclear quanto na plastidial estão de acordo com a hipótese inicial de origem hibrida de *D. julianae*, agrupando

essa espécie com *D. hebdingii* e *D. choristaminea*. Por fim, como uma terceira abordagem, foram realizados experimentos de biologia reprodutiva *in situ* para averiguar o sistema de cruzamento, sucesso reprodutivo, e germinação do pólen das três espécies, além de confirmar a compatibilidade de *D. julianae* com as outras duas espécies. Os resultados dessa abordagem indicaram que *D. choristaminea* e *D. julianae* têm um sistema de cruzamento misto, gerando frutos nos tratamentos de polinização cruzada e autopolinização. Curiosamente, *D. hebdingii* só produziu frutos quando submetida à autopolinização. Com o experimento de crescimento de tubo polínico, obtivemos resultados concordantes, no qual *D. hebdingii* apresentou crescimento do tubo somente no tratamento de autopolinização manual, enquanto *D. julianae* e *D. choristaminea* tiveram crescimento do tubo com autopolinização e polinização cruzada. *Dyckia julianae* apresentou o menor sucesso reprodutivo entre as espécies, mas a proporção de sementes viáveis ainda foi menor em *D. choristaminea*. Cruzamentos interespecíficos mostraram compatibilidade entre *D. julianae* e ambas espécies parentais, porém os frutos só foram gerados quando as espécies parentais agiram como doadoras de pólen. Os resultados obtidos através das abordagens utilizadas nessa tese, permitiram propor a origem híbrida de *D. julianae*. Os resultados apresentados não estão de acordo com os padrões esperados para hibridação recente, pelo contrário, indicam que *D. julianae* teria surgido por hibridação, mas se manteve ao longo das gerações como uma espécie independente de *D. hebdingii* e *D. choristaminea*, embora proximamente relacionadas.

Abstract

Hybridization is an important element to understand the processes that lead to speciation, especially in plants, since natural hybrids are common for this group. *Dyckia hebdingii*, *D. julianae* and *D. choristaminea* are endangered species, endemic to Campos sulinos ecozone, which occur in sympatry. *Dyckia julianae* has intermediate morphology between the other two species and the issue of hybridization has been raised. Studies using microsatellite markers have generated results compatible with hybrid speciation. This thesis contemplates three different approaches used to discuss the possible origin of *D. julianae* by hybrid speciation. First, we analyzed these species with a cytogenetic approach, investigating the chromosomal numbers, their meiotic regularity, the morphology and viability of the pollen grains and the size of the genome. The results showed that the three species are diploids 2n = 50, with a very similar genome size (2C = ~1.71 pg); all have high meiotic regularity and high pollen viability. Based exclusively on these data, the hybridization hypothesis could not yet be confirmed or refuted, but indicating a possible homoploid hybrid. Molecular analyzes using sequencing of plastid and nuclear regions were performed to test the hypothesis of hybrid origin of *D. julianae* and to infer the biparental (nuDNA) and maternal (cpDNA) inheritance. The results indicate that *D. choristaminea* probably contributed maternally to the origin of *D. julianae*, with *D. hebdingii* being the probable paternal parent. In addition, the relationship patterns in both nuclear and plastid phylogeny are in accordance with the initial hypothesis of hybrid origin of *D. julianae*, grouping this species with *D. hebdingii* and *D. choristaminea*. Finally, as a third approach, *in situ* reproductive biology experiments were carried out to investigate the mating system, reproductive success, and pollen germination of the three species, in

addition to confirming the compatibility of *D. julianae* with the other two species. The results of this approach indicate that *D. choristaminea* and *D. julianae* have a mixed mating system, generating fruits in cross-pollination and self-pollination treatments. Curiously, *D. hebdingii* only produced fruit when subjected to self-pollination. With the experiment of tube pollinic growth, we obtained concordant results, in which *D. hebdingii* showed tube growth only in the treatment of manual self-pollination, while *D. julianae* and *D. choristaminea* had tube growth with self-pollination and cross-pollination. *Dyckia julianae* had the lowest reproductive success among species, but the proportion of viable seeds was still lower in *D. choristaminea*. Interspecific crosses showed compatibility among *D. julianae* and both parental species, however the fruits were only generated when the parental species acted as pollen donors. The results obtained through the approaches used in this thesis, allowed to propose the hybrid origin of *D. julianae*. The results presented are not in accordance with the patterns expected for recent hybridization, on the contrary, they indicate that *D. julianae* emerged through hybridization, but has remained throughout the generations as an independent species from *D. hebdingii* and *D. choristaminea*, although closely related.

Capítulo 1

Introdução Geral

1. *Introdução*

1.1 Família Bromeliaceae

Os ancestrais das bromélias surgiram no escudo das Guianas há cerca de 100 milhões de anos, mas apenas durante o Mioceno (~20 milhões de anos) após mudanças ambientais associadas a elevação dos Andes, os taxa atuais começaram a se diversificar na América do Sul (Givnish et al., 2007; 2011).

Atualmente, a família Bromeliaceae se distribui amplamente nos Neotrópicos (Smith, 1934; Smith e Downs, 1974), ocorrendo nos Estados da Virgínia, Texas e Califórnia, nos Estados Unidos, até o norte da Patagônia, na Argentina. Essa família é praticamente endêmica das Américas, com apenas uma espécie ocorrendo no Oeste da África, na região da Guiné (Porembsky e Barthlott, 1999), ocupando diferentes ambientes, desde mesofítico a xerofítico, do nível do mar a altas altitudes, podendo ter hábitos terrestre, rupículo ou epifítico. Bromeliaceae é uma das famílias de plantas não-lenhosas com maior riqueza de espécies e reportada como um ótimo exemplo de radiação adaptativa (Benzing, 2000; Givnish et al., 2014). O sucesso evolutivo da família está associado ao surgimento repetido de características-chave, tanto em sistemas fisiológicos (metabolismo Ácido das Crassuláceas - CAM) como na morfologia (presença de tanques de armazenamento de água e de tricomas de absorção de água e nutrientes; Crayn et al., 2004; Silvestro et al., 2014).

Tradicionalmente Bromeliaceae estava dividida em três subfamílias: Pitcairnioideae que agrupava espécies com sementes aladas, Tillandsioideae

com suas sementes com apêndices plumosos e Bromelioideae com frutos tipo baga e sementes com mucilagem (Smith e Downs, 1974, 1977, 1979; Smith e Till, 1998). Bromelioideae e Tillandsioideae são monofiléticas, entretanto Pitcairnioideae se mostrava um grupo parafilético (Gilmartin e Brown, 1987; Clark e Clegg, 1990; Givnish e Smith, 1990, Givnish e Hanm, 1992; Givnish et al., 2007; Ranker et al., 1990). Atualmente Bromeliaceae está dividida em oito subfamílias monofiléticas (Brocchinioideae, Lindmanioideae, Tillandsioideae, Hechtioideae, Navioideae, Pitcairnioideae, Puyoideae e Bromelioideae; Givinish et al., 2007), compreendendo 3630 espécies distribuídas em 77 gêneros (Gouda, Butcher e Gouda, cont. updated). 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).

Bromeliaceae possui espécies de notória importância econômica, como o abacaxi (*Ananas comosus* L. Merr); diversas ornamentais empregadas em paisagismo, além de algumas espécies que são usadas como fonte de fibras ou na medicina popular. As bromélias também desempenham um importante papel ecológico uma vez que através da estrutura de tanque atuam como reservatório de água e abrigo, além de fonte de alimentação através de seus frutos e néctar (Benzing, 2000).

1.2 Gênero *Dyckia*

Dyckia Schult. e Schult.f. é um dos maiores grupos dentro da subfamília Pitcairnioideae, com 176 espécies descritas, a maioria ocorrendo no Brasil

(Gouda, Butcher e Gouda, cont. updated). O Cerrado brasileiro é considerado o centro de diversidade de *Dyckia*, de onde o gênero se dispersou em direção a Mata Atlântica e Caatinga à leste, para o Chaco na Bolívia e Paraguai à oeste e para os Pampas do Uruguai e Argentina, ao sul (Smith e Downs, 1974). A maioria das espécies ocorre em ambientes conhecidos como campos rupestres, nos Estados de Minas Gerais e Bahia (Versieux e Wendt, 2006, 2007; Versieux, 2008). Os ambientes em que tipicamente ocorrem espécies de *Dyckia* são secos e rochosos, como penhascos, encostas e "inselbergs", que geralmente são pobres em nutrientes, com pouca disponibilidade de água e alta exposição solar (Smith e Downs, 1974), embora existam espécies de *Dyckia* em solos arenosos (por exemplo *Dyckia maritima*; Winkler, 1980) e leitos de rios (por exemplo *Dyckia ibiramensis*; Hmeljevski et al., 2011).

Morfologicamente as espécies desse gênero são caracterizadas por ter formato de roseta e não apresentar o tanque de armazenamento de água, estrutura bem característica das bromélias. Suas folhas são coriáceas com espinhos marginais com diferentes níveis de suculência e a inflorescência, simples ou ramificada, se insere lateralmente na roseta, caráter este que diferencia *Dyckia* de *Encholirium*, um gênero proximamente relacionado (Smith e Downs, 1974; Forzza, 2005). As flores podem ser sésseis a pediceladas com pétalas e sépalas tipicamente amarelas, laranjas ou vermelhas (Smith e Downs, 1974), tendo como polinizadores beija-flores, borboletas, abelhas e outros insetos (Bernadello et al., 1991). Os frutos são do tipo capsulado e liberam sementes aladas que não são tão eficientes na dispersão anemocórica por seu tamanho relativamente grande. Todas as espécies de *Dyckia* investigadas até o momento apresentam metabolismo ácido das crassuláceas – CAM (Givnish et al., 2007; Santos-Silva et al., 2013).

Dyckia é conhecido como um gênero com alto grau de plasticidade morfológica intraespecífica (Leme et al., 2012), tornando a delimitação de espécies e reconstrução filogenética um desafio (Versieux e Wendt, 2007; Krapp et al., 2014; Hirsch et al., 2020; Pinangé et al., 2020). Já em 1934, Smith declarou que *Dyckia* é um verdadeiro pesadelo para os sistematas, devido à forma fluida que uma espécie passa a ser outra.

Em *Dyckia* podem ser encontrados desde indivíduos únicos gerados a partir da dispersão de sementes até densos aglomerados resultantes de propagação clonal (Smith e Downs, 1974). As espécies de *Dyckia* exibem propagação vegetativa por meio de rizomas subterrâneos curtos ou longos (Givnish et al., 2007), sendo a propagação clonal considerada uma estratégia vantajosa para ocupação de novos habitats (Lenzi e Orth, 2012). Algumas espécies podem se reproduzir preferencialmente por propagação vegetativa, enquanto que outras não se utilizam dessa estratégia (Reis et al., 2005; Zanella et al., 2012; Lenzi e Paggi, 2020). Muitas espécies possuem os dois modos de reprodução: sexual e assexual. Por exemplo *D. tuberosa* (Vell.) Beer e *D. brevifolia* Baker, a primeira apresentando reprodução basicamente por propagação clonal e a outra por polinização cruzada ou autopolinização (Vosgueritchian e Buzato, 2006; Rogalski et al., 2009; 2017). *Dyckia* pode apresentar reprodução sexuada via polinização cruzada, autopolinização ou sistemas mistos, nos quais ambas as estratégias podem ser utilizadas (Vosgueritchian e Buzato, 2006; Rogalski et al., 2009; 2017; Hmeljevski et al., 2011; Pinangé et al., 2020).

Dyckia hebdingii Smith, *D. choristaminea* Smith e *D. julianae* Strehl são espécies que podem ocorrer em simpatria, saxícolas e endêmicas de afloramentos rochosos da ecoregião Campos Sulinos. *Dyckia hebdingii* é uma

espécie com inflorescência ramificada e pode atingir 1 m de altura, as sementes possuem uma asa estreita apical e 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. Entretanto, 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". Além disso, em 2014 a "Fundação Zoobotânica do Rio Grande do Sul" (FZB/RS) tornou pública uma 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" (Decreto Nº 52.109).

1.3 Hibridação e Especiação

Hibridação é o cruzamento entre grupos ou taxa geneticamente distintos, levando à produção de híbridos viáveis (Mallet, 2005). Esse processo deve ocorrer em grande parte das especiações, com exceção de casos onde ocorre especiação alopátrica ou instantânea (Abbott et al., 2013). Estima-se

que 30 - 70% das plantas com flores experimentaram hibridação em algum momento de suas histórias evolutivas (Rieseberg, 1995; Soltis e Soltis, 2009).

A hibridação em plantas tem sido vista como uma força evolutiva, capaz de gerar novidades pela recombinação de dois genomas distintos. Quando o principal fator no surgimento da nova linhagem é o cruzamento de espécies diferentes, temos, então, um caso de especiação híbrida. (Mallet, 2007). Outra possibilidade é o aparecimento de indivíduos híbridos que se retrocruzam com um, ou mesmo ambos, parentais. Esse processo é conhecido como introgressão e, embora não resulte em uma nova linhagem permanente, permite a transferência de material genético de uma espécie para a outra (Folk et al., 2018). Esses eventos são responsáveis por gerar variação, transferir adaptações, eliminar ou mesmo reforçar as barreiras reprodutivas entre espécies relacionadas, podendo ou não levar ao surgimento de uma nova espécie (Soltis et al., 2003; Slotte et al., 2008).

A hibridação pode seguir uma rota homoploide ou alopoliploide. A primeira ocorre quando as espécies parentais têm o mesmo número de cromossomos e a prole híbrida mantem o mesmo nível de ploidia. Os híbridos homoploides, muitas vezes possuem problemas de fertilidade devido à formação de gametas desbalanceados, havendo a manutenção dos indivíduos por propagação clonal (Soltis e Soltis, 2000).

Na alopoliploidia, as espécies parentais podem ter o mesmo número cromossômico ou números cromossômicos diferentes, porém, neste caso o híbrido gerado passará por um evento de duplicação de seu genoma, geralmente devido à formação de gametas não reduzidos. Com isso, o híbrido alopoliploide poderá produzir gametas balanceados e viáveis (Leitch e Bennett, 1997; Soltis e Soltis, 2000). A poliploidia isola o híbrido dos parentais de

imediato, possibilitando o estabelecimento da nova linhagem. Esse isolamento imediato não ocorre na hibridação homoploide e a nova linhagem depende de outros tipos de isolamento para evitar a homogeneização, se tornando com o tempo indistinguível, genética e fenotipicamente, dos parentais (Rieseberg, 1997; Buerkle et al., 2000; Gross e Rieseberg, 2005; Abbott et al., 2010).

Em muitos trabalhos a hibridação é estudada a fim de se compreender o isolamento reprodutivo das espécies, entretanto existe um número crescente de estudos discutindo o papel da hibridação na diversificação de espécies ou populações (Hersch e Roy, 2007). Como hibridação é um processo relativamente comum em plantas, é importante diferenciar entre taxa reprodutivamente isolados que foram originados por hibridação (espécies hibridas) e híbridos que surgem continuamente (Rieseberg, 1997; Hegarty e Hiscock, 2004). Embora não haja dúvidas de que existam espécies híbridas, é importante avaliar diversos caracteres (morfológicos, ecológicos, reprodutivos e genéticos) dos híbridos, a fim de estabelecer uma delimitação taxonômica clara para a sua descrição como uma nova espécie (Marczewski et al., 2016).

1.4 Citogenética

Análises cariológicas constituem uma valiosa estratégia para investigar questões evolutivas (Gitaí et al., 2014). A comparação do número cromossômico de diferentes espécies de um determinado táxon possibilita a inferência de seu número cromossômico ancestral, definindo as possíveis linhagens cromossômicas derivadas durante a história evolutiva do grupo e ainda correlacionar essas diferentes linhagens com determinados grupos taxonômicos (Sybenga, 1992).

A meiose é o caminho natural para a formação dos gametas sexuais sendo também uma fonte de variabilidade genética (Fachinetto e Tedesco, 2009). Erros meióticos podem ocorrer por mutações em genes que controlam a gametogênese ou como resultado de efeitos ambientais. (Karsburg e Battistin, 2006; Belo et al., 2018). A análise do comportamento meiótico em indivíduos híbridos é especialmente importante, tendo em vista a presença do genoma de duas espécies distintas. Uma baixa homologia entre os genomas das duas espécies pode resultar em problemas no pareamento cromossômico e na segregação, gerando tétrades anormais e grãos de pólen com baixa viabilidade. Portanto, a fertilidade do híbrido pode ser avaliada com base na regularidade do processo meiótico (Granato, 2010). A viabilidade do pólen produzido pelas espécies é um parâmetro importante na avaliação do sucesso reprodutivo (Dafni e Firmage, 2000). Se os cromossomos têm comportamento irregular durante a meiose, uma baixa proporção de pólen inviável é esperado, embora outras causas possam resultar em baixa viabilidade, incluindo a idade do pólen, problemas estruturais no desenvolvimento da antera e a exposição a fatores ambientais (Stone et al., 1995; Kelly et al., 2002; Palma-Silva et al., 2008; Mendes et al., 2016). Estudos de comportamento meiótico foram realizados em Bromeliaceae, por exemplo *Aechmea* (Palma-Silva et al., 2004), *Vriesea* (Palma-Silva et al., 2008) e *Orthophytum* (Louzada et al., 2010), todos revelando meioses regulares e alta viabilidade do pólen.

Os cromossomos de Bromeliaceae costumam ser pequenos, variando de 0,21 a 2,72 µm (Zanella et al., 2012), tornando as técnicas de citogenética desafiadoras e os trabalhos para a família com essa abordagem ainda escassos (Gitaí et al., 2014). O número básico para Bromeliaceae é x=25 sendo a maior parte das espécies diploides com 2n=50. (Marchant, 1967; Brown e Gilmartin, 1986, 1989). Embora haja pouca variação quanto ao número

cromossômico dentre as espécies, a evolução de Bromeliaceae tem sido caracterizada por múltiplos eventos de hibridação, poliploidização e disploidia (Marchant, 1967; Brown e Gilmartin, 1986; Gitaí et al., 2005).

A citometria de fluxo é uma metodologia que vem sendo largamente empregada para estimar o tamanho do genoma (conteúdo de DNA) e avaliar o nível de ploidia, uma vez que gera informações de forma rápida e precisa (Doležel et al., 2007). A combinação de dados de número cromossômico e de tamanho do genoma pode trazer informações valiosas para um melhor entendimento da evolução dos genomas (Silva et al., 2016). A estimativa do conteúdo de DNA tem sido utilizada como uma ferramenta importante em estudos de biossistemática, podendo prover informações úteis com propósitos filogenéticos e taxonômicos, provando ser útil na delimitação e/ou diferenciação de espécies (Tacuatiá et al., 2012). De acordo com as estimativas de tamanho de genoma disponíveis para Bromeliaceae, o valor 2C varia de 0.60 a 3.34 pg enquanto para *Dyckia* o conteúdo 2C varia de 1.58 a 1.60 pg (Leitch et al., 2019). De acordo com Moura et al., (2018), das 176 espécies pertencentes a *Dyckia*, apenas 19 tiveram o tamanho de seus genomas investigados.

1.5 Filogenética e Biologia Reprodutiva

A observação de morfologia intermediária de certos indivíduos em relação a duas espécies estabelecidas, muitas vezes leva à suspeita de hibridação. Apesar disso, sabe-se que a identificação e confirmação de status híbrido, bem como a ocorrência de introgressão, não podem ser fundamentados somente em caracteres morfológicos, tendo em vista que

muitos taxa apresentam uma ampla plasticidade fenotípica (Rieseberg et al., 1993; Harding e Millam, 2000). Análises moleculares têm se mostrado úteis na identificação da hibridação e introgressão, assim como para investigar espécies de origem híbrida (Fogelqvist et al., 2015).

Determinadas técnicas moleculares permitem avaliar a arquitetura genética dos indivíduos híbridos (Deacon et al., 2017). O modo de herança do DNA permite reconstruir filogenias e discutir sobre genomas híbridos. Acessando os genomas nucleares (herança biparental) e plastidiais (herança uniparental) é possível identificar a direção da hibridação, assim como a espécie materna e paterna da progênie híbrida (Isoda et al., 2000; Hersch-Green et al., 2014). Regiões plastidiais podem ser derivadas de uma única espécie parental (Hamzeh et al., 2007; Hamilton and Aitken, 2013), ou então, de ambas (Hersch-Green et al., 2014; Floate et al., 2016). Como as regiões nucleares são herdadas de maneira biparental, híbridos F_1 (primeira geração) devem possuir dois alelos parentais em casa sítio. Quando o híbrido não é recente a evidencia molecular se torna menos elucidativa. Neste caso, há três cenários possíveis: 1) os indivíduos analisados podem ter os dois alelos de cada parental distinguíveis devido à pouca conversão gênica (Campbell et al., 1997); 2) apenas um alelo parental é mantido, devido a conversão gênica enviesada (Wendel et al., 1995); 3) nenhum alelo parental é distinguível, no caso de extensa recombinação e divergência.

Muitos estudos moleculares têm tentado elucidar as relações filogenéticas dentro da família Bromeliaceae (Horres et al., 2000, 2007; Givnish et al., 2004, 2007; Barfuss et al., 2005), entretanto, pouco se sabe sobre as relações infra genéricas, fluxo gênico e mecanismos de especiação de *Dyckia* (Pinangé et al., 2020).

Hirsch et al. (2020), relataram que *D. julianae* apresenta morfologia e perfil molecular intermediários entre *D. hebdingii* e *D. choristaminea* propondo uma origem híbrida de *D. julianae.* Os resultados indicam uma hibridação antiga entre as duas espécies, uma vez que *D. hebdingii* e *D. choristaminea* parecem não ter fluxo gênico contemporâneo. Verificar a origem híbrida de uma taxa e quais são as espécies parentais envolvidas na hibridação, são questões importantes para estudos na área de taxonomia, evolução e conservação.

Em plantas, a formação de híbridos F_1 depende, primordialmente, que o pólen de uma espécie seja transferido para o estigma de outra espécie. Para espécies que são polinizadas por animais, a hibridação é influenciada pelo comportamento do polinizador (Campbell et al., 1997; Wesselingh e Arnold, 2000). Um polinizador que tem preferência por certa espécie e dispensa as flores das demais espécies promove uma polinização assertiva, enquanto que um polinizador generalista movendo-se randomicamente entre diferentes espécies promove a troca de pólen interespecífico, podendo ter como consequência a formação de híbridos (Wesselingh e Arnold, 2000).

Em populações simpátricas, o movimento do polinizador entre diferentes espécies pode estar ligado às características morfológicas e ecológicas das plantas. Entretanto, tem sido documentado que espécies simpátricas filogeneticamente próximas, inclusive em Bromeliaceae, possuem morfologia floral e síndrome de polinização semelhantes, com sobreposição do período de floração e compartilhamento de polinizadores (García-Franco et al., 2001; Field et al., 2008; Zanella et al., 2016; Aguilar-Rodríguez et al., 2019), favorecendo a hibridação.

Estudos quanto à variação dos traços reprodutivos dentro das espécies podem contribuir grandemente na compreensão da evolução e da radiação de

grupos recentes dos Neotrópicos (Barret et al., 2008; Levin, 2012; Paggi et al., 2015). Alguns estudos de biologia reprodutiva em Bromeliaceae mostraram que as estratégias de cruzamento podem variar entre espécies do mesmo gênero e entre as subfamílias (Wendt et al., 2001, 2002, 2008; Cascante-Marín et al., 2005; Barbará et al., 2008, 2009). Muitas características associadas à fertilidade da planta têm sido usadas para determinar a viabilidade das populações, como o tamanho das plantas/inflorescência, produção de flores, produção de frutos e sementes, padrões de produção de frutos, limitações do pólen e viabilidade das sementes (McIntosh, 2002; Burne et al., 2003; Kéry e Matthies, 2004; Sampaio et al., 2012; Paggi et al., 2015).

A família Bromeliaceae é um modelo interessante para o estudo de hibridação e manutenção das espécies, por ser uma família que sofreu uma radiação adaptativa recente. Considerando que as espécies de *Dyckia* são proximamente relacionadas (Krapp et al., 2014) torna-se de grande interesse evolutivo definir a origem e composição genética dos híbridos e sua viabilidade, além de verificar quais são as barreiras reprodutivas entre *D. hebdingii*, *D. choristaminea* e *D. julianae* e seu papel no processo de especiação dessas entidades bem definidas. Além disso, visto que as três espécies estão ameaçadas de extinção e têm distribuição restrita é de extrema importância levantar informações acerca da especiação, reprodução e barreiras de isolamento entre elas.

Capítulo 2

Objetivos

1. Objetivos

Geral

Esta tese tem como objetivo geral testar a hipótese de origem híbrida de *D. julianae,* avaliando seu posicionamento filogenético, além de suas relações com as espécies consideradas putativos parentais (*D. hebdingii* e *D. choristaminea*). Além disso, investigar aspectos citogenéticos, da biologia reprodutiva e dos mecanismos de isolamento reprodutivo das espécies, confrontando com a hipótese de hibridação.

Objetivos Específicos

Para confirmar ou refutar a origem híbrida de *D. julianae* essa tese tem como objetivos específicos:

a) Determinar o posicionamento das três espécies em uma árvore filogenética de espécies de *Dyckia*;

b) Determinar as espécies parentais de *D. julianae*;

c) Determinar se *D. julianae* sofreu especiação homoploide ou alopoliploide;

d) Analisar a viabilidade das três espécies;

f) Determinar o sistema de cruzamento das três espécies;

h) Determinar o sucesso reprodutivo das três espécies;

i) Determinar a compatibilidade interespecífica das três espécies.

Capítulo 3

ARTIGO PUBLICADO NA REVISTA TAXON

IAPT chromosome data 31/9

Cuscuta sect. *Indecorae* has such large chromosomes. Most species in *Cuscuta* subg. *Grammica* have either $2n = 30$ or $2n = 60$ chromosomes, but we report for the first time $2n = 150$ (decaploid) chromosome number for the Hawaiian endemic *C. sandwichiana* (Fig. 17D), a species of probable allopolyploid origin (Stefanović & Costea, 2008; García & al., 2014). This finding is remarkable because the highest known chromosome number for the genus was $2n = 60$.

Hybridization and polyploidy have likely played a significant role in *Cuscuta* species diversification (Stefanović & Costea, 2008; Costea & Stefanović, 2010; García & al., 2014, 2018). The enormous variation in chromosome type, number, size, and DNA amounts, along with a well-resolved phylogeny at multiple levels, makes *Cuscuta* a great model to study genome and chromosome evolution in plants generally, and the transition from monocentric to holocentric chromosomes in particular.

LITERATURE CITED

Aryavand, A. 1987. The chromosome numbers of some *Cuscuta* L. (Cuscutaceae) species from Isfahan, Iran. *Iran. J. Bot.* 3: 177–182.

Costea, M. & Stefanović, S. 2009. *Cuscuta jepsonii* (Convolvulaceae): An invasive weed or an extinct endemic? *Amer. J. Bot.* 96: 1744–1750. https://doi.org/10.3732/ajb.0800425

Costea, M. & Stefanović, S. 2010. Evolutionary history and taxonomy of the *Cuscuta umbellata* complex (Convolvulaceae): Evidence of extensive hybridization from discordant nuclear and plastid phylogenies. *Taxon* 59: 1783–1800. http://www.jstor.org/stable/41059873

Costea, M. & Tardif, F.J. 2006. Biology of Canadian weeds: *Cuscuta campestris* Yuncker, *C. gronovii* Willd. ex Schult., *C. umbrosa* Beyr. ex Hook., *C. epithymum* (L.) L. and *C. epilinum* Weihe. *Canad. J. Pl. Sci.* 86: 293–316. https://doi.org/10.4141/P04-077

Costea, M., García, M.A. & S. Stefanović, S. 2015. A phylogenetically based infrageneric classification of the parasitic plant genus *Cuscuta* (dodders, Convolvulaceae). *Syst. Bot.* 40: 269–285. https://doi.org/10.1600/036364415X686567

Fogelberg, S.O. 1938. The cytology of *Cuscuta*. *Bull. Torrey Bot. Club* 65: 631–645.

García, M.A. 2001. A new western Mediterranean species of *Cuscuta* (Convolvulaceae) confirms the presence of holocentric chromosomes in subgenus *Cuscuta*. *Bot. J. Linn. Soc.* 135: 169–178. https://doi.org/10.1111/j.1095-8339.2001.tb01089.x

García, M.A. & Castroviejo, S. 2003. Estudios citotaxonómicos en las especies ibéricas del género *Cuscuta* (Convolvulaceae). *Anales Jard. Bot. Madrid* 60: 33–44.

García, M.A., Costea, M., Kuzmina, M. & Stefanović, S. 2014. Phylogeny, character evolution and biogeography of *Cuscuta* (dodders; Convolvulaceae) inferred from coding plastid and nuclear sequences. *Amer. J. Bot.* 101: 670–690. https://doi.org/10.3732/ajb.1300449

García, M.A., Stefanović, S., Weiner, C., Olszewski, M. & Costea, M. 2018. Cladogenesis and reticulation in *Cuscuta* sect. *Denticulatae* (Convolvulaceae). *Organisms Diversity Evol.* 18: 383–398. https://doi.org/10.1007/s13127-018-0383-5

McNeal J., Arumugunathan, K., Kuehl, J., Boore, J. & dePamphilis, C. 2007. Systematics and plastid genome evolution of the cryptically photosynthetic parasitic plant genus *Cuscuta* (Convolvulaceae). *B. M. C. Biol.* 5: 55. https://doi.org/10.1186/1741-7007-5-55

Pazy, B. & Plitmann, U. 1994. Holocentric chromosome behaviour in *Cuscuta* (Cuscutaceae). *Pl. Syst. Evol.* 191: 105–109. https://doi.org/10.1007/BF00985345

Pazy, B. & Plitmann, U. 1995. Chromosome divergence in the genus *Cuscuta* and its systematic implications. *Caryologia* 48: 173–180. https://doi.org/10.1080/00087114.1995.10797327

Stefanović, S. & Costea, M. 2008. Reticulate evolution in the parasitic genus *Cuscuta* (Convolvulaceae): Over and over again. *Botany* 86: 791–808. https://doi.org/10.1139/B08-033

IAPT chromosome data 31/9

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* First chromosome count for the taxon.

BROMELIACEAE

Subfamily Pitcairnioideae

**Dyckia choristaminea* Mez

$2n = 50$, CHN. Brazil, Rio Grande do Sul, Barra do Ribeiro, 30°18′52″S, 51°30′14″W, 228 m, 26 Oct 2017, *L.D. Hirsch s.n.* (ICN) [Fig. 19].

**Dyckia hebdingii* L.B.Sm.

$2n = 50$, CHN. Brazil, Rio Grande do Sul, Barra do Ribeiro, 30°18′52″S, 51°30′21″W, 239 m, 07 Oct 2015, *C.J. Breitsameter, L.D. Hirsch, F. Bered & C. Aguiar-Melo s.n.* (ICN) [Fig. 20].

**Dyckia julianae* Strehl

$2n = 50$, CHN. Brazil, Rio Grande do Sul, Barra do Ribeiro, 30°18′52″S, 51°30′14″W, 228 m, 20 Oct 2016, *L.D. Hirsch s.n.* (ICN) [Fig. 21].

Bromeliaceae is one of the most diverse families of the Neotropics (Benzing, 2000; Martinelli & al., 2008; Givnish & al., 2011), comprising approximately 75 genera and 3552 species (Gouda & al., 2018). *Dyckia* Schult. & Schult.f. is a large genus of the Pitcairnioideae subfamily (Givnish & al., 2007; Krapp & al., 2014), with 171 species (Gouda & al., 2018). Twenty-eight species of *Dyckia* have been described for the southernmost portion of Brazil, of which several are endemics (Strehl, 2004). *Dyckia choristaminea*, *D. hebdingii* and *D. julianae* are endemic to southern Brazil with restricted distribution, often occurring in sympatry (Smith & Downs, 1974; Strehl, 2004). Hirsch & al. (2019), analyzing sympatric populations of these three species through SSR markers, found an intermediate molecular profile for *D. julianae* when compared to the other two species. Such data strongly suggests a hybrid origin for *D. julianae* resulting from an ancient crossing between *D. hebdingii* and *D. choristaminea*. To better understand this issue, other approaches have to

be used, including cytogenetic analysis. In Bromeliaceae, cytogenetic studies have so far been carried out for approximately 10% of the species. The basic chromosome number of this family is $x = 25$ (Marchant, 1967), with prevalence of diploid species. Chromosome counts for *Dyckia* are reported for only 14 species (Rice & al., 2015), most of them presenting $2n = 50$. Considering the three species investigated in the present study, no previous data have been found. Determination of chromosome number/ploidy level and evaluation of meiotic stability are especially important when hybrid status is investigated, since homoploid or allopolyploid pathways give rise to individuals with different ploidy levels and irregular meiosis is expected.

Aiming to shed some light on the occurrence of hybridization events among these three *Dyckia* species, a cytogenetic characterization was carried out. Therefore, the goals of the present study were: (1) to determine chromosome numbers; (2) to analyze the meiotic behavior and pollen viability, and (3) to estimate the genome size of the three studied species.

The chromosome number and meiotic behavior were analyzed by young inflorescences previously fixed (3 : 1 ethanol : acetic acid). Anthers were squashed in 1% propionic carmine for slides preparation. Chromosome counts were performed in diakinesis. All available phases of meiosis I and II were analyzed for meiotic stability. Meiotic indexes were calculated from 200 tetrads per plant using the formula: MI = (number of normal tetrads / total of tetrads) × 100. Pollen viability estimation and pollen morphology evaluation were carried out by Alexander method (Alexander, 1980). Categorization of pollen morphology followed the classification of Erdtman (1971). For genome size analysis, approximately 25 mg of leaf tissue from standard (*Zea mays* L. 'CE-777' 2C = 5.43 pg; Doležel & al., 2007) and from each species was chopped in 1 ml of cold nuclear-isolation buffer LB01. The suspension was filtered through a 40 µm mesh nylon filter, and nuclei were stained with 50 µl propidium iodide. The DNA content of 10,000 stained nuclei for each sample was estimated using a FACSAria II (Becton Dickinson, Franklin Lakes, New Jersey, U.S.A.) flow cytometer.

Cytogenetic data obtained for the three sympatric species of *Dyckia* are presented in Table 4 and discussed taking into account the recent study of our team (Hirsch & al., 2019) involving molecular markers and hybridization of these species.

All species, *Dyckia choristaminea*, *D. hebdingii* and *D. julianae* have their chromosome number determined for the first time, all being diploids with $2n = 2x = 50$ (Figs. 19A, 20A, 21A). Only three tetraploid species (*D. argentea*, *D. lorentziana*, *D. remotiflora*) have been reported for this genus (Sharma & Ghosh, 1971; Baracho & Guerra, 2000; Cotias-de-Oliveira & al., 2004). The chromosomes are small, as generally found in bromeliads, and similar in size among the three species.

The genome sizes for the three species were very similar, around 1.7 pg (Table 4), corroborating the same ploidy level of them. DNA content is available only for *D. choristaminea*; Moura & al. (2018) reported 2C = 1.78 pg for this species, therefore an equivalent result. Bromeliaceae species present a narrow range for DNA content, with

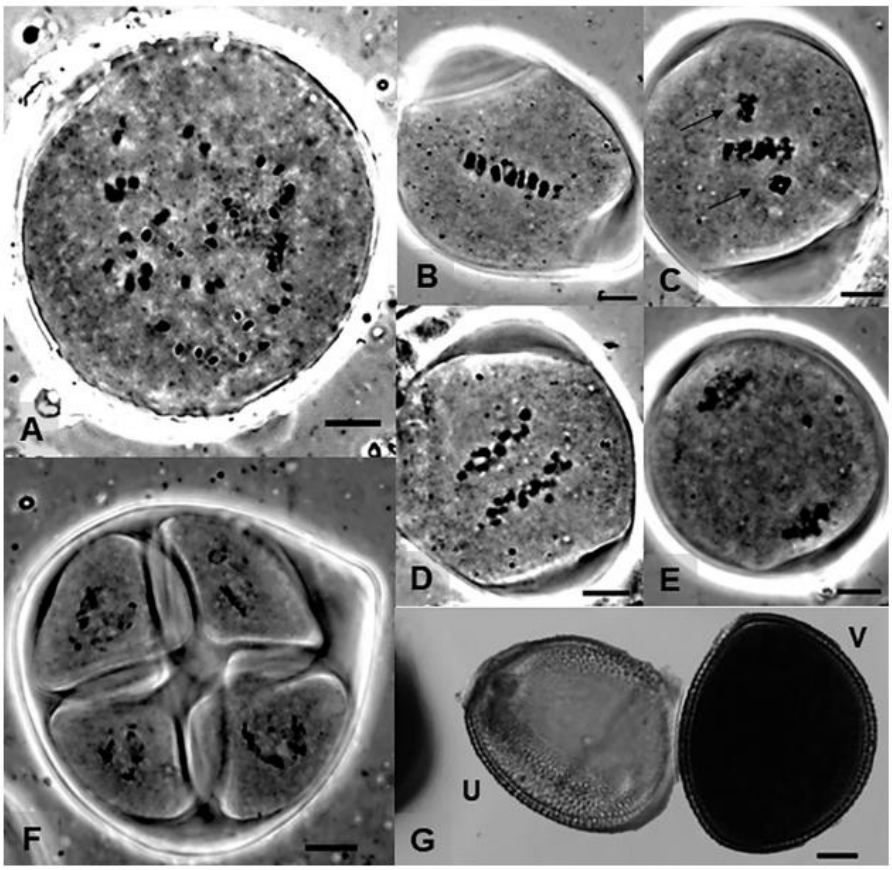

Fig. 19. Cytogenetic analysis of *D. choristaminea*. **A,** Diakinesis with 25 bivalents; **B,** Metaphase I; **C,** Metaphase I with non-oriented chromosomes (arrows); **D,** Anaphase I; **E,** Telophase I; **F,** Tetrade; **G,** Viable pollen (V) and unviable (U) pollen grains. — Scale bar = 5 µm.

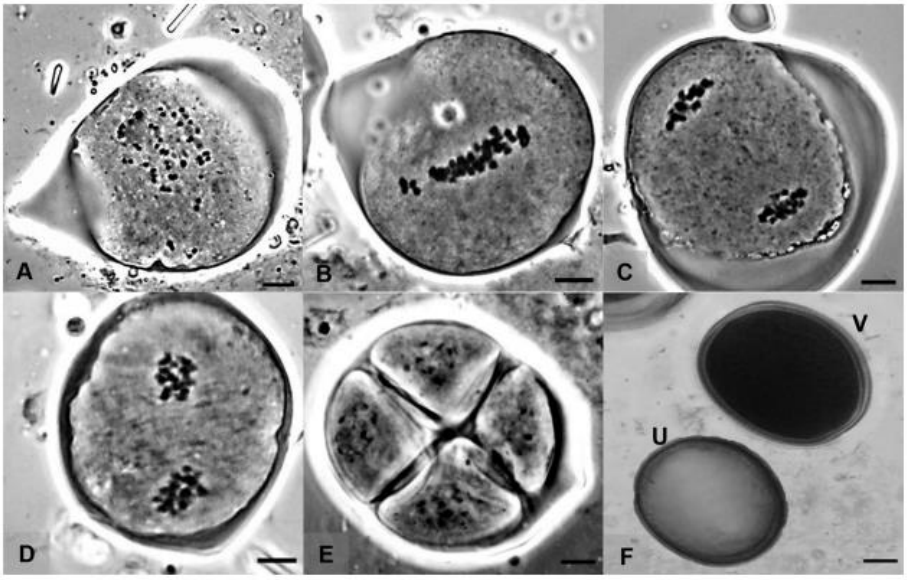

Fig. 20. Cytogenetic analysis of *D. hebdingii*. **A,** Diakinesis with 25 bivalents; **B,** Metaphase I; **C,** Anaphase I; **D,** Telophase I; **E,** Tetrade; **F**, Viable pollen (V) and unviable (U) pollen grains. — Scale bar = 5 µm.

2C values between 0.6 and 2.52 pg (Angiosperm DNA C-values Database; Bennett & Leitch, 2012).

Concerning meiotic behavior, the three species showed high stability with bivalents pairing and regular segregation at anaphase I and II. Elevated meiotic indexes (MI) and pollen viability for all species reinforce the regular meiotic behavior. The lowest viability (92.64%) was observed in *D. hebdingii*. The pollen morphology was subprolate for all species. Of the species studied, *D. choristaminea* showed the largest pollen, whereas *D. hebdingii* had the smallest ones (Table 4).

As mentioned above, Hirsch & al. (2019) showed that *D. julianae* has an intermediate molecular profile when compared to *D. hebdingii* and *D. choristaminea* (both with pure molecular profiles), raising questions about the origin of this pattern. One of the possibilities involves hybridization. *Dyckia julianae* could be a contemporary hybrid between the other two species or a newly discovered established taxon originated by hybrid speciation. Interspecific crosses can produce two kinds of hybrids: homoploids and allopolyploids. Depending on the hybrid status of *D. julianae*, cytogenetics results can result in interesting hints to clarify these evolutionary pathways.

In this study, we observed that *D. julianae* has the same chromosome number as *D. hebdingii* and *D. choristaminea* ($2n = 50$). The same chromosome number by itself does neither confirm nor discard the possibility of hybridization. Nevertheless, in this case, allopolyploidy is refuted. Possibly, we are facing homoploid hybridization, a situation in which parental species and hybrid have the same chromosome number. Despite a homoploid hybridization scenario, surprisingly, *D. julianae* has a regular meiosis and produces highly fertile pollen grains. Although the three species have the same chromosomal number, depending on the similarity of the parental species, an instable meiosis is expected, considering the inability of the chromosome sets to pair and segregate adequately (Ramsey & Schemske, 1998; Rounsaville & al., 2011). Although the present study clearly shows that *D. julianae* is not an allopolyploid, more than one hypothesis can be raised to explain its origin: (1) *Dyckia julianae* may have had a hybrid origin in sufficient time to overcome compatibility problems and present dissomic behavior; (2) *Dyckia hebdingii* and *D. choristaminea* could have very similar and compatible chromosomes, reducing errors at meiosis, once they are closely related (Krapp & al., 2014) species and occur in sympatry (Strehl, 2004); (3) The intermediate molecular profile obtained for *D. julianae* may be due to a reason other than hybridization, such as retention of ancestral polymorphism. To elucidate these questions and better understand the genome contribution of each parental species in the formation of this taxon, other analyzes are necessary, such as *in situ* hybridization (FISH and GISH).

Table 4. Cytogenetic analyzis.

Species	Chromo-some number *n*	Meiosis I and II N	Meiosis I and II %	Meiotic index %	Pollen viability N	Pollen viability %	Pollengrains N	P (µm) Mean	E (µm) Mean	P/E Mean	Pollen morphology	Genome size 2C (pg)
DC	25	11 (2053)	99.90	100	10 (5078)	98.88	10 (200)	30.43	24.04	1.27	Subprolate	1.67
DH	25	23 (1780)	99.89	100	10 (5109)	92.64	10 (200)	26.70	21.75	1.23	Subprolate	1.72
DJ	25	19 (2105)	99.95	100	10 (5542)	93.28	10 (200)	28.93	23.68	1.22	Subprolate	1.75

DC = *D. choristaminea*; DH = *D. hebdingii*; DJ = *D. julianae*
N, Number of individuals (number of analyzed cells); P, Polar diameter; E, Equatorial diameter

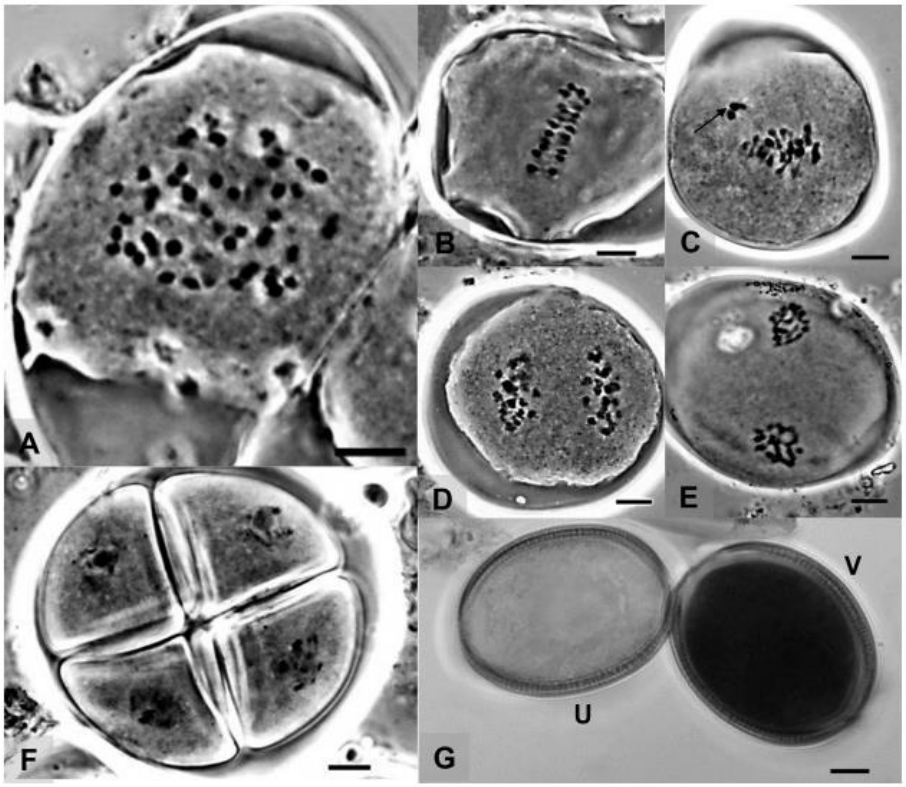

Fig. 21. Cytogenetic analysis of *D. julianae*. **A,** Diakinesis with 25 bivalents; **B,** Metaphase I; **C,** Metaphase I with non-oriented bivalents (arrow); **D,** Anaphase I; **E,** Telophase I; **F,** Tetrade; **G,** Viable pollen (V) and unviable (U) pollen grains. — Scale bar = 5 μm.

LITERATURE CITED

Alexander, M.P. 1980. A versatile stain for pollen fungi, yeast and bacteria. *Stain Technol*. 55: 13–18. https://doi.org/10.3109/10520298009067890

Baracho, G.S. & Guerra, M. 2000. Números cromossômicos em espécies brasileiras de Bromeliaceae. P. 317 in: *Anais do 51° Congresso Nacional de Botânica*. Sociedade de Botânica do Brasil.

Bennett, M.D. & Leitch, I.J. 2012. Plant DNA C-values database. Release 8.0. Available at: http://data.kew.org/cvalues (accessed 9 Nov 2018).

Benzing, D.H. 2000. *Bromeliaceae: Profile of an adaptative radiation*. Cambridge: Cambridge University Press.

Cotias-de-Oliveira, A.L., Assis, J.G.A., Ceita, G.O., Palmeira, A.C.L. & Guedes, M.L. S. 2004. Chromosome number for Bromeliaceae species occurring in Brazil. *Cytologia* 69: 161–166. https://doi.org/10.1508/cytologia.69.161

Doležel, J., Greilhuber, J. & Suda, J. 2007. Estimation of nuclear DNA content in plants using flow cytometry. *Nature, Protoc*. 2: 9. https://doi.org/10.1038/nprot.2007.310

Erdtman, G. 1971. *Pollen morphology and plant taxonomy: Angiosperms; An introduction to palynology*. New York: Hafner.

Givnish, T.J., Milliam, K.C., Berry, P.E. & Sytsma, K.J. 2007. Phylogeny, adaptive radiation, and historical biogeography of Bromeliaceae inferred from *ndhF* sequence data. *Aliso* 23: 3–26. https://doi.org/10.5642/aliso.20072301.04

Givnish, T.J., Barfuss, M.H.J., Ee, B.V., Riina, R., Schulte, K., Horres, R., Gonsiska, P.A., Jabaily, R.S., Crayn, D.M., Smith, J.A.C., Winter, K., Brown, G.K., Evans, T.M., Holst, B.K., Luther, H., Till, W., Zizka, G., Berry, P.E. & Sytsma, K.J. 2011. Phylogeny, adaptive radiation, and historical biogeography in Bromeliaceae: Insights from an eight-locus plastid phylogeny. *Amer. J. Bot*. 98: 872–895. https://doi.org/10.3732/ajb.1000059

Gouda, E.J., Butcher, D. & Gouda, C.S. 2018 (continuously updated). Encyclopaedia of Bromeliads, version 4. University Botanic Gardens, Utrecht. http://bromeliad.nl/encyclopedia/ (accessed 11 Jun 2018).

Hirsch, L.D., Zanella, C.M., Aguiar-Melo, C., Costa, L.M. & Bered, F. 2019. Interspecific gene flow and an intermediate molecular profile of *Dyckia julianae* (Bromeliaceae), an endemic species from southern Brazil. *Bot. J. Linn. Soc*. https://doi.org/10.1093/botlinnean/boz037

Krapp, F., Pinangé, D., Benko-Iseppon, A.M., Leme, E. & Weising, K. 2014. Phylogeny and evolution of *Dyckia* (Bromeliaceae) inferred from chloroplast and nuclear sequences. *Pl. Syst. Evol*. 300: 1591–1614. https://doi.org/10.1007/s00606-014-0985-0

Marchant, C.J. 1967. Chromosome evolution in Bromeliaceae. *Kew Bull*. 21: 161–170. https://doi.org/10.2307/4108461

Martinelli, G., Vieira, C.M., Gonzalez, M., Leitman, P., Piratininga, A., Costa, A. & Forzza, R.C. 2008. Bromeliaceae da Mata Atlântica: Lista de espécies, distribuição e conservação. *Rodriguésia* 59: 209–258. https://www.jstor.org/stable/23499386

Moura, M.N., Forzza, R.C. & Cristiano, M.P. 2018. Reconstruction of ancestral genome size in Pitcairnioideae (Bromeliaceae): What can genome size tell us about the evolutionary history of its five genera? *Bot. J. Linn. Soc*. 186: 321–333. https://doi.org/10.1093/botlinnean/box101

Ramsey, J. & Schemske D.W. 1998. Pathways, mechanisms, and rates of polyploidy formation in flowering plants. *Annual Rev. Ecol. Syst*. 29: 467–501. https://doi.org/10.1146/annurev.ecolsys.29.1.467

Rice, A., Glick, L., Abadi, S., Einhorn, M., Kopelman, N.M., Salman-Minkov, A., Mayzel, J., Chay, O. & Mayrose, I. 2015. The Chromosome Counts Database (CCDB) – A community resource of plant chromosome numbers. *New Phytol*. 206: 19–26.

Rounsaville, T.J., Touchell, D.H. & Ranney T.G. 2011. Fertility and reproductive pathways in diploid and triploid *Miscanthus sinensis*. *HortScience* 46: 1353–1357.

Sharma, A.K. & Ghosh, I. 1971. Cytotaxonomy of the Bromeliaceae. *Cytologia* 36: 237–247. https://doi.org/10.1508/cytologia.36.237

Smith, L.B. & Downs R.J. 1974. *Pitcairnioideae*. Flora Neotropica Monograph 14(1). New York: The New York Botanical Garden.

Strehl, T. 2004. Novas espécies de Bromeliaceae do Rio Grande do Sul, Brasil. *Vidalia* 2: 19–25.

IAPT chromosome data 31/10

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* First chromosome count for the species.
** New chromosome report (cytotype) for the species.
• First chromosome count for an Indian accession.

BALSAMINACEAE

**Impatiens badrinathii* Pusalkar & D.K.Singh

n = 10, CHN. India, Uttarakhand, Uttarkashi, Har-ki-Dun Trek, 31°07′57.35″N, 78°23′32.12″E, 3300 m, isolated individuals in moist places along streams and grassy slopes, *Rohit Kumar 34392* (PUN 61649) [Fig. 22A].

**Impatiens devendrae* Pusalkar

n = 7, CHN. India, Uttarakhand, Uttarkashi, Rarhi, 30°46′15.67″N, 78°15′17.19″E, 2230 m, shaded or partly shaded moist places in forests and along forest edges, *Rohit Kumar 30727* (PUN 60222) [Fig. 22B].

**Impatiens leggei* Pusalkar & D.K.Singh

n = 7, CHN. India, Uttarakhand, Uttarkashi, Har-ki-Dun, 31°08′51.20″N, 78°25′25.85″E, 3600 m, moist, open or partly shaded places along streams in meadows, *Rohit Kumar 34810* (PUN 61666) [Fig. 22C].

CARYOPHYLLACEAE

**Silene gangotriana* Pusalkar, D.K.Singh & Lakshmin.

n = 36, CHN. India, Uttarakhand, Uttarkashi, Near Gomukh glacier, 30°55′58.85″N, 79°04′22.59″E, 3900 m, sandy places along river banks, on rock walls and open dry slopes, *Rohit Kumar 34645* (PUN 61603) [Fig. 22D].

FABACEAE

***Astragalus melanostachys* Benth. ex Bunge

n = 8, CHN. India, Uttarakhand, Uttarkashi, Near Gomukh glacier, 30°55′44.56″N, 79°04′38.65″E, 3900 m, open dry, sandy or gravely slopes, *Rohit Kumar 34855* (PUN 61713) [Fig. 22E].

Previously, 2*n* = 12 was reported for this species (Ashraf & Gohil, 1986, 1988; Kumar & Singhal, 2011).

**Astragalus sanjappae* L.B.Chaudhary & Z.H.Khan

n = 8, CHN. India, Uttarakhand, Uttarkashi, Near Gomukh glacier, 30°55′44.56″N, 79°04′38.65″E, 3900 m, open dry, sandy or gravely slopes, *Rohit Kumar 34861* (PUN 61719) [Fig. 22F].

POACEAE

***Agrostis griffithiana* (Hook.f.) Bor

n = 21, CHN, India, Himachal Pradesh, Kullu, Kothi, 32°18′52.77″N, 77°11′24.76″E, 2500 m, mountain slopes, *Vandna Kumari 34292* (PUN 61496) [Fig. 22G].

Previously, 2*n* = 28 was reported for this species (Mehra & Sood, 1974; Parkash, 1979; Mehra, 1982).

***Arthraxon hispidus* (Thunb.) Makino

n = 15, CHN. India, Himachal Pradesh, Kullu, Malana Village, 32°03′45″N, 77°15′38″E, 2652 m, along road sides, among crops in fields, *Vandna Kumari 32475* (PUN 61379) [Fig. 22H].

The species is having several base numbers (*x* = 5, 8, 9, 13, 19). Previous reports for the species are 2*n* = 10 (Sindhe & al., 1975), 2*n* = 18 (Gosavi & Yadav, 2011), 2*n* = 36 (Gould & Soderström, 1970; Pohl & Davidse, 1971; Mehra & Sharma, 1975; Dujardin, 1979), 2*n* = 38 (Gill & al., 1980) and 2*n* = 40 (Avdulov, 1931; Sindhe, 1967). So, based on *x* = 5, the present gametic count is the first report of hexaploid cytotype for the species.

***Brachypodium sylvaticum* (Huds.) P.Beauv.

n = 18, CHN. India, Himachal Pradesh, Kullu, Kothi, 32°18′52.77″N 77°11′24.76″E, 2400 m, along slopes and along roadsides, *Vandna Kumari 34627* (PUN 61358) [Fig. 22I].

Previous reports for the species were 2*n* = 14, 18, 42 (Mehra & Sunder, 1969), 2*n* = 18 (Tischler, 1934; Larsen, 1960; Mehra & al., 1968; Saxena & Gupta, 1970; Mehra & Remanandan, 1973; Mehra & Sharma, 1975, 1977; Strid & Franzén, 1981; Baltisberger & Leuchtmann, 1991; Dobeš & al., 1997; Lövkvist & Hultgård, 1999; Gupta & al., 2014), 2*n* = 18, 28, 44, 56 (Kozuharov & Petrova, 1973) and 2*n* = 28 (Mehra & Sood, 1974). The present meiotic count of 2*n* = 36 is the first report of 4*x* cytotype for the species.

***Cymbopogon olivieri* (Boiss.) Bor

n = 20, CHN. India, Himachal Pradesh, Kullu, Palchan, 32°18′35″N, 77°10′31″E, 2400 m, moist places and along roads, *Vandna Kumari 34201* (PUN 61405) [Fig. 22J].

Previous reports for the species were 2*n* = 20 (Gupta, 1969, 1970; Quraish & Faruqi, 1979). The present meiotic count of the species is the first report of 4*x* cytotype for the species.

**Danthonia cachemyriana* Jaub. & Spach

n = 14, CHN. India, Himachal Pradesh, Kullu, Jalori Pass, 31°32′14″N, 77°22′26″E, 3223 m, dry rocky slopes, rock crevices, *Vandna Kumari 34710* (PUN 61259) [Fig. 22K].

Capítulo 4

ARTIGO A SER SUBMETIDO PARA A REVISTA

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Hybrid origin of *Dyckia julianae* Strehl (Bromeliaceae) investigated by molecular phylogeny and reproductive biology analyses

Abstract

Dyckia julianae is an endemic species from Campos Sulinos ecozone and found in sympatry with *D. hebdingii* and *D. choristaminea*. A recent hypothesis suggests its origin through hybridization events. To test this hypothesis, three plastid regions (*rps16-trnK*, *psbA-trnH* and *rpl32-trnL*) and one nuclear region (*ITS1*) were sequenced, and *in situ* experiments of reproductive biology were carried out. The studied species were evaluated to determine its mating system, reproductive success, tube pollinic growth and interspecific compatibility. Pointing to the hybrid origin of *D. julianae*, our results indicated *D. choristaminea* as maternal species and *D. hebdingii* as paternal. Moreover, *D. julianae* was shown to be more related to *D. hebdingii* and *D. choristaminea* than to any of the other species used in our phylogenies. The reproductive experiments showed that *D. julianae* and *D. choristaminea* can reproduce by cross and self-pollination, whereas *D. hebdingii* generated fruits only in self-pollination. As expected, *D. julianae* present lower reproductive success than *D. hebdingii* and *D. choristaminea*. Likewise, *D. julianae* is able to reproduce with the parental species, however only receiving interspecific pollen. The data generated in this study point to the confirmation of the hypothesis of a hybrid origin of *D. julianae* with *D. hebdingii* and *D. choristaminea* as parental species.

Key words: hybrid speciation; interspecific crosses; mating system; plastidial inheritance; reproductive success.

Introduction

It is widely known that speciation is a continuous and complex process that results in emergence of new lineages (Darwin, 1859; Mallet, 2007). A crucial point in speciation is the arising of reproductive isolation and the consequent accumulation of differences in populations. Emergence of reproductive barriers that isolate these groups can be a slow process, in which species can occupy different positions in a continuous process (Coyne and Orr, 2004; Baack et al., 2015). Nonetheless, strong barriers can appear rapidly, even in a single generation, in the case of ploidy change and chromosomal rearrangements, for example (Soltis et al., 2003; Kulmuni et al., 2020). Even in the presence of evolutionary barriers, genetic flow can still occur and speciation may be incomplete (Nosil et al., 2009; Osborne et al., 2020).

Since Darwin (1859), the diversification of species has been thought as a bifurcating tree, in which one species is divided into two new ones. However, the tree of life branches can be porous, with exchanges between species at some biological level and, when it comes to plants, many taxa are not distributed according to a tree, but forming a network (Goulet et al., 2017; Dunning and Christin, 2020). This network model occurs due to reticulated evolution, in which two independent lineages generates a new one (Gontier, 2015). At a species level, two different taxa can exchange information by hybridization (that plays a central role in the evolution of plants) or by horizontal gene transfer, both result in reticulation (Gontier, 2015).

When reproductively compatible species occupy the same environment, hybridization can occur. This can lead to genetic outcomes with potentially important consequences for adaptation and evolution (Baack et al., 2015). When the hybrid presents reproductive barriers at least partial with the parents, hybrid speciation can occur (Ungerer et al., 1998; Rieseberg et al., 2006). On the other hand, if there are no reproductive barriers between hybrid and parental species, introgression may occur, often increasing the genetic diversity of parental species (Ellstrand and Elan, 1993).

Phylogenetic methods can help elucidate speciation patterns and track hybridization footprints at putative hybrid taxa (Linder and Rieseberg, 2004). Morphology is often not enough to determine the origin of a taxa, in this way characterizing plastid and nuclear genomes can be very useful. However, comparing biparental nuclear and uniparental plastid phylogenies may present inconsistencies between them (Isoda et al., 2000; Linder and Rieseberg, 2004). These conflicts may be due to rapid diversification, lineage sorting, horizontal transfer or hybridization/introgression (Wendel and Doyle, 1998). Even so, the identification of the hybrid origin of a given species has been facilitated by advances in the field of molecular biology.

Several studies have shown encouraging results in this regard, using internal transcribed spacer (*ITS*) region of nuclear ribosomal DNA (nrDNA), due to maintenance of both parental *ITS* sequences (Kim and Jansen, 1994; Mummenhoff et al., 2009; Wendel et al., 1995). The use of *ITS* has been shown to be suitable for the reconstruction of phylogenies at the level of species in different groups of flowering plants (Baldwin et al., 1995; Meerow et al., 2000; Cheng et al., 2016). Also, chloroplast DNA is an extremely valuable molecule

for phylogenetic studies, foremost for its conservative mode of evolution (Palmer et al., 1992) that in most plants is purely maternal (Reboud and Zeyl, 1994).

In natural plant communities, the species' life histories and aspects related to its reproductive biology such as pollinators, flowering periods and mating system, are tightly linked with the possibility of hybridization (Carney et al., 1996; Ramírez-Rosas et al., 2020). This is very true for sympatric species that display similarities in floral morphology (Field et al., 2008), overlapping flowering periods and shared pollinators (García-Franco et al., 2001), breaking down reproductive isolation (Ramírez-Rosas et al., 2020). The maintenance of hybrids in a population depends on their fertility that can be evaluated by several features, such as the height of the inflorescence, number of flowers, fruit set and the seed germination. The measurement of these characteristics allows comparatively evaluate the reproductive success among species and populations, in order to assess fertility (McIntosh, 2002; Burne et al., 2003; Buide, 2004; Clark-Tapia and Molina-Freaner, 2004; Kéry and Matthies, 2008; Hampe, 2005; Paggi et al., 2007; 2015; Sampaio et al., 2012). Together with reproductive success, knowledge of species reproduction is essential to understand the evolution of breeding systems in the context of the speciation process (Wendt et al. 2002; Sakai and Wright 2008; Barbosa et al. 2009; Matallana et al., 2010). Many studies have shown that in populations of species with restricted distribution, self-pollination is more frequent than in those species with wide distribution (Karron 1991; Hamrick et al. 1991; Wyatt et al. 1992; Wendt et al., 2002; Paggi et al., 2015). In this sense, selfing ability (both autonomous and geitonogamous) is expected in these taxa.

Bromeliaceae is a monocotyledons family known for high diversity and endemism, with large number of sympatric-related species (Versieux and Wendt

2007; Martinelli et al. 2008; Matallana et al., 2010). Bromeliads are traditionally considered as predominant outcrossers (Benzing 2000), with some self-incompatible species; however, autogamy have been reported also (Martinelli 2008). *Dyckia* Schult. E Schult.f. (Pitcairnioideae) is the most diverse genus in the southernmost exoregion of Brazil (Campo Sulinos), with 28 recorded species, of which 17 are possibly endemics of this biome (Strehl, 2004). *Dyckia hebdingii* L.B. Smith, *D. choristaminea* Mez and *D. julianae* Strehl are closely related (Krapp et al., 2014) and endemic species found in a small region of the south of Brazil, and they can occur in sympatry (Smith and Downs, 1974; Strehl, 2004; Hirsch et al., 2020; Figure 1). As far as we know, until the present study, *Dyckia julianae* was found only in one locality (Barra do Ribeiro municipality), occurring in sympatry with *D. hebdingii* and *D. choristaminea*. Previous studies revealed that *D. julianae* has an intermediate molecular profile and possibly originated from the two ancestors, *D. hendingii* and *D. choristaminea*, through homoploid hybridization, since the three species have the same number of chromosomes (2n = 2x = 50) and have similar genome size (Hirsch et al., 2019; 2020).

However, some questions about the origin of *D. julianae* remain unclear. Here we aimed to: a) confirm or reject the hybrid origin hypothesis, investigating if *D. hebdingii* and *D. choristaminea* are suitable to be the parental species of *D. julianae*; b) discuss about the possible contribution of each parent (maternal or paternal); and c) answer what are the reproductive system of these species, their reproductive success and how these factors can be involved in the hybrid origin *D. julianae*.

Material and methods

Taxon sampling

Dyckia choristaminea, *D. hebdingii* and *D. julianae* are endemic species of rocky outcrops found in Campos Sulinos ecozone. To carry out the reproductive biology experiments and the reconstruction of phylogenetic trees, these three species were sampled in sympatric population in Barra do Ribeiro municipality (30°20‟S; 51°18‟W). In addition to these samples, we conducted the phylogenetic analysis with five others *Dyckia* species collected from the greenhouse of the Botanical Department (Universidade Federal do Rio Grande do Sul - UFRGS, RS, Brazil): *Dyckia brevifolia* Baker, *Dyckia distachya* Hassler, *Dyckia maritima* Baker, *Dyckia delicata* Larocca e Sobral and *Dyckia elisabethae* Winkler. *Deuterocohnia meziana* Kuntze ex Mez was used as outgroup. Also, in order to position *Dyckia julianae* for the first time in a more extensive phylogeny of *Dyckia* (Krapp et al., 2014), we included 97 accessions of *Dyckia* plus five of *Deuterocohnia* species from GeneBank (Table S1).

DNA extraction and sequencing

For DNA extraction, a leaf from each specimen was collected both in the wild and in the greenhouse. Leaves were dried and then macerated following the Doyle and Doyle (1990) protocol for DNA extraction. The PCR reactions for *ITS1* were run using the following parameters: denaturation at 94 °C for 5 min, followed by 35 cycles of 94 °C for 50 seconds, 54 °C for 50 seconds, and 72 °C 50 seconds, and a final extension for 8 min at 72 °C, using ITS1F-ITS2R (5’-GGA AGT AAA AGT CGT AAC G-3’ / 5’-TCC TCC TCC GCT TAT TGA TAT GC-3’) primers. Amplification of plastid regions followed the parameters: denaturation at 94 °C for 3 min, followed by 35 cycles of 94 °C for 1 min, 58 °C for *rsp16-trnK* (5’-AAA GTG GGT TTT TAT GAT CC / TTA AAA

GCC GAG TAC TCT ACC-3'), 50 °C for *psbA-trnH* (5'-TCT TCC TTT GTT TGA TTC ACA TC / GAA TTC GCG CCT ACT CTG AC-3') and 54 °C for 1 min for *rpl32-trnL* (5'-CAG TTC CAA AAA AAC GTA CTT C / CTG CTT CCT AAG AGC AGC GT-3') and 72 °C 50 seconds, and a final extension for 8 min at 72 °C. All PCR reactions were carried out in a total volume of 20 µL containing 10 ng DNA template, 1X *GoTaq* buffer, 2 mM MgCl2, 250 µM dNTP mix, 5 ρmol forward and reverse primers and 1 U *GoTaq* DNA polymerase (Promega, Madison, WI, USA). The nuclear and plastid DNA PCR products were sequenced from both ends using a BigDye kit (Applied Biosystems) at Macrogen Inc. (South Korea). To check for the presence and quality of amplicons, aliquots of PCR products were electrophoresed on agarose gels and stained with GelRed.

DNA analysis

The sequences obtained were checked using CHROMAS Lite version 2.6.6 and were aligned to obtain consensus using MUSCLE (Edgar, 2004) implemented in MEGA5 (Tamura et al., 2011) and were then manually edited. Sequences were aligned and polymorphisms at the mononucleotide microsatellites were excluded due to ambiguous alignment and higher mutation rates. Long insertions/deletions (indels; usually > 5 bp) were manually coded as one evolutionary event. All phylogenetic reconstructions were performed separately for the combined plastid data set and for the *ITS1* sequence alignment. Due to considerable degree of incongruence, no combined analysis of nuclear and plastid data sets was attempted.

For Bayesian inference (BI) analyses the data sets were tested for the best-fit model of evolution with Jmodeltest v2.1.4 (Darriba et al., 2012) using the Akaike information criterion (AIC). BI analyses were done using Beast v1.8.4

(Drummond et al., 2007). The cpDNA trees were generated using the GTR+G+I substitution model. The tree prior was set to the Yule process. Markov chains were run for 10,000,000 steps, with sampling performed every 1000 steps. To control convergence and to ensure a sufficient effective sample size of 200 and above for all parameters, the log files were analyzed with Tracer 1.6 (Rambaut and Drummond 2009). HKY+G+I was used as a substitution mode for *ITS1* tree. All other priors' conditions were the same, except for the substitution rate. TreeAnnotator 1.8.4, part the Beast software package, was used to summarize the trees, and the statistical support for all branches was measured as Bayesian posterior probability (PP). The software FigTree 1.4.2 was used to draw the tree (Rambaut, 2014).

Reproductive biology

Manual pollination experiments and reproductive success

All hand-pollination experiments were performed *in situ* in Barra do Ribeiro municipality throughout the species' flowering and fruiting seasons (September-February 2018/2019). To investigate the mating systems, 308 flowers from 82 individuals of *D. hebdingii*, *D. julianae* and *D. choristaminea* were tagged in the field and were undergone to the following treatments: Cross-pollination (CP); Autocompatibility (AC); Agamospermy (AG) and Control (C). In CP treatment emasculated flowers received pollen from another plant and in AC treatment received its own pollen. To test AG, the flowers were emasculated and bagged. The flowers used as controls were under natural conditions and were tagged for identification. All flowers from the CP, AC and AG experiments were bagged with paper bags to avoid pollinator visits, except in the C treatment. Two to five flowers per individual were used in treatments for the three species

(Table 1). Differences among treatments for each species were detected using Kruskal-Wallis test (5%) (SAS Studio, version 5.2).

Reproductive success was assessed through the following measures: (1) plant height; (2) inflorescence height; (3) reproductive potential (total number of flowers per plant); (4) fruiting (total number of fruits per plant); (5) number of seeds per fruit. To test the significance of differences between species for each variable the Kruskal-Wallis test (5%; SAS Studio, version 5.2) was performed (Table 2).

Pollen tube growth and Interspecific compatibility

Manual self-pollination (MSP) and manual cross-pollination (MCP) crosses were performed to analyze the growth of the pollen tube and the number of penetrated and non-penetrated ovules in each treatment. The hand-pollinated flowers were fixed in 3:1 ethanol: glacial acetic acid in 48h, 72h and 96h after manipulation. A total of 31 flowers were used for each treatment for the three species. The proportion of pollen tubes was evaluated in flowers subjected to MSP and MCP by scraping the ovule to allow observation of tubes growing using fluorescence microscopy and aniline blue staining (Martin, 1959). Differences in the proportion of pistils with tube growth in the treatments were detected using Kruskal-Wallis test (5%) with SAS Studio, version 5.2 (Table 3).

With the intention of confirming the genetic compatibility between the three species studied, interspecific artificial crosses were performed in glasshouse of the Botanical Department (UFRGS, RS, Brazil). Manual crossings were made following Cafasso et al., (2005). In total, we used 61 flowers and all crosses were conducted in both directions (each plant provided and received pollen), and were performed between different individuals (Table 4). A viability test was performed with the seeds, following Hirsch et al., (2020).

DNA analysis

The aligned matrix for *ITS1* consisted of 247 total characters, 24 heterozygous sites. The total number of SNPs was 99 for all species. Considering only *D. hebdingii*, *D. julianae* and *D. choristaminea* we obtained 61 SNPs. Three polymorphic sites are present only in *D. julianae*, resulting in 95% of additive polymorphic sites, relative to *D. hebdingii* and *D. choristaminea*. Moreover, *D. julianae* was recovered more closely related to *D. choristaminea* than to *D. hebdingii* (Figure 2). Two independent analyses yielded identical relationships with high posterior probability support and resolution to interpret interspecific relationships (data not shown).

trnH-psbA region showed 161 sites of which only four were polymorphic. On the opposite, 731 sites and 24 SNPs were obtained in *rps16-trnK* region. *rpL32-trnL* region showed 854 sites and 14 polymorphisms. Plastid regions were aligned and concatenated, totaling 1746 sites and 42 SNPs. Thirteen differences were found between *D. julianae* and *D. choristaminea* as well as between *D. julianae* and *D. hebdingii*. Same pattern was recovery with cpDNA, with *D. julianae* more closely related to *D. choristaminea* (Figure 3).

Adding GenBank accessions to reconstruct a more extensive cpDNA tree using *rps16-trnK* and *rpL32-trnL*, it was observed that individuals of *D. julianae* form a clade with *D. hebdingii* and *D. choristaminea*, although these two last species were also recovered in different positions in the tree (Figure 4). Interestingly, *D. hebdingii* and *D. choristaminea* samples that are in the clade

highlighted in Figure 4 come from the sympatric population of Barra do Ribeiro municipality.

No inconsistencies were observed in the genetic pattern of the species comparing nuclear and plastid phylogenetic trees. Both analyzes resulted in a closer relationship between *D. julianae* and *D. choristaminea* than between *D. julianae* and *D. hebdingii*. *Dyckia hebdingii* is more closely related to *D. julianae and D. choristaminea* than with the others *Dyckia* species analyzed (Figure 2 and 3).

Reproductive biology

Manual pollination experiments and reproductive success

Manual pollination experiments showed different results for each studied species, except for the agamospermy treatment in which all species did not produce any fruit.

Dyckia hebdingii showed 30% of fruit set in AC and 56% in C, although the seed viability for these two treatments were high and do not showed significant differences. No fruit was generated in the CP treatment (Table 1). On the opposite, *D. choristaminea* had the highest fruit set rates when undergone to CP (91%) followed by control treatment (80%). Considering flowers tested for AC only 17% developed into fruits. Despite the high proportion of fruit set, the seeds viability of *D. choristaminea* was low for all treatments, ranging from 22 to 224 seeds (Table 1). In *D. julianae*, the three treatments CP, AC and C did not differ significantly regarding to fruit set rates. All treatments differ from AG that did not produce fruits. The seed viability ranged from 57% to 71% among treatments, but not differing significantly (Table 1).

The overall means of plant and inflorescence height did not differ significantly between *D. hebdingii* (67 ± 2.26; 53 ± 2.16) and *D. julianae* (70.1 ± 6.85; 59.5 ± 6.52), whilst *D. choristaminea* differs from these two species for both characters (27.6 ± 1.4; 24.4 ± 1.33; Table 2). The means of flowers was significantly higher for *D. hebdingii* (88.9 ± 7.87) and lower for *D. choristaminea* (11 ± 1.12), being *D. julianae* intermediate (30.4 ± 0.17). However, the number of fruits did not differ between *D. julianae* (10.3 ± 1.59) and *D. choristaminea* (6.4 ± 1.41), but was significantly higher in *D. hebdingii* (48.9 ± 7.27). The average number of seeds per fruit ranged from 52.75 in *D. julianae* to 115.4 in *D. choristaminea*, with significant differences. Despite presenting more seeds, *D. choristaminea* had the lowest proportion of viable seeds among the three species (39.7%), followed by *D. julianae* (53.3%) and then *D. hebdingii*, with the highest viability (81.4%; Table 2).

Pollen tube growth and Interspecific compatibility

Different times in which the flowers were collected and fixed showed no differences in the length of the pollen tube and the results were presented combined (Table 3). Fluorescence microscopy analysis showed that in both treatments, manual self-pollination (MSP) and manual cross-pollination (MCP), the pollen grains germinated on stigma and produced tubes in *D. julianae* (Figure 5) and *D. choristaminea*, with no statistical difference. On the other hand, *D. hebdingii* had statistical differences between the treatments. No tubes were generated in the MCP treatment, while 10 of 15 flowers had tubes in MSP. Until 96h no pollen tube was able to reach the ovary in any of the analyzed species, for this reason only the presence or absence of the tube was evaluated.

Considering the interspecific crosses performed, *D. julianae* was able to produce fruits and viable seeds when received pollen from parental species. The number of fruits produced between *D. julianae* and each parental species, when *D. julianae* act as maternal species, did not show statistical differences. Although compatible, both crosses had low fruit production but high seeds viability. Crossings between *D. hebdingii* and *D. choristaminea* did not generated fruits (Table 4).

Discussion

Phylogenetic reconstruction and parental inheritance

Dyckia julianae was described as a species within the genus (Strehl, 2004), however, its morphology and presence exclusively in sympatry raised questions about its origin. In a recent study using microsatellites loci, *D. julianae* showed intermediate molecular patterns between *D. hebdingii* and *D. choristaminea* and shared haplotypes among them (Hirsch et al., 2020). All these facts together pointed to the hypothesis of a hybrid origin of *D. julianae*. At the same time, *D. julianae* has the same chromosome number of *D. hebdingii* and *D. choristaminea* (2n = 50), suggesting a case of homoploid hybridization, situation in which parental species and hybrid have the same chromosome number (Hirsch et al., 2019).

The nuclear phylogenetic tree showed that *D. julianae* is sister species of *D. choristaminea* and that these two species, together, are sister species of D*. hebdingii*. Because nuclear genes are generally biparentally inherited, hybrids must have both parental alleles at each locus. In later generation hybrids, molecular evidence of parentage may be less evident because

recombination (Frajman and Oxelman, 2007). However, in cases of recent hybridization both parental alleles are present and the relationship of species is easily accessed in phylogeny (Barkman and Simpson, 2002; Bendiksby et al., 2011). Our results showed that in three different sites, *D. julianae* did not show addition patterns. If we were facing recent hybridization events, we would expect that *D. julianae* showed all sites with additivity, indicating inheritance of alleles from both parental species. According to that, the hypothesis of an ancient hybridization event between *D. hebdingii* and *D. choristaminea* that generated *D. julianae* (Hirsch et al., 2020) cannot be rejected.

Although our hypothesis is that *D. hebdingii* and *D. choristaminae* are the parents of *D. julianae*, the occurrence of other close species in sympatry does not allow us to rule out their participation in this speciation process. According to Sthrel (2004), *D. elisabethae* is one of the taxa that may occur in sympatry with our target species. However, it has not been recovered as a species close to *D. julianae* (Figure 2), fact that allows us to discard the option of being *D. elisabethae* a parental species of *D. julianae*.

Chloroplast DNA is maternally inherited in the majority of angiosperms (Corriveau and Coleman, 1988) and in Bromeliaceae inheritance seems to be maternal as well (Wagner et al., 2015). Our phylogenetic tree based on three chloroplast regions did not show any inconsistencies with the nuclear DNA tree, concerning relationship of *D. julianae* with de parental species. Again, *D. julianae* and *D. choristaminea* seem to be more related to each other than both to *D. hebdingii*. However, individuals of *D. julianae* were recovered in different clades, showing discordance with nuDNA at this point (Figure 3). This result may be due to the nature of the cpDNA that describes historical episodes because it is inherited as a fixed unit (Palmé et al., 2004; Heuertz et al., 2006;

Palma-Silva et al., 2011) and because haplotype sharing of *D. hebdingii*, *D. julianae* and *D. choristaminea* (Hirsch et al., 2020). Further on to being closely related to *D. hebdingii* and *D. choristaminea*, *D. julianae* also groups with *D. elisabethae* and *D. maritima*, but with low support (Figure 3).

When we insert *D. julianae* sequences in an extensive plastid phylogeny, this species forms a clade separately, with high posterior probability. This *Dyckia* phylogeny had low posterior probability in most branches when *D. julianae* is added (Figure 4 and 5). Possible causes for low support in phylogenetic trees are lineage sorting, horizontal transfer, rapid diversification and hybridization (Wendel and Doyle, 1998). Plant families that suffered fast diversification and frequent hybridization events used to show incongruencies in phylogenies, probably due to these phenomena (for example, Hewitt, 2001; Gobert et al., 2008; Wang et al., 2014). This also true for Bromeliaceae, (Benzing, 2000; Barfuss et al., 2005; Wendt et al., 2008; Goetze et al., 2017).

In previous study, plastid SSR indicated a long history of gene exchange between *D. hebdingii* and *D. choristaminea*, with low genetic differentiation between species (Hirsch et al., 2020). Here, haplotypes present in *D. julianae* were grouped with those found in *D. choristaminea*, indicating a close relationship between these two species. This similarity of cpDNA can be explained by many processes, as hybridization, ancestral polymorphism and homoplasy and can effectively be related with the hybrid origin of *D. julianae* as hypothesized here (Palma-Silva et al., 2011; Zanella et al., 2016).

Phylogenetic studies have frequently recovered nuclear trees consistent with morphology, taxonomy and biogeography of species. When comparing nuclear and plastid phylogenetic trees may occur inconsistencies, usually related to past events of hybridization with chloroplast capture

(Rieseberg et al., 2006; Frajman and Oxelman, 2007; Bendiksby et al., 2011). In this sense, despite the plausibility of hybridization, our study cannot reject more complex models involving the origin of *D. julianae*. Another model possible would be *D. julianae* has diverged from *D. hebdingii* or *D. choristaminea* and underwent an instantaneous expansion after divergence. Such proposal seems not very robust taking into account the intermediate molecular profile of *D. julianae* (Hirsch et al., 2020). Still, the three species could have a common ancestor and diverged simultaneously. This model is in conflict with *ITS1* phylogeny, because *D. julianae* initially diverged from a recent common ancestor with *D. choristaminea* (Figure 2). Finally, *D. julianae* could have originated by homoploid hybrid speciation from multiple crosses between *D. hebdingii* and *D. choristaminea* (Figure 6). This hypothesis seems to be the most parsimonious because it can explain the phylogenetic relationships observed for both cpDNA and *ITS1* data. The shared cpDNA may be due to recent radiation of *Dyckia* and, since organellar genomes typically evolve as a single nonrecombining unit (Doyle, 1993), it is straightforward to fix the same divergent history for different species. Another explanation is that both parental taxa may have served as maternal donors multiple times during the initial establishment of *D. julianae*, as has been found in numerous hybrid species (Rieseberg, 1997; Buerkle et al., 2000; Gross and Rieseberg, 2005; Abbott et al., 2010).

Although *D. julianae* was recovered in different clades by cpDNA phylogeny, in all these clades *D. julianae* was more related to *D. choristaminea* than to any other species, indicating that *D. choristaminea* probably was the maternal species of *D. julianae*. This approach has been used successfully in different groups of plants, although in some cases the branches have been left with low statistical support (Barkman and Simpson, 2002; Lubinsky et al., 2008;

Sun, 2014). When we are facing a case of recent hybridization, it is common to recover nuclear phylogenetic trees with the supposed hybrid grouped closely with both different species (parental species; Shi et al., 2006; Lubinsky et al., 2008; Bendiksby et al., 2011). Here, *D. julianae* formed a sister clade of *D. choristaminea* and *D. hebdingii*. This result is in agreement with the observed through nuSSR, in which *D. julianae* presented twice as many alleles shared with *D. choristaminea* as with *D.* hebdingii (Hirsch et al., 2020). A possible explanation is that *D. julianae* maintained gene flow with *D. choristaminea* while *D. hebdingii* underwent reproductive isolation of these two species (as discussed later in this article, see Figure 6).

Reproductive biology

Manual pollination experiments and reproductive success

In studies on hybridization and speciation, the approach on the reproductive biology of the target species is essential for a better understanding of the processes involved in these mechanisms. Self-incompatibility in homoploid hybrids has usually been found to be dominant over self-compatibility, although there are exceptions (Zeng and Cheng, 2014). To understand the patterns found in molecular and phylogenetic analyzes we need to know how these species reproduce.

In the present study we tested the mating systems of *D. hebdingii*, *D. julianae* and *D. choristaminae* aiming to contribute to the understanding of the probable hybrid origin of *D. julianae*. All three species do not generated fruits in agamospermy treatment, showing the clear need for fertilization in these species (Table 1). Also, there is a clear difference between the mating system of *D. hebdingii* in relation to the other two species. While *D. choristaminae* and

D. julianae produce fruit with both pollen from the same flower (selfing) and pollen from different individuals (crossing), and therefore fit into the mixed mating system, *D. hebdingii* is only able to produce fruits when the pollen comes from the same individual (flower), not accepting foreign pollen. (Table 1). This unusual and unexpected result had already been observed previously for this species when we carried out manual pollination experiments in a greenhouse (Hirsch et al., 2020) and is now maintained with *in situ* experiments. Cases of intraspecific cross-incompatibility, in with pollen is unable to germinate under the stigma of another plant of the same species, are very rare, although there are a few reports in the literature showing cases where the crosses are compatible in one direction but reciprocals are incompatible (Rashid et al., 1991). Although our results cannot affirm that *D. hebdingii* does spontaneous selfing, they indicate that this is a species that reproduces exclusively by self-pollination. Selfing syndrome has been reported in other species of bromeliads, including *Dyckia* species (Matallana et al., 2010; Zanella et al., 2012), however, no other study has shown this particularity of *D. hebdingii* in not accepting pollen from another individual of the same species. *Dyckia hebdingii* has flowers features (small and inconspicuous flowers and reduced herkogamy) that influence pollen dispersion and are characteristics of self-fertilizing plants (Barret, 2003; Sicard et al., 2011; Carleial et al., 2017). It is not yet clear whether self-fertilization is spontaneous or whether pollinating agents are involved, because the design our experiment did not allow us to verify this. However, during our field expeditions we observed at least one species of floral visitor in the flowers of *D. hebdingii* (data not shown). Despite the clear participation of selfing in *D. hebdingii* reproductive biology, the selfing treatment generated significantly less fruit than the control treatment (Table 1). This result may be due to the technique of handling the flower (emasculation and bagging),

because the flowers are delicate and can be damaged by the technique. Another possibility is the unintentionally use of immature pollen or even geitonogamy, that has not been tested here.

On the other hand, *D. choristaminea* seems to reproduce preferentially by cross-pollination. This species showed high fruit production when subjected to cross-pollination and in the control treatment (Table 1), however the selfing treatment had a much lower fruit production. Despite this difference between treatments, all had low seed viability. Personal observations were made during the expeditions regarding insects such as ants and Hemiptera, causing damage to the flowers and larvae in the fruits, facts that may be involved in this low seed viability.

The three species had several characters relative to their reproductive success evaluated (Table 2). Regarding to the height of the plant and the inflorescence no differences were found between *D. hebdingii* and *D. julianae*, as expected, considering the morphological description of the species (Smith and Downs, 1974; Strehl, 2004). On the other hand, both species differed significantly from *D. choristaminea*, which is the smaller species. In relation to the number of flowers per individual, the obtained results are in agreement with the description for the species, with *D. hebdingii* presenting a greater number of flowers, followed by *D. julianae* and then *D. choristaminea*, with the lowest average.

The differences observed in the number of fruits, seeds produced and viable seeds are due to the morphological differences between species. *Dyckia hebdingii* produces more flowers and consequently has a higher number of fruits. In the same way, *D. julianae* produces an intermediate number of flowers and *D. choristaminea* produces few flowers per individual, this is reflected in the

number of fruits produced. However, *D. choristaminea* has the highest number of seeds per fruit compared to the other two species. This may be the result of a superior number of ovules per flower for this species. Despite the high number of seeds produced, *D. choristaminea* has the lowest viability while *D. hebdingii* has high seed viability. As expected, *D. hebdingii* and *D. choristaminea* have high reproductive success, despite the differences in strategies, since the number of viable seeds produced by these species does not differ statistically.

In agreement with our hypothesis of hybrid origin *D. julianae* had lower reproductive success than *D. hebdingii* and *D. choristaminea*. Also, was observed in expeditions, flowers and, sometimes, whole inflorescences aborted in this species (data not shown). Traditionally hybrid individuals were thought to have less reproductive success due to the incompatibility of parental genomes (Mayr 1947). However, in some cases, hybrid genotypes may exhibit a similar or higher fitness than both parental species (Mallet 2005, Wendt et al., 2001; Leinonen et al., 2010; Arnold et al., 2012). The fitness of hybrid individuals appears to be dependent on the environment in which they establish (López-Caamal et al., 2014). The reproductive success of the hybrid species can be higher than that of the parental species when it colonizes a new habitat, where the parental are not adapted and the hybrid does not compete for nutrients and floral visitors (Welch and Rieseberg, 2007; Arnold and Martin, 2010).

Pollen tube growth and Interspecific compatibility

Stigma receptivity is fundamental for the effectiveness of reproduction in plants (Souza et al., 2016). Pollen source has a significant influence on pollen germination on the stigmatic surface, number of pollen tubes penetrating the stigma, distance of pollen tube growth down the style, and pollen tubes reaching the base of the style (Jahed and Hirst, 2017). Confirming the other experiments,

D. hebdingii does not show pollen tube growing in any flower under MCP (cross-pollination), evidencing once again that this species seems to use a selfing strategy for reproduction. *Dyckia julianae* and *D. choristaminea* also generated expected results, with both treatments generating pollinic tubes (Table 3; Figure 5). No pollen tube reached the ovary until the longest time tested (96 hours). Possibly tube growth speed is lower than other bromeliads (Paggi et al., 2015, Matallana et al., 2016), abiotic factors such as temperature may be related to this difference (Hebbar et al., 2018).

Hybrid origin hypothesis

According to Hirsch et al (2020), our main hypothesis is that *D. hebdingii* and *D. choristaminae* crossed in the past to give rise to *D. julianae* (Figure 6). Our results show that currently *D. hebdingii* and *D. choristaminae* are no longer able to breed (Hirsch et al., 2020; Table 4). However, *D. julianae* is able to cross both *D. hebdingii* and *D. choristaminea*, and apparently, has no preference between the two species because there were no statistical differences between the crossings (Table 4). Interestingly, the crosses only generated fruits when *D. julianae* received pollen from both *D. hebdingii* and *D. choristaminea*. Although genetic flow between *D. julianae* and these two species has been demonstrated (Hirsch et al., 2020), apparently this reflects the similarity between them, since they have diverged recently. In fact, there seems to be only one-way genetic flow. This result agrees with other experiments showing that *D. hebdingii* only seems to reproduce by autogamy, so it would not be plausible to generate fruit through the pollen of another species.

Despite the history of hybridization between *D. hebdingii* and *D. choristaminea*, these species seem to have developed pre-zygotic reproductive barriers, since they do not produce fruits at interspecific crosses (Hirsch et al.,

2020; Table 4). Two main issues can lead to this result. *D. hebdingii* is a species that reproduces exclusively from selfing, not accepting foreign pollen, feature that facilitates reproductive isolation (Wendt et al., 2002). In addition, *D. hebdingii* has the flowering period with little overlap with *D. choristaminea* and *D. julianae*, which trigger led to the reproductive isolation between *D. hebdingii* and *D. choristaminea*, since they seem to have crossed in the past. The difference in the flowering period leading to reproductive isolation has already been seen in other species of bromeliads (Wendt et al., 2001; Matallana et al., 2010; Zanella et al., 2016).

The results obtained in our different approaches were consistent with the hypothesis of a hybrid origin of *D. julianae* and that *D. choristaminea* and *D. hebdingii* were the parental species. The characteristics of *D. julianae* suggest its origin was possibly by homoploid hybridization, being in agreement with cytogenetic data of these species (Hirsch et al., 2019).

References

- Abbott RJ, Hegarty MJ, Hiscock SJ and Brennan AC. 2010. Homoploid hybrid speciation in action. *Taxon* 59: 1375–1386.
- Arnold M, Ballerini E and Brothers A. 2012. Hybrid fitness, adaptation and evolutionary diversification: lessons learned from Louisiana Irises. *Heredity* 108: 159–166.
- Arnold ML and Martin NH. 2010. Hybrid fitness across time and habitats. *Trends in Ecology & Evolution* 25(9): 530-536.
- Baack E, Melo MC, Rieseberg LH and Ortiz-Barrientos D. 2015. The origins of reproductive isolation in plants. *New Phytologist* 207(4): 968-984.
- Barfuss MHJ, Samuel R, Till W and Stuessy TF. 2005. Phylogenetic relationships in subfamily Tillandsioideae (Bromeliaceae) based on DNA sequence data from seven plastid regions. *American Journal of Botany* 92(2): 337-351.
- Baldwin BG, Sanderson MJ, Porter JM, Wojciechowski MF, Campbell CS and Donoghue MJ. 1995. The ITS Region of Nuclear Ribosomal DNA: A Valuable Source of Evidence on Angiosperm Phylogeny. *Annals of the Missouri Botanical Garden* 82(2): 247-27.
- Barbosa SS, Byrne M and Kelaher BP. 2009. Reproductive periodicity of the tropical intertidal chiton acanthopleura gemmata at one tree Island, great barrier reef, near its southern latitudinal limit. *Journal of the Marine Biological Association of the United Kingdom* 89(2): 405 – 411.
- Barkman TJ and Simpson BB. 2002. Hybrid origin and parentage of *Dendrochilum acuiferum* (Orchidaceae) inferred in a phylogenetic context using nuclear and plastid DNA sequence data. *Systematic Botany* 27(2): 209-220.
- Barrett SCH. 2003. Mating strategies in flowering plants: the outcrossing–selfing paradigm and beyond. Philosophical Transactions of the Royal Society of London, B, *Biological Sciences* 358: 991–1004.
- Bendiksby M, Thorbek L, Scheen A, Lindqvist C, Ryding O. 2011. An updated phylogeny and classification of Lamiaceae subfamily Lamioideae. *Taxon* 60(2): 471-484.
- Benzing DH. 2000. Bromeliaceae: Profile of an adaptative radiation. Cambridge University Press, Cambridge, 689 p.
- Buerkle AC, Morris RJ, Asmussen MA and Rieseberg LH. 2000. The likelihood of homoploid hybrid speciation. *Heredity* 84: 441–451.
- Buide ML. 2004. Intra-inflorescence variation in floral traits and reproductive success of the hermaphrodite *Silene acutifólia*. *Annals of Botany* 94(3): 441–448.
- Burne HE, Yates CJ and Ladd PG. 2003. Comparative population structure and reproductive biology of the critically endangered shrub *Grevillea althoferorum* and two closely related more common congeners. *Biological Conservation.* 114: 53–65.
- Cafasso D, Widmer A and Cozzolino S. 2005. Chloroplast DNA inheritance in the orchid *Anacamptis palustris* using single seed polymerase chain reaction. *Journal of Heredity.* 96: 66–70.
- Carleial S, van Kleunen M and Stift M. 2017. Small reductions in corolla size and pollen: ovule ratio, but no changes in flower shape in selfing populations of the North American *Arabidopsis lyrata*. *Oecologia.* 183: 401–413.
- Carney SE, Hodges AS and Arnold ML. 1996. Effects of differential pollen-tube growth on hybridization in the *Louisiana irises*. *Evolution.* 50(5): 1871-1878.

- Cheng T, Xu C, Lei L, Li C, Zhang Yand and Zhou S. 2016. Barcoding the kingdom Plantae: new PCR primers for ITS regions of plants with improved universality and specificity. *Molecular Ecology.* 16(1): 138-149.
- Clark-Tapia R and Molina-Freaner F. 2004. Reproductive ecology of the rare clonal cactus *Stenocereus eruca* in the Sonoran desert. *Plant Systematics and Evolution.* 247: 155–164.
- Corriveau JL and Coleman AW. 1988. Rapid screening method to detect potential biparental inheritance of plastid DNA and results for over 200 angiosperm species. *American Journal of Botany.* 75(10): 1443-1458.
- Coyne JA and Orr HA. 2004. Speciation. Sinauer, Sunderland de Sousa LOF, Silva BR, de Sousa RCOS. 2003. x *Hohenmea*, a new natural intergeneric hybrid in the Bromelioideae. *Journal Bromeliad Society* 53:71–77.
- Darwin C. 1859. The origin of species. New York, NY: The Modern Library Darriba D, Taboada G, Doallo R et al. 2012. jModelTest 2: more models, new heuristics and parallel computing. *Nat Methods* 9: 772.
- Doyle JJ and Doyle JL. 1990. Isolation of plant DNA from fresh tissue. Focus 12: 13-15.
- Doyle JJ. 1993. DNA, Phylogeny, and the Flowering of Plant Systematics. *BioScience* 43(6): 380-389.
- Drummond AJ and Rambaut A. 2007. BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evol Biol* 7:214
- Dunning LT and ChristinPA. 2020. Reticulate evolution, lateral gene transfer, and innovation in plants. *American Journal of Botany.* 107(4): 541–544.
- Gontier N. 2015. Reticulate Evolution. *Interdisciplinary Evolution Research* v3.
- Edgar RC. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research* 32: 1792–1797.
- Ellstrand NC and Elam DR. 1993. Population genetic consequences of small population size: implications for plant conservation. *Annual Review of Ecology and Systematics.* 24: 217–242.
- Field D, Ayre DJ, Whelan RJ and Young AG. 2008. Relative frequency of sympatric species influences rates of interspecific hybridization, seed production and seedling performance in the uncommon *Eucalyptus aggregate*. *Journal of Ecology.* 96: 1198–1210.
- Frajman B and 1Oxelman B. 2007. Reticulate phylogenetics and phytogeographical structure of *Heliosperma* (*Sileneae*, Caryophyllaceae) inferred from chloroplast and nuclear DNA sequences. *Molecular Phylogenetics and Evolution.* 43(1): 140-155.
- García-Franco JG, Burgoa DM and Pérez TM. 2001. Hummingbird Flower Mites and Tillandsia spp. (Bromeliaceae): Polyphagy in a Cloud Forest of Veracruz, Mexico. *Biotropica.* 33(3): 538-542.
- Gobert V, Moja S, Taberlet P and Wink M. 2008. Heterogeneity of Three Molecular Data Partition Phylogenies of Mints Related to M. ×*piperita* (*Mentha*; Lamiaceae). *Plant Biology.* 8(4): 470-485.
- Goetze M, Zanella CM, Palma-Silva C, Büttow MV and Bered F. 2017. Incomplete lineage sorting and hybridization in the evolutionary history of closely related, endemic yellow-flowered Aechmea species of subgenus Ortgiesia (Bromeliaceae). *American Journal of Botany.* 104(7): 1073-1087.
- Goulet BE, Roda F and Hopkins R. 2017. Hybridization in Plants: Old Ideas, New Techniques. *Plant Physiology.* 173(1) 65-78.
- Gross BL and Rieseberg LH. 2005. The Ecological Genetics of Homoploid Hybrid Speciation. *Journal of Heredity* 96(3): 241–252.
- Hampe A and Petit RJ. 2005. Conserving biodiversity under climate chenge: the rear edge matters. *Ecology.* 8: 461-467.
- Hamrick JL, Godt MJW, Murawski DA and Loveless MD. 1991. Correlations between species traits and allozyme diversity: implication for conservation biology. In: Falk D. A. S., Holsinger K. E. (eds.) Genetics and conservation of rare plants. Oxford University Press, Oxford, pp. 75–86.

- Hebbar KB, Rose HM, Nair AR, Kannan S, Niral V, Arivalagan M, Gupta A, Samsudeen K, Chandran KP, Chowdappa P and Prasa PVV. 2018. Differences in in vitro pollen germination and pollen tube growth of coconut (*Cocos nucifera* L.) cultivars in response to high temperature stress. *Environmental and Experimental Botany*. 153: 34-44.
- Heuertz M, Carnevale S, Fineschi S et al. 2006. Chloroplast DNA phylogeography of European ashes, *Fraxinus* sp. (Oleaceae): roles of hybridization and life history traits. *Molecular Ecology.* 15, 2131–2140.
- Hewitt GM. 2001. Speciation, hybrid zones, and phylogeography – or seeing genes in space and time. *Molecular Ecology.* 10: 537–549.
- Hirsch LD, Bered F, Aguiar-Melo C, Carvalho L and Kaltchuk-Santos E. 2019. IAPT chromosome data 31. *Taxon.* 68(6): E32-E36.
- Hirsch LD, Zanella CM, Aguiar-Melo C, Costa LM and Bered F. 2020. Interspecific gene flow and an intermediate molecular profile of *Dyckia julianae* (Bromeliaceae), an endemic species from Southern Brazil. *Botanical Journal of the Linnean Society.* 192:675–690.
- Isoda KS, Shiraishi S, Watanabe and Kitamura K. 2000. Molecular evidence of natural hybridization between *Abies veitchii* and *A. homolepis* (Pinaceae) revealed by chloroplast, mitochondrial and nuclear DNA markers*. Molecular Ecology.* 9:1965-1974.
- Jahed KR and Hirst PM. 2017. Pollen tube growth and fruit set in apple. *American Society of Horticultural Science.* 52(8):1054-1059.
- Karron JD. 1991. Patterns of genetic variation and breeding systems in rare plant species. In: Falk D. A. S., Holsinger K. E. (eds.) *Genetics and conservation of rare plants*. Oxford University Press, Oxford, pp. 87–98.
- Kéry M and Matthies D. 2008. Reduced fecundity in small populations of the rare plant *Gentianopsis ciliate* (Gentianaceae). *Plant Biology.* 6(6):683-688.
- Kim KJ and Jasen RK. 1994. Comparisons of phylogenetic hypotheses among different data sets in dwarf dandelions (Krigia, Asteraceae): Additional information from internal transcribed spacer sequences of nuclear ribosomal DNA. *Plant Systematics Evolution.* 190:157–185.
- Krapp F, Pinangé D, Benko-Iseppon AM, Leme E and Weising K. 2014. Phylogeny and evolution of *Dyckia* (Bromeliaceae) inferred from chloroplast and nuclear sequences. *Plant Systematic and Evolution.* 300:1591–1614.
- Kulmuni J, Butlin RK, Lucek K, Savolainen V and Westram AM. 2020. Towards the completion of speciation: the evolution of reproductive isolation beyond the first barriers. *Philosophycal Transaction of Royal Society B*. 375: 20190528.
- Leinonen PH, Remington DL and Savolainen O. 2010. Local adaptation, phenotypic differentiation, and hybrid fitness in diverged natural populations of *Arabidopsis lyrata. Evolution* 65(1):90-107.
- Linder CR and Rieseberg LH. 2004. Reconstructing patterns of reticulate evolution in plants. *American Journal of Botany.* 91(10):1700-1708.
- López-Caamal A and Tovar-Sánchez E. 2014. Genetic, morphological, and chemical patterns of plant hybridization. *Revista Chilena de História Natural.* 87: 16.
- Loren H. Rieseberg LH, Sinervo B, Linder CR, Ungerer MC and Arias DM. 1996. Role of Gene Interactions in Hybrid Speciation: Evidence from Ancient and Experimental Hybrids. *Science.* 272(5262):741-745.
- Lubinsky P, Cameron KM, Molina MC, Wong M, Lepers-Andrzejewski S, Gómez-Pompa A and Kim S. 2008. Neotropical roots of a polynesian spice: The hybrid origin of *Tahitian vanilla*, *Vanilla tahitensis* (Orchidaceae). *American Journal of Botany.* 95(8):1040–1047.
- Mallet J. 2005. Hybridization as an invasion of the genome. *Trends in Ecology and Evolution.* 20:229–237.
- Mallet J. 2007. Hybrid speciation. *Nature.* 446.
- Martin FW. 1959. Stainingand observing pollen tubes in the style by means of fluorescence. *Stain Technology.* 34(3):125-128.

- Martinelli G. 1997. Biologia reprodutiva de bromeliaceae na reserva ecológica de Macaé de Cima. In: Lima H. C., Guedes-Bruni R. R. (eds.) Serra de Macaé de Cima: diversidade florística e conservação em Mata Atlântica. Jardim Botânico do Rio de Janeiro, Rio de Janeiro, pp. 213– 250.
- Martinelli G, Vieira CM, Gonzalez M, Leitman P, Piratininga A, Costa A and Forzza RC. 2008. Bromeliaceae da Mata Atlântica: lista de espécies, distribuição e conservação. *Rodriguésia.* 59: 209–258.
- Matallana G, Godinho MAS, Guilherme FAG, Belisario M, Coser TS and Wendt T. 2010. Breeding systems of Bromeliaceae species: evolution of selfing in the context of sympatric occurrence. *Plant Systematic and Evolution.* 289:57–65.
- Matallana G, Oliveira PE, Silva PRR and Wendt T. 2016. Post-pollination barriers in an assemblage of Bromeliaceae in south-eastern Brazil. *Botanical Journal of the Linnean Society.* 181:521–531.
- Mayr E. 1947. Ecological factors in speciation. *Evolution*. 1(4): 263-288.
- McIntosh ME. 2002. Plant size, breeding system, and limits to reproductive success in two sister species of *Ferocactus* (Cactaceae). *Plant Ecology.* 162: 273-288.
- Meerow AW, Guy CL, Li Q and Yang S. 2000. Phylogeny of the American Amaryllidaceae Based on nrDNA ITS Sequences. *Systematic Botany.* 25(4):708-726.
- Mummenhoff K, Linder P, Friesen N, Bowman JL, Lee J, Franzke A, Nosil P, Funk Dj and Ortiz-Barrientos D. 2009. Divergent selection and heterogeneous genomic divergence. *Molecular Ecology.* 18 375–402.
- Nosil P. 2008. Speciation with gene flow could be common. News and Viewa, Journal compilation.
- Osborne OG, Kafle T, Brewer T, Dobreva MP, Hutton I and Savolainen V. 2020. Sympatric speciation in mountain roses (Metrosideros) on an oceanic island. *Philosophycal Transactions of Royal Society* B. 375:20190542.
- Paggi GM, Palma-Silva C, Silveira LCT, Kaltchuk-Santos E, Bodanese-Zanettini MH and Bered F. 2007. Fertility of *Vriesea gigantea* Gaud. (Bromeliaceae), in SouthernBrazil. *American Journal of Botany.* 94(4):683-689.
- Paggi GM, Palma-Silva C, Bodanese-Zanettini MH, Lexer C, Bered F. 2015. Limited pollen flow and high selfing rates towards geographic range limit in an Atlantic forest bromeliad. *Flora.* 211:1–10.
- Palma-Silva C, Wendt T, Pinheiro F, Barbará T, Fay MF, Cozzolino S and Lexer C. 2011. Sympatric bromeliad species (Pitcairnia spp.) facilitate tests of mechanisms involved in species cohesion and reproductive isolation in neotropical inselbergs. *Molecular Ecology.* 20:3185–3201.
- Palmé AE, Su Q, Palsson S and Lascoux M. 2004. Extensive sharing of chloroplast haplotypes among European birches indicates hybridization among *Betula pendula*, *B. pubescens* and *B. nana*. *Molecular Ecology.* 13: 167–178.
- Palmer JD. 1992. Mitochondrial DNA in Plant Systematics: Applications and Limitations. *Molecular Systematics of Plants*. Springer, Boston, MA.
- Rashid KY, BernierCC and Conner RL. 1991. Genetics of Resistance in Faba Bean Inbred Lines to Five Isolates of Ascochyta Fabae. *Canadian Journal of Plant Pathology.* 13(3):218-225.
- Rambaut A and Drummond AJ. 2009. Tracer 1.5. Program distributed by the author.
- Rambaut A. 2014. *Figtree*. Institute of Evolutionary Biology, Univ. Edinburgh.
- Ramírez-Rosas K, Aguirre-Jaimes A, Ramírez-Morillo IM and García-Franco JG. 2020. Floral biology and potential hybridization of three sympatric epiphytic bromeliads in Veracruz, Mexico. *Plant Species Biology.* 35:197–209.
- Reboud X and Zeyl C. 1994. Organelle inheritance in plants. *Heredity.* 72:132–140.
- Rieseberg LH. 1997. Hybrid origins of plant species. *Annual Revision Ecology* Systematics. 28:359–389.

- Rieseberg LH, Wood TE and Baack EJ. 2006. The nature of plant species. *Nature.* V 440.
- Sakai S and Wright SJ. 2008. Reproductive ecology of 21 coexisting *Psychotria* species (Rubiaceae): when is heterostyly lost? *Botanical Journal of the Linnean Society.* 93(1):125–134.
- Sampaio JAT, GM Paggi, CM Zanella, M Bruxel, C Palma-Silva, M Goetze, MV Büttow e F Bered. 2012. Inbreeding depression in *Vriesea gigantea*, a perennial bromeliad from Southern Brazil. *Botanical Journal of the Linnean Society.* 169:312-319.
- Sicard A, Stacey N, Hermann K, Dessoly J, Neuffer B, Bäurle I and Lenhard M. 2011. Genetics, 58epresent, and adaptive significance of the selfing syndrome in the genus *Capsella*. *The Plant Cell.* 23:3156–3171.
- Shi S, Qiu Y, Li E, Wu L, and Fu C. 2006. Phylogenetic Relationships and Possible Hybrid 58epresent *Lycoris* Species (*Amaryllidaceae*) Revealed by ITS
- Sequences. *Biochemical Genetics.* 44:5/6.
- Smith LB and Downs RJ. 1974. *Pitcairnioideae* (Bromeliaceae). *Flora Neotropica Monograph.* 14:1–660.
- Soltis SP, Soltis ED, Bennett MD and Leitch IJ. 2003. Evolution of genome size in the angiosperms. *American Journal of Botany.* 90(11):1596–1603.
- Souza EH, Carmello-Guerreiro SM, Souza FVD, Ross ML and Martinelli AP. 2016. Stigma structure and receptivity in Bromeliaceae. *Scientia Horticulturae.* 203(12):118-125.
- Strehl T. 2004. Novas espécies de Bromeliaceae do Rio Grande do Sul, Brasil. *Vidalia* 2(2):19-25.
- Sun G. 2014. Molecular phylogeny revealed complex evolutionary process in *Elymus* species. *Journal of Systematics and Evolution.* 52(6):706-711.
- Tamura K, Peterson D, Peterson N, Stecher G, Nei M and Kumar S. 2011. MEGA5: Molecular.
- Ungerer Mc, Baird Sje, Pan J and Rieseberg Lh. 1998. Rapid hybrid speciation in wild sunflowers. *PNAS.* 95:11757–11762.
- Versieux LM and Wendt T. 2007. Bromeliaceae diversity and conservation in Minas Gerais state, Brazil. *Conservations Genetic.s* 16:2989-3009.
- Wagner ND, Wöhrmann T, Öder V, Burmeister A and Weising K. 2015. Reproduction biology and chloroplast inheritance in Bromeliaceae: a case study in *Fosterella* (Pitcairnioideae). *Plant Systematics and Evolution.* 301:2231-2246.
- Wang Z, Du S, Dayanandan S, Wang D, Zeng Y and Zhang J. 2014. Phylogeny reconstruction and hybrid analysis of *Populus* (*Salicaceae*) based on nucleotide sequences of multiple single-copy nuclear genes and plastid fragments. PloS ONE 9(8):e103645.
- Welch ME and Rieseberg LH. 2007. Patterns of genetic variation suggest a single, ancient origin for the diploid hybrid species *Helianthus paradoxos*. *Evolution.* 56(11):2126-2137.
- Wendel J and Doyle J. 1998. Phylogenetic Incongruence: Window into Genome *History and Molecular Evolution.* v 10.
- Wendel Jf, Schnabel A and Seelanan T. 1995. Bidirectional interlocus concerted evolution following allopolyploid speciation in cotton (*Gossypium*). PNAS. V 92:280-284.
- Wendt T, Canela MBF, Faria APG and Rios RI. 2001. Reproductive biology and natural hybridization between two endemic species of *Pitcairnia* (Bromeliaceae). *American Journal of Botany.* 88:1760–1767.
- Wendt T, Canela MBF, Klein DE and Rios RI. 2002. Selfing facilitates reproductive isolation among three sympatric species of *Pitcairnia* (Bromeliaceae). *Plant Systematics and Evolution.* 232:201–212.
- Wendt T, Coser TS, Matallana G and Guilherme FAG. 2008. An apparent lack of prezygotic reproductive isolation among 42 sympatric species of

Bromeliaceae in southeastern Brazil. *Plant Systematic and Evolution.* 275:31-41.

- Wyatt R, Evans EA and Sorenson JC. 1992. The evolution of self-pollination in granite outcrop species of *Arenaria* (Caryophyllaceae). VI. Electrophoretically detectable genetic variation. *Systematics Botany.* 17:201–209.
- Zanella CM, Janke A, Palma-Silva C, Kaltchuk-Santos E, Pinheiro FG, Paggi GM, Soares LE, Goetze M, Büttow MV and Bered F. 2012. Genetics, Evolution and conservation of Bromeliaceae. *Genetics and Molecular Biology.* 35:1020–1026.
- Zanella CM, Palma-Silva C, Goetze M and Bered F. 2016. Hybridization between two sister species of Bromeliaceae: *Vriesea carinata* and *V. incurvata*. *Botanical Journal of the Linnean Society.* 181:491–504.
- Zeng F and Cheng B. 2014. Self-(in)compatibility inheritance and allele-specific marker development in yellow mustard (*Sinapis alba*). *Molecular Breeding*. 33:187–196.

Table 1. Mating systems: fruit set, number of flowers, number of seeds and viable seeds for each species. Number of individuals (*N*); Cross-pollination (CP); Auto-compatibility (AC); Agamospermy (AG); Control (C).

		Treatments							
		Fruit set/Number of flowers				Number of viable seeds/Seed set			
Species	*N*	CP	AC	AG	C	CP	AC	AG	C
D. hebdingii	40	0/50[a]	15/50[b]	0/50[a]	28/50[c]	0/0[a]	203/256[b]	0/0[a]	490/526[b]
Rate (%)		0	30	0	56	0	79	0	93
D. julianae	17	7/14[a]	11/14[a]	0/10[b]	9/13[a]	94/143[a]	111/194[a]	0/0[b]	111/156[a]
Rate (%)		50	79	0	69	66	57	0	71
D. choristaminea	25	10/11[a]	2/12[b]	0/9[c]	20/25[a]	132/275[a]	22/41[a]	0/0[b]	224/504[a]
Rate (%)		91	17	0	80	46	54	0	44

Means with the same letter in the rows are not significantly different by Kruskal-Wallis Test (5%).

Table 2. Reproductive success: means ± SE of plant and inflorescence height, number of flowers, fruits, seeds and seeds viability of the three *Dyckia* species.

Species	Number of plants	Plant height (cm)	Inflorescence height (cm)	Flowers (*N*)	Fruit (*N*)	Seeds (*N*)	Viable seeds (*N*/%)
D. hebdingii	10	67[a] ± 2.26	53[a] ± 2.16	88.9[a] ± 7.87	48.9[a] ± 7.27	74.3[ab] ± 13.02	60.5[a]/81.4 ± 13.41
D. julianae	8	70.1[a] ± 6.85	59.5[a] ± 6.52	30.4[b] ± 0.17	10.3[b] ± 1.59	52.75[a] ±10.91	28.1[b]/53.3 ± 0.12
D. choristaminea	10	27.6[b] ± 1.4	21.4[b] ± 1.33	11[c] ± 1.12	6.4[b] ± 1.41	115.4[b] ± 22.11	45.8[ab]/39.7 ± 10.33

Means with the same letter in the columns are not significantly different by Kruskal-Wallis (5%).
SE: Standart error.
N: Number

Table 3. Pollen tube growth: number of flowers manipulated and number of flower with pollen tube growth for each *Dyckia* species.
Manual cross-pollination (MCP); manual selfing pollination (MSP).

Species	**Treatments**			
	MCP		MSP	
	Number of flowers	Flower with pollen tube	Number of flowers	Flower with pollen tube
D. hebdingii	12	0*	15	10*
D. julianae	8	4	7	3
D. choristaminea	11	4	9	6

*Treatments significantly different by the Kruskal-Wallis Test (5%).

Table 4. Artificial crosses, including number of pollinated flowers, number of fruits produced and the percentage of viable seeds.

Interespecific crosses				
Pollen donor	Pollen receptor	Number of flowers	Number of fruits	Viable seeds (%)
D. hebdingii	*D. julianae*	15	6*	80.7
D. julianae	*D. hebdingii*	9	0	0
D. choristaminea	*D. julianae*	7	5*	78.9
D. julianae	*D. choristaminea*	10	0	0
D. hebdingii[1]	*D. choristaminea*	10	0	0
D. choristaminea[1]	*D. hebdingii*	10	0	0

*Number of fruits and viable seeds did not differ significantly between crosses by Kruskal-Wallis Test (5%).
[1] Data published in Hirsch et al., 2020

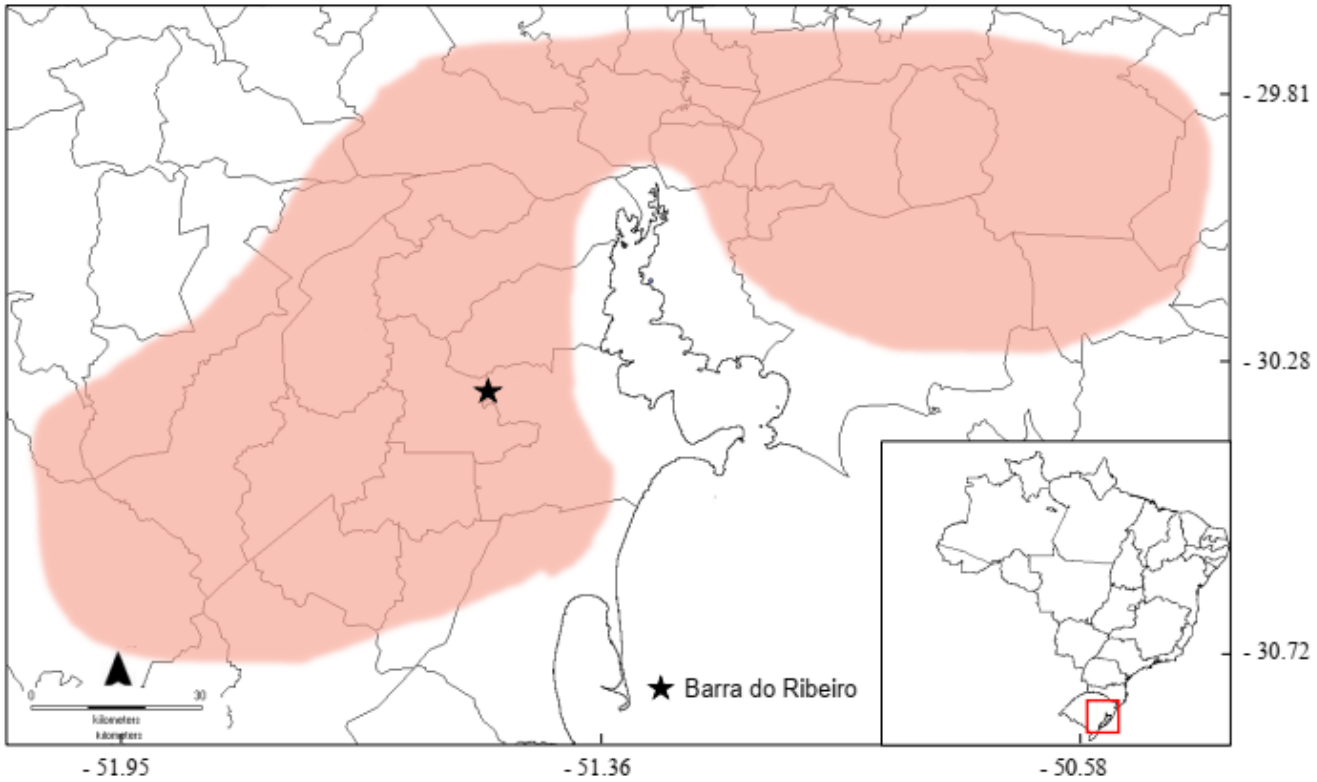

Figure 1. Geographical distribution range of *D. hebdingii*, *D. julianae* and *D. choristaminea* (red shaded area) and sympatric populations used in this study (star).

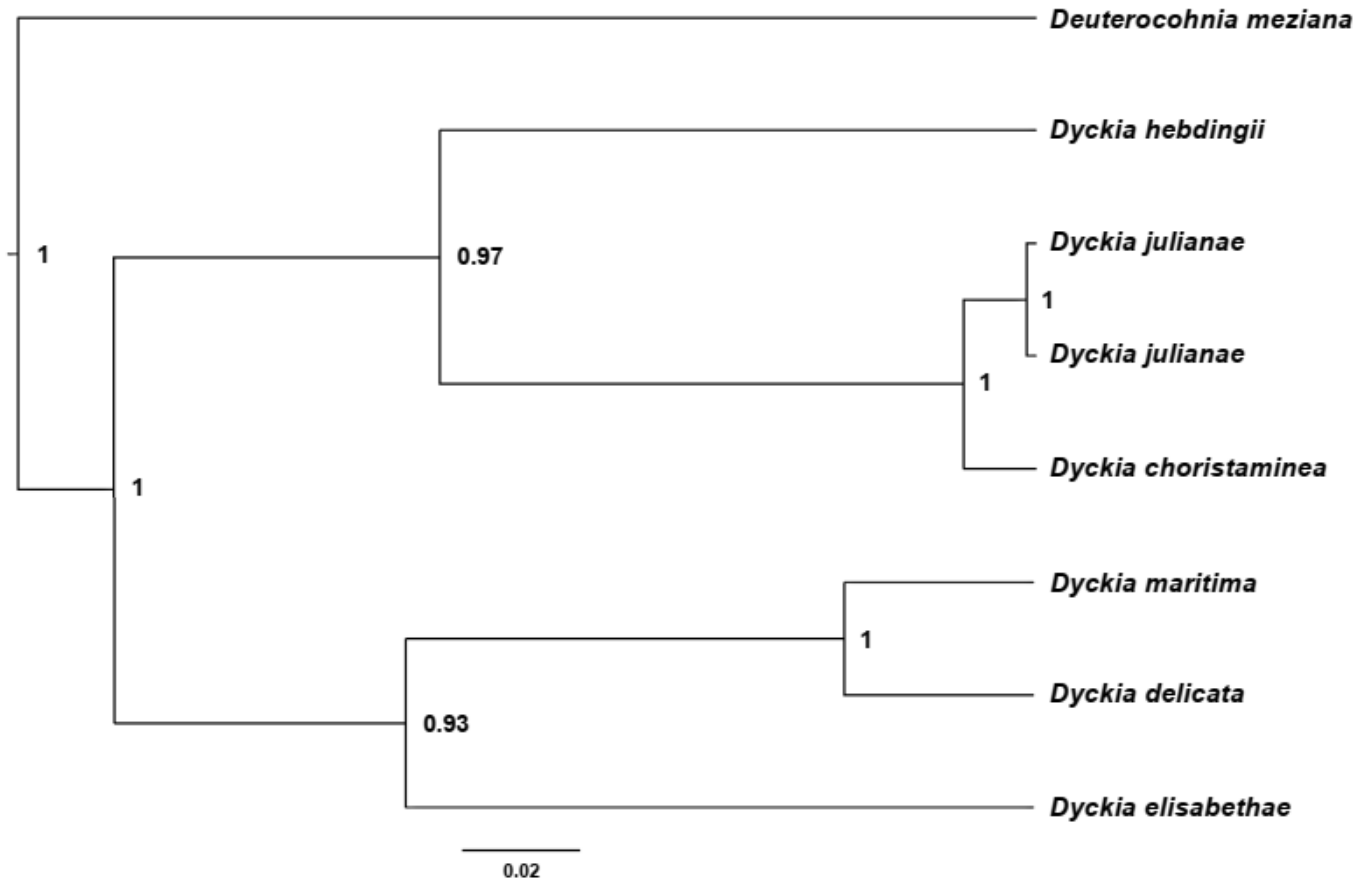

Figure 2: Phylogenetic tree based on Bayesian relaxed analysis showing placement of *D. julianae* relative to its parental species *D. choristaminea* and *D. hebdingii* in nuclear *ITS1* reconstruction with BEAST 1.8.4. Posterior probabilities are given in nodes.

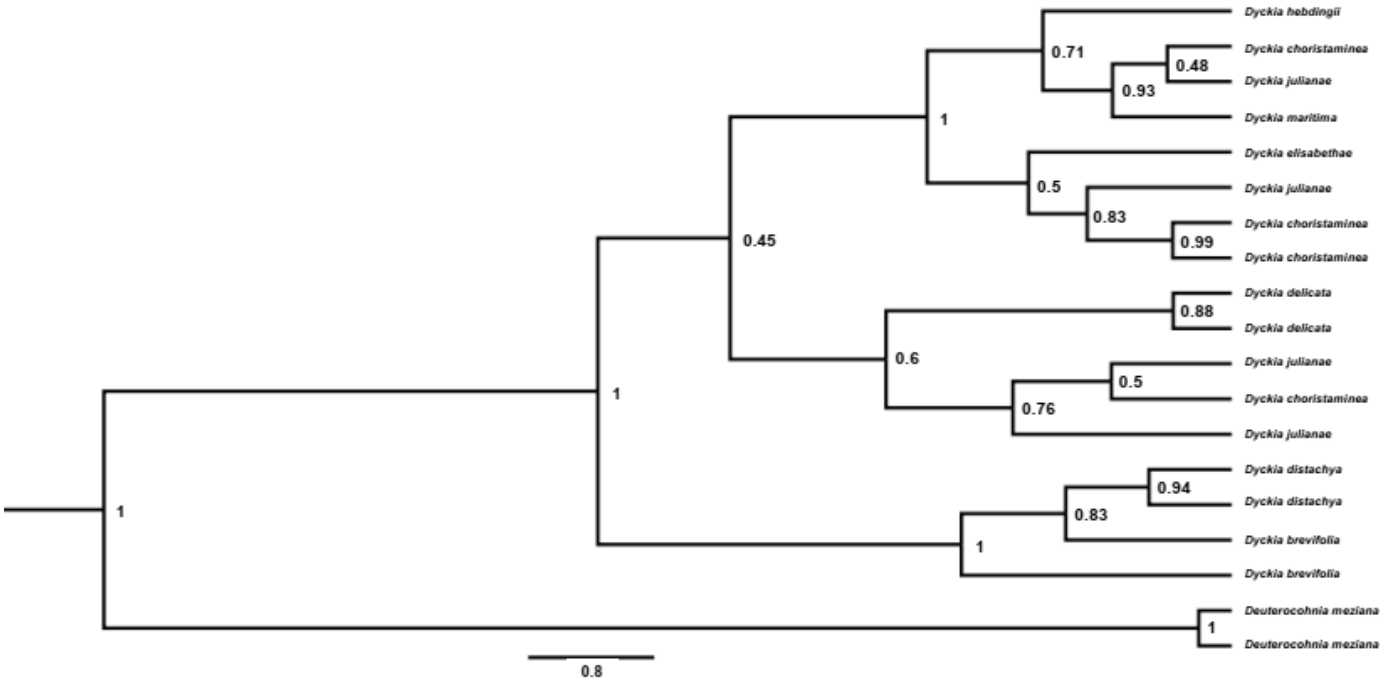

Figure 3: Phylogenetic tree based on Bayesian relaxed analysis of the combined plastid data, showing placement of *D. julianae* relative to its parental species *D. choristaminea* and *D. hebdingii*, with BEAST 1.8.4. Posterior probabilities are given in nodes.

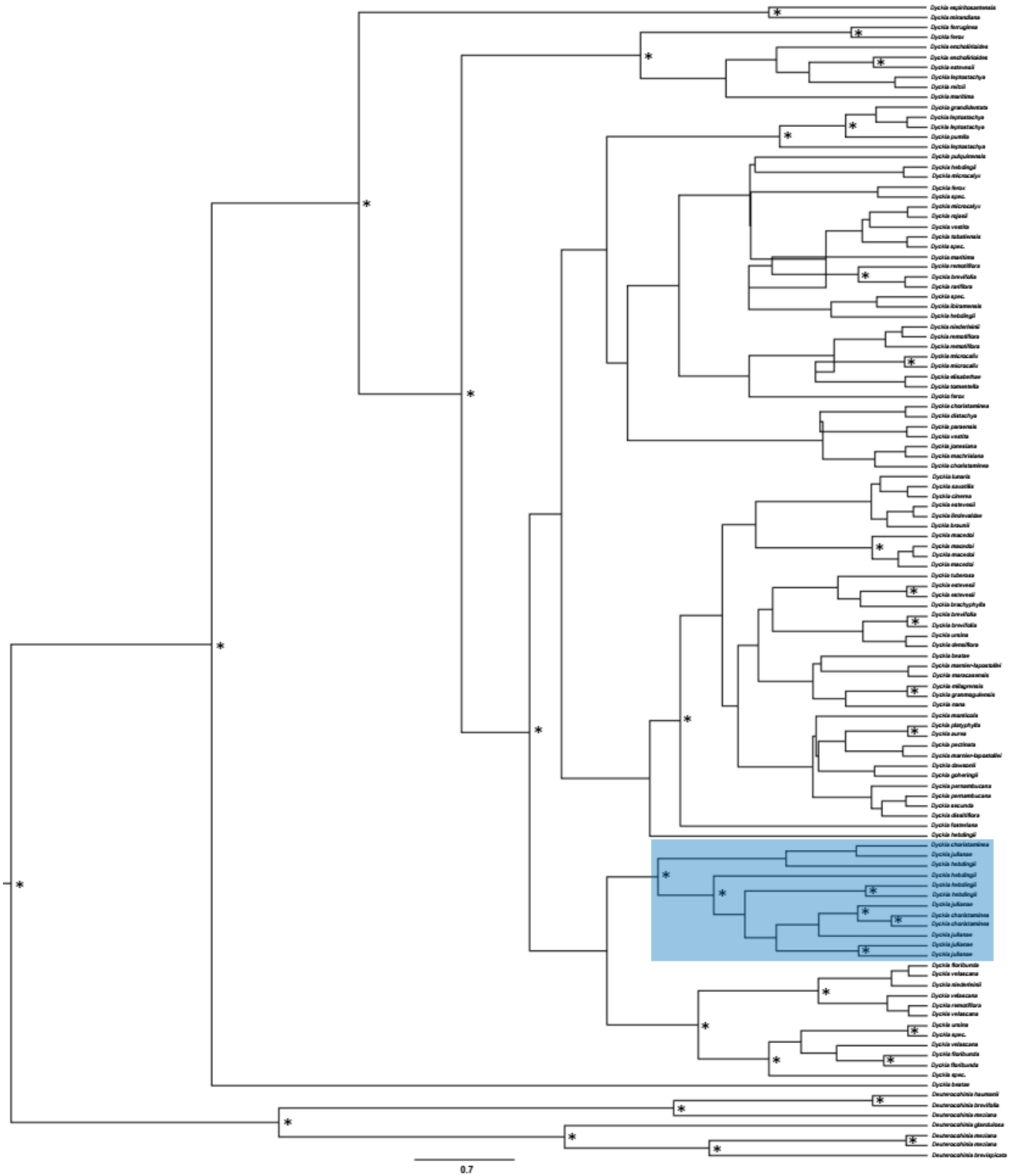

Figure 4. Plastid phylogeny of *Dyckia*, based on a Bayesian relaxed phylogenetic analysis of the combined plastid regions (*rps16-trnK* and *rpl32-trnL*) with BEAST 1.8.4. Posterior probabilities values greater than 0.90 are represented with asterisk. The shaded area represents the species collected in Barra do Ribeiro.

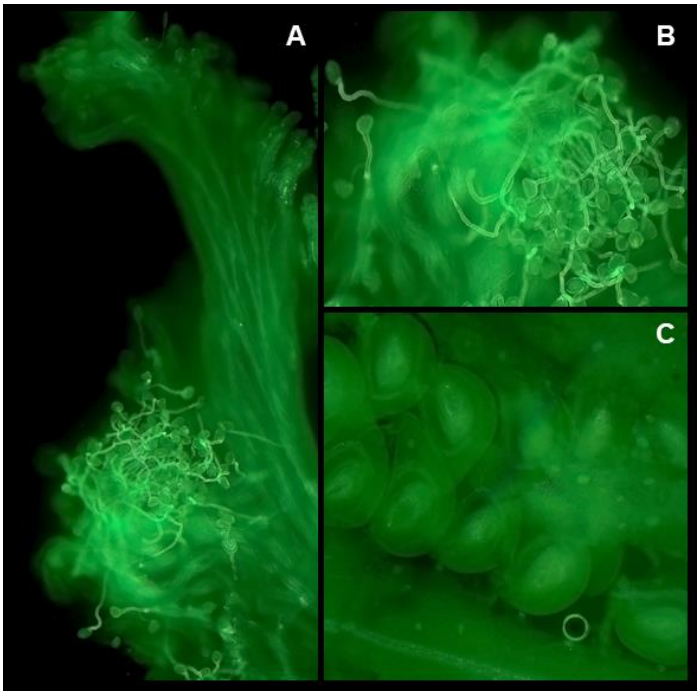

Figure 5. Pollen tube growth in *D. julianae*. (A) Stigma showing germinated pollen grains; (B) Detail of pollen grains and pollen tubes; (C) Ovules with no pollen tube penetration.

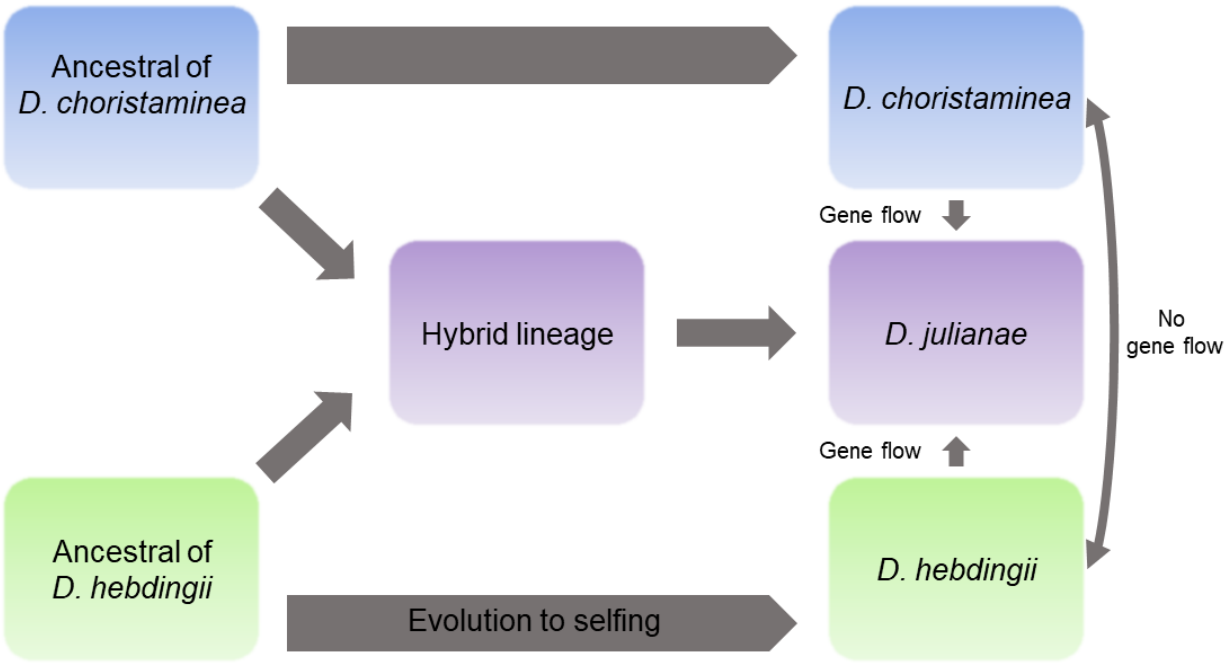

Figure 6: Scheme showing the hypothesis of hybrid origin for *D. julianae* and the gene flow between the three species.

Table S1. Studied plant material, including collected samples, greenhouse samples and GenBank accessions.

Species

Field*	**Sample identification**				
Dyckia hebdingii	48	154	372		
Dyckia julianae	111	142	148	188	304
Dyckia choristaminea	68	198	253	256	
Dyckia elisabethae	412				
Deuterocohnia meziana	316D	317D			
Greenhouse*	**Registration number**				
Dyckia brevifolia	CV0303	CV0305			
Dyckia distachya	CV0869	CV0885			
Dyckia maritima	CV1332	CV1333			
Dyckia delicata	CV1321	CV1322			
GenBank	**Accession number**				
Dyckia aurea	FK0194				
Dyckia beateae	FK0044	FK0091			
Dyckia brachyphylla	FK0045				
Dyckia braunii	FK0042				
Dyckia brevifolia	FK0010	FK0067			
Dyckia choristaminea	FK0021	FK0040			
Dyckia cinerea	FK0047				
Dyckia dawsonii	FK0043				
Dyckia delicata	FK0184				
Dyckia densiflora	FK0213				
Dyckia dissitiflora	FK0141				
Dyckia distachya	FK0126				
Dyckia elisabethae	FK0219				
Dyckia encholirioides	FK0095				
Dyckia espiritosantensis	FK0207				
Dyckia estevesii	FK0001	FK0004	FK0005	FK0033	
Dyckia ferox	FK0020	FK0027	FK0056		
Dyckia ferruginea	FK0090				
Dyckia floribunda	FK0052	FK0107	FK0108		
Dyckia fosteriana	FK0094				
Dyckia goehringii	FK0031				
Dyckia grandidentata	FK0183				
Dyckia granmogulensis	FK0007				
Dyckia hebdingii	FK0112	FK0013	FK0038		
Dyckia aff. Ibiramensis	FK0025				
Dyckia jonesiana	FK0217				
Dyckia leptostachya	FK0016	FK0035	FK0053	FK0115	
Dyckia lindevaldae	FK0019				
Dyckia lunaris	FK0193				
Dyckia macedoi	FK0099	FK0100	FK0101	FK0102	

Dyckia machrisiana	FK0189				
Dyckia maracasensis	FK0087				
Dyckia maritima	FK0092	FK0113			
Dyckia marnier-lapostollei	FK0029	FK0030			
Dyckia microcalyx	FK0009	FK0054	FK0111	FK0051	
Dyckia milagrensis	FK0096				
Dyckia mirandiana	FK0202				
Dyckia monticola	FK0088				
Dyckia nana	FK0191				
Dyckia niederleinii	FK0103	FK0110			
Dyckia paraensis	FK0190				
Dyckia pectinata	FK0188				
Dyckia pernambucana	FK0097	FK0098			
Dyckia platyphylla	FK0209				
Dyckia pulquinenses	FK0199				
Dyckia pumila	FK0017	FK0093			
Dyckia rariflora	FK0039				
Dyckia reitzii	FK0050				
Dyckia remotiflora	FK0011	FK0015	FK0055	FK0068	
Dyckia rojasii	FK0195				
Dyckia saxatilis	FK0036				
Dyckia secunda	FK0192				
Dyckia spec.	FK0023	FK0024	FK0049	FK0086	FK0116
Dyckia tobatiensis	FK0018				
Dyckia tomentella	FK0114				
Dyckia tuberosa	FK0206				
Dyckia ursina	FK0012	FK0089			
Dyckia velascana	FK0006	FK0104	FK0105	FK0106	
Dyckia vestita	FK0032	FK0109			
Deuterocohnia brevifolia	FK0074				
Deuterocohnia brevispicata	FK0071				
Deuterocohnia glandulosa	FK0072				
Deuterocohnia haumanii	FK0075				
Deuterocohnia meziana	FK0073				

*Leaf sampling for DNA extraction

Capítulo 5

Considerações finais

A presente tese é constituída de dois artigos que abordam a genética, evolução e biologia reprodutiva de três espécies ameaçadas de extinção, simpátricas e endêmicas dos Campos Sulinos: *Dyckia hebdingii*, *D. julianae* e *D. choristaminea*. Além das três espécies terem uma distribuição restrita, até o presente momento *D. julianae* só foi encontrada em simpatria com *D. hebdingii* e *D. choristaminea*, no município de Barra do Ribeiro, onde este estudo foi realizado.

Estudos realizados anteriormente, utilizando marcadores microssatélites revelaram padrões moleculares puros para *D. hebdingii* e *D. choristaminea* e perfil molecular intermediário para *D. julianae*. A partir desses resultados outras análises foram feitas, incluindo cruzamentos interespecíficos, indicando que *D. julianae* poderia ser uma espécie de origem híbrida.

Os resultados desse primeiro estudo impulsionaram a proposta para a presente tese a fim de elucidar outras questões envolvendo a origem de *D. julianae*. Com o objetivo de testar a hipótese de especiação hibrida, três abordagens foram utilizadas: citogenética, biologia molecular e biologia reprodutiva, todas apresentadas nesta tese.

O capítulo 3 desta tese apresenta o artigo gerado a partir dos dados de citogenética, incluindo número cromossômico, comportamento meiótico, viabilidade de pólen e tamanho de genoma. Neste estudo foi descartada a origem de *D. julianae* por alopoliploidia (hibridação seguida de poliploidia), uma vez que as três espécies apresentaram o mesmo número cromossômico (2n = 50). Esse dado não descarta e nem confirma hibridação, uma vez que poderíamos estar diante de um evento de hibridação homoploide. A alta regularidade meiótica, tanto no pareamento quanto na segregação sugere que

esse evento de hibridação não seja contemporâneo ou que as três espécies tenham uma alta homologia.

O capítulo 4 desta tese é dedicado as análises moleculares e de biologia reprodutiva. Árvores filogenéticas foram reconstruídas para três marcadores plastidiais e um nuclear com o objetivo de inferir qual a contribuição de cada espécie parental e confirmar *D. hebdingii* e *D. choristaminea* como as espécies que deram origem a *D. julianae*. Além disso, utilizando sequencias depositadas no banco de dados GenBank, pudemos reconstruir uma extensa filogenia de *Dyckia* e, pela primeira vez, posicionar *D. julianae* em uma filogenia. Ainda nesse capítulo são apresentados experimentos de biologia reprodutiva para avaliar o sistema de cruzamento, sucesso reprodutivo, crescimento do tubo polínico e cruzamento interespecífico.

Embora *D. julianae* apresente características que sugiram um status híbrido, aparentemente não se trata de um hibrido contemporâneo, mas uma espécie resultante de diversos eventos de hibridação no passado.

A proposta de especiação hibrida em *D. julianae* como um evento antigo foi baseada em diversos resultados obtidos nessa tese: a) o baixo fluxo gênico entre as espécies parentais e barreiras reprodutivas encontradas entre as espécies; b) *D. julianae* apresentou baixa taxa de erros meióticos, alta viabilidade de pólen e sementes; c) a espécie possui um sistema misto de cruzamento, sendo capaz de se reproduzir tanto por autogamia como por alogamia. Nada disso seria esperado para um hibrido recente, de primeira ou segunda geração. As análises filogenéticas confirmaram *D. hebdingii* e *D. choristaminea* como prováveis espécies parentais, possivelmente sendo *D. choristaminea* a espécie doadora do DNA plastidial. Quando uma extensa filogenia de espécies de *Dyckia* foi realizada, adicionando *D. julianae*, esses

indivíduos formaram um clado com alta suporte estatístico. Sendo assim, somando-se todas as abordagens e conjuntos de dados gerados nesta tese, nós propusemos a confirmação da hipótese de especiação híbrida de *Dyckia julianae*.

Capítulo 6

Referências Bibliográficas

- Abbott R, Albach D, Ansell S, Arntzen JW, Baird, SJE, Bierne N, Boughman J, Brelsford A, Buerkle A, Buggs R et al. 2013. Hybridization and speciation. *Journal of Evolutionary Biology.* 26:229-246.
- Abbott, RJ, Hegarty, MJ, Hiscock, SJ e Brennan, AC. 2010. Homoploid hybrid speciation in action. *Taxon.* 59:1375–1386.
- Aguilar-Rodríguez PA, Krömer T, Tschapka M, García-Franco JG, Escobedo-Sarti J e MacSwiney CMG. 2019. Bat pollination in Bromeliaceae, *Plant Ecology & Diversity.* 12 (1):1-19.
- Barbará T, Lexer C, Martinelli G, Mayo S.J, Fay MF e Heuertz M. 2008. Within-population spatial genetic structure in four naturally fragmented species of a neotropical inselberg radiation *Alcantarea imperialis*, *A. geniculata*, *A. glaziouana* and *A. regina* (Bromeliaceae). *Heredity*. 101:285–289.
- Barbará T, Martinelli G, Palma-Silva C, Fay MF, Mayo S e Lexer C. 2009. Genetic relationships and variation in reproductive strategies in four closely related bromeliads adapted to neotropical "inselbergs": *Alcantarea glaziouana*, *A. regina*, *A. geniculata* and *A. imperialis* (Bromeliaceae). *Annals of Botany.* 103:65–77.
- Barrett SHC, Colautti RI e Eckert CG. 2008. Plant reproductive systems and evolution during biological invasion. *Molecular Ecology.* 17:373–383.
- Barfuss MHJ, Samuel R, Till W e Stuessy TF. 2005. Phylogenetic relationships in subfamily *Tillandsioideae* (Bromeliaceae) based on DNA sequence data from seven plastid regions. *American Journal of Botany.* 92 (2):337-351.
- Belo GO, Souza MM, Silva GS e Lavinscky MP. 2018. Hybrids of Passiflora: *P. gardneri* versus *P. gibertii*, confirmation of paternity, morphological and cytogenetic characterization. *Euphytica*. 214:2.
- Benzing DH. 2000. Bromeliaceae: Profile of an adaptative radiation. Cambridge University Press, Cambridge, 689 p.
- Bernadello LM, Galetto L e Juliani HR. 1991. Floral nectar, nectary structure and pollinators in some Argentinean Bromeliaceae. *Annals of Botany.* 67:401–411.
- Brown GK e Gilmartin AJ. 1986. Chromosomes of the Bromeliaceae. *Selbyana.* 9:88-93.
- Brown GK e Gilmartin AJ. 1989. Chromosome numbers in Bromeliaceae. *American Journal of Botany.* 76:657-665.
- Buerkle AC, Morris RJ, Asmussen MA and Rieseberg LH. 2000. The likelihood of homoploid hybrid speciation. *Heredity.* 84:441–451.
- Burne HE, Yates CJ e Ladd PG. 2003. Comparative population structure and reproductive biology of the critically endangered shrub *Grevillea althoferorum* and two closely related more common congeners. *Biological Conservation.* 114:53–65.
- Campbell CS, Wojciechowski MF, Baldwin BG, Alice LA e Donoghue MJ. 1997. Persistent nuclear ribosomal DNA sequence polymorphism in the Amelanchier agamic complex (Rosaceae). *Molecular Biology and Evolution.* 14:81-90.
- Cascante-Marín A, Oostermeijer JGB, Wolf JHD e den Nijs JCM. 2005. Reproductive biology of the epiphytic bromeliad *Werauhia gladioliflora* in a premontane tropical forest. *Plant Biology.* 7:203-209.
- Clark WD e Clegg MT. 1990. Phylogenetic comparisons among *rbcL* sequences in the Bromeliaceae. *American Journal of Botany.* 71:115.
- Crayn DM, Winter K e Smith JAC. 2004. Multiple origins of crassulacean acid metabolism and the epiphytic habit in the Neotropical family Bromeliaceae. *Proceedings of the National Academy of Sciences of the United States of America.* 101(10):3703–3708.
- Dafni A e Firmage D. 2000. Pollen viability and longevity: pratical, ecological and evolutionary implications. In: Dafni A, Hesse M, Pacini E (eds) Pollen and Pollination. Springer, Vienna. 113-132.

- Deacon NJ, Grossman JJ, Schweiger AK, Armour I e Cavender-Bares J. 2017. Genetic, morphological, and spectral characterization of relictual Niobrara River hybrid aspens (*Populus* ×*smithii*). *American Journal of Botany.* 104(12):1-13.
- Diário oficial, Porto Alegre, terça-feira, 02 de dezembro de 2014. *Lista da Flora Ameaçada do Rio Grande do Sul* (Fundação Zoobotânica). Decreto Nº 52.109, de 19 de dezembro de 2014.
- Doležel J, Greilhuber J e Suda J. 2007. Estimation of nuclear DNA content in plants using flow cytometry. *Nature Protocols.* 2(9).
- Fachinetto JM e Tedesco SB. 2009. Número cromossômico, análise meiótica e estimativa da viabilidade polínica em populações de *Hyptis mutabilis* (Rich.) *Revista Brasileira de Plantas Medicinais.* 11(1):110-116.
- Field D, Ayre DJ, Whelan RJ e Young AG. 2008. Relative frequency of sympatric species influences rates of interspecific hybridization, seed production and seedling performance in the uncommon *Eucalyptus aggregate*. *Journal of Ecology.* 96:1198–1210.
- Floate KD, Godbout J, Lau MK, Isabel N e Whitham TG. 2016. Plant–herbivore interactions in a trispecific hybrid swarm of Populus: Assessing support for hypotheses of hybrid bridges, evolutionary novelty and genetic similarity. *The New Phytologist.* 209:832-844.
- Fogelqvist J, Verkhozina AV, Katyshev AI, Pucholt P, Dixelius C, Christin A, Rönnberg-Wästljung, Lascoux M e Berlin S. 2015. Genetic and morphological evidence for introgression between three species of willows. *BMC Evolutionary Biology.* 15:193.
- Folk RA, Soltis PS, Soltis DE e Guralnick R. 2018. New prospects in the detection and comparative analysis of hybridization in the tree of life. *American Journal of Botany*. 105:364–375.
- Forzza RC. 2005. Revisão taxonômica de *Encholirium* Mart. ex. Schult. & Schult. f. (Pitcairnioideae—Bromeliaceae). *Boletim de Botânica Universidade de São Paulo* 23(1):1–49.
- García-Franco JG, Burgoa DM e Pérez TM. 2001. Hummingbird Flower Mites and Tillandsia spp. (Bromeliaceae): Polyphagy in a Cloud Forest of Veracruz, Mexico. *Biotropica.* 33(3):538-542.
- Gilmartin, A. J., e G. K. Brown. 1987. Bromeliales, related monocots, and resolution of relationships among Bromeliaceae subfamilies. *Systematics Botany.* 12:493–500.
- Gitaí J, Horres R e Benko-Iseppon AM. 2005. Chromosome features and evolution of Bromeliaceae. *Plant Systematics and Evolution.* 253:65–80.
- Gitaí J, Paule J, Zizka G, Schulte K e Benko-Iseppon AM. 2014. Chromosome numbers and DNA content in Bromeliaceae: additional data and critical review. *Botanical Journal of the Linnean Society.* 176:349–368.
- Givnish TJ, Barfuss MH, Van Ee B, Riina R, Schulte K, Horres R, Gonsiska PA, Jabaily RS, Crayn DM, Smith JA, Winter K, Brown GK, Evans TM, Holst BK, Luther H, Till W, Zizka G, Berry PE, Sytsma KJ. 2011. Phylogeny, adaptive radiation, and historical biogeography in Bromeliaceae: insights from an eight-locus plastid phylogeny. *American Journal of Botany.* 98:872–895.
- Givnish TJ, Barfuss MH, van Ee B, Riina R, Schulte K, Horres R, Gonsiska PA, Jabaily RS, Crayn DM, Smith JA, Winter K. 2014. Adaptive radiation, correlated and contingent evolution, and net species diversification in Bromeliaceae. *Molecular Phylogenetics and Evolution.* 71:55–78.
- Givnish TJ e Hahn WJ. 1992. Molecular evolution, phylogeny, and geography in the *Pitcairnioideae* (Bromeliaceae). *American Journal of Botany.* 79:145.
- Givnish TJ, Milliam KC, Millam TM, Evans JC, Hall JC, Pires PE, Berry e Sytsma KJ. 2004. Ancient vicariance or recent long-distance dispersal? Inferences about phylogeny and South American-African disjunction in Rapateaceae and Bromeliaceae based on *ndhF* sequence data. *International Journal Plant Scienc*e. 165:S35–S54.

- Givnish TJ, Milliam KC, Berry PE e Sytsma KJ. 2007. Phylogeny, adaptive radiation, and historical biogeography of Bromeliaceae inferred from *ndhF* sequence data. *Aliso.* 23:3–26.
- Givnish TJ e Smith JF. 1990. A re-examination of phylogenetic relationships among Bromeliaceae subfamilies. *American Journal of Botany*. 77:133.
- Gouda EJ, Butcher D e Gouda CS (cont.updated) Encyclopaedia of Bromeliads, Version 4. http://bromeliad.nl/encyclopedia/ University Botanic Gardens, Utrecht (accessed: 11/08/2020).
- Granato LM .2010. Comportamento meiótico em híbridos de café arabusta (*C. arabica* cv *Bourbon Vermelho* X *C. canephora* cv *Robusta*). Dissertation, Instituto Agronômico de Campinas, Campinas, São Paulo.
- Gross BL e Rieseberg LH. 2005. The Ecological Genetics of Homoploid Hybrid Speciation. *Journal of Heredity.* 96(3):241–252.
- Hamilton JA e Aitken SN. 2013. Genetic and morphological structure of a spruce hybrid (*Picea sitchensis* × *P. glauca*) zone along a climatic gradient. *American Journal of Botany.* 100:1651-1662.
- Hamzeh M, Sawchyn C, Perinet P e Dayanandan S. 2007. Asymmetrical natural hybridization between *Populus deltoides* and *P. balsamifera* (Salicaceae). *Botany.* 85:1227-1232.
- Harding K e Millam S. 2000. Analysis of chromatin, nuclear DNA and organelle composition in somatic hybrids between *Solanum tuberosum* and *Solanum sanctae-rosae*. *Theory Applications Genetics.* 101:939–947.
- Hegarty MJ e Hiscock SJ. 2004. Hybrid speciation in plants: new insights from molecular studies. *New Phytologist.* 165:411–423.
- Hersch-Green EI, Allan GJ e Whitham TG. 2014. Genetic analysis of admixture and patterns of introgression in foundation cottonwood trees (Salicaceae) in southwestern Colorado, USA. *Tree Genetics & Genomes.* 10:527-539.
- Hersch EI e Roy BA. 2007. Context-dependent pollinator behavior: an explanation for patterns of hybridization among three species of indian paintbrush. *Evolution.* 61:111–124.
- Hirsch LD, Zanella CM, Aguiar-Melo C, Costa LM e Bered F. 2020. Interspecific gene flow and an intermediate molecular profile of *Dyckia julianae* (Bromeliaceae), an endemic species from southern Brazil. *Botanical Journal of the Linnean Society.* 192:675–690.
- Hmeljevski KV, Reis A, Montagna T e dos Reis MS. 2011. Genetic diversity, genetic drift and mixed mating system in small subpopulations of *Dyckia ibiramensis*, a rare endemic bromeliad from Southern Brazil. *Conservation Genetics.* 12:761–769.
- Horres R, Zizka G, Kahl G e Weising K. 2000. Molecular Phylogenetics of Bromeliaceae: Evidence from *trnL* (UAA) Intron Sequences of the Chloroplast Genome. *Plant biology.* 2:306-315.
- Horres R, Schulte K, Weising K e Zizka G. 2007. Systematics of Bromelioideae (Bromeliaceae) – evidence from molecular and anatomical studies. *Aliso.* 23(1):27–43.
- Isoda KS, Shiraishi S, Watanabe e Kitamura K. 2000. Molecular evidence of natural hybridization between *Abies veitchii* and *A. homolepis* (Pinaceae) revealed by chloroplast, mitochondrial and nuclear DNA markers. *Molecular Ecology.* 9:1965-1974.
- Karsburg IV e Battistin A. 2006. Meiose e número cromossômico de cinco espécies da família Urticaceae do Rio Grande do Sul. *RCAA* 4:47–60.
- Kelly JK, Rasch A e Kalisz S. 2002. A method to estimate pollen viability from pollen size variation. *American Journal of Botany.* 89:1021–1023.
- Kéry M e Matthies D. 2004. Reduced Fecundity in Small Populations of the Rare Plant *Gentianopsis ciliate* (Gentianaceae). *Plant Biology.* 6:683-688.
- Krapp F, Pinangé D, Benko-Iseppon AM, Leme E e Weising K. 2014. Phylogeny and evolution of *Dyckia* (Bromeliaceae) inferred from chloroplast and nuclear sequences. *Plant Systematic and Evolution.* 300:1591–1614.

- Leitch IJ e Bennett MD. 1997. Polyploidy in angiosperms. *Trends Plant Science.* 12:470–476.
- Leitch IJ, Johnston E, Pellicer J, Hidalgo O e Bennett MD. 2019. Angiosperm DNA C-values database (release 9.0, Apr 2019) https://cvalues.science.kew.org/
- Leme EMC e Marigo LC. 1993. Bromélias na natureza. Marigo Comunicação Visual Ltda Rio de Janeiro 183 pp.
- Leme EMC, Ribeiro OBC e Miranda ZJG. 2012. New species of *Dyckia* (Bromeliaceae) from Brazil. *Phytotaxa.* 67:9–37.
- Lenzi, M e Orth, AI. 2012. Mixed reproduction systems in *Opuntia monacantha* (Cactaceae) in Southern Brazil. *Brazilian Journal of Botany.* 35:1.
- Lenzi M e Paggi GM. 2019. Reproductive biology of *Dyckia excelsa* Leme (Bromeliaceae): a xerophyte species from ironstone outcrops in central-western Brazil. *Plant Species Biology.* 35:97–108.
- Levin DA. 2012. Mating system shifts on the trailing edge. *Annals of Botany.* 109:613-620.
- Louzada RB, Palma-Silva C, Corrêa AM, Kaltchuk-Santos E e Wanderley MGL. 2010. Chromosome number of *Orthophytum* species (Bromeliaceae). *Kew Bulletin.* 65(1).
- Mallet J. 2005. Hybridization as an invasion of the genome. *Trends in Ecology and Evolution.* 20:229–237.
- Mallet J. 2007. Hybrid speciation. *Nature.* 446.
- Marchant CJ. 1967. Chromosome evolution in Bromeliaceae*. Kew Bull.* 21:161-170.
- Marczewski T, Ma YP, Zhang XM, Sun WB e Marczewski AJ. 2016. Why is population information crucial for taxonomy? A case study involving a hybrid swarm and related varieties. *AoB Plants.* 8: plw070.
- Martinelli G e Moraes MA. 2013. O livro vermelho da flora do Brasil. NCFLORA – Centro Nacional de Conservação da Flora p. 1055 – 1056.
- McIntosh ME. 2002. Plant size, breeding system, and limits to reproductive success in two sister species of *Ferocactus* (Cactaceae). *Plant Ecology.* 162:273-288.
- Mendes SP, Duarte-Silva E, Kaltchuk-Santos E, Mariath JEA, Vieira RC, and De Toni KLG. 2016. A case of male sterility in the endangered endemic species *Pitcairnia encholirioides* L.B.SM. (Bromeliaceae) of brazilian atlantic forest inselbergs. *International Journal of Plant Science.* 177(6):498–510.
- Moura MN, Forzza FC e Cristiano MP. 2018. Reconstruction of ancestral genome size in *Pitcairnioideae* (Bromeliaceae): what can genome size tell us about the evolutionary history of its five genera? *Botanical Journal of the Linnean Society.* 186:321–333.
- Paggi GM, Palma-Silva C, Bodanese-Zanettini MH, Lexer C, Bered F. 2015. Limited pollen flow and high selfing rates towards geographic range limit in an Atlantic forest bromeliad. *Flora.* 211:1–10.
- Palma-Silva C., Dos Santos D. G., Kaltchuk-Santos E. e BodaneseZanettini M. H. 2004. Chromosome numbers, meiotic behavior and pollen viability of species of *Vriesea* and *Aechmea* genera (Bromeliaceae) native to Rio Grande do Sul, Brazil. *American Journal of Botany.* 91:804–807.
- Palma-Silva C, Paggi GM, Felicetti RA, Ferraz R, KaltchukSantos E, Bered F et al. 2008. Meiotic behavior and pollen viability of wild populations of the neotropical species *Vriesea gigantea* (Bromeliaceae). *Plant Species Biology.* 23:.217–221.
- Pinangé DSB, Louzada RB, Wöhrmann T, Krapp F, Weising K, Zizka G, Polo EM, Wanderley MGL e Benko-Iseppon AM. 2020. Population genetics shed light on species delimitation and life history of the *Dyckia pernambucana* complex (Bromeliaceae). *Botanical Journal of the Linnean Society.* XX: 1–20.
- Porembski S e Barthlott W. 1999. *Pitcairnia feliciana*: the only indigenous African bromeliad. *Harvard Papers in Botany.* 4:175-184.

- Ranker TA, Soltis DE, Soltis PS e Gilmartin AJ. 1990. Subfamilial relationships of the Bromeliaceae: evidence from chloroplast DNA restriction site variation. *Systematics Botany.* 15:425–434.
- Reis A, Rogalski J, Veira NK e Berkenbrock IS. 2005. Conservação de espécies reófitas de *Dyckia* no Sul do Brasil: *Dyckia distachya*. Relatório técnico 2. Florianópolis, UFSC.
- Rieseberg LH. 1995. The role of hybridization in evolution: old wine in new skins. *American Journal of Botany.* 82(7):944-953.
- Rieseberg LH. 1997. Hybrid origins of plant species. *Annual Revision Ecology Systematics*. 28:359–389.
- Rieseberg LH, Ellstrand NC e Arnold M. 1993. What Can Molecular and Morphological Markers Tell Us About Plant Hybridization? *Critical Reviews in Plant Sciences.* 12(3):213-241.
- Rogalski JM, Reis A, Reis MS e Hmeljevski KV. 2009. Reproductive biology of the ryophyte *Dyckia brevifolia* Baker (Bromeliaceae) in the Itajaí-Açu River, Santa Catarina, Brazil. *Brazilian Journal of Botany.* 32:691–702.
- Rogalski JM, Reis A, Rogalski M, Montagna M e Reis MS. 2017. Mating System and Genetic Structure Across All Known Populations of *Dyckia brevifolia*: A Clonal, Endemic, and Endangered Rheophyte Bromeliad. *Journal of Heredity.* 108(3):299–307.
- Sampaio JAT, GM Paggi, CM Zanella, M Bruxel, C Palma-Silva, M Goetze, MV Büttow e F Bered. 2012. Inbreeding depression in *Vriesea gigantea*, a perennial bromeliad from Southern Brazil. *Botanical Journal of the Linnean Society.* 169:312-319.
- Santos-Silva F, Saraiva DP, Monteiro RF, Pita P, Mantovani A, Forzza RC. 2013. Invasion of the South American dry diagonal: what can the leaf anatomy of *Pitcairnioideae* (Bromeliaceae) tell us about it? *Flora.* 208:508–521.
- Silva HML, Vasconcelos EV, Benko-Iseppon AM, Wagner N, Weising K e Brasileiro-Vidal AC. 2016. Chromosomal features of *Fosterella* species (Bromeliaceae, Pitcairnioideae). *Botanical Journal of the Linnean Society.* 181(3):532–541.
- Silvestro D, Zizka G e Schulte K. 2014. Disentangling the effects of key innovations on the diversification of Bromelioideae (Bromeliaceae). *Evolution.* 68(1):163–175.
- Slotte T, Huang H, Lascoux M e Ceplitis A. 2008. Polyploid Speciation Did Not Confer Instant Reproductive Isolation in *Capsella* (Brassicaceae). *Molecular Biology and Evolution.* 25(7):1472–1481.
- Smith LB. 1934. Geographical evidence on the lines of evolution in the Bromeliaceae. *Botanische Jahrbücher für Systematik* 66: 446–468.
- Smith LB e Downs RJ. 1974. *Pitcairnioideae* (Bromeliaceae). *Flora Neotropica Monograph* 14: 1–660.
- Smith LB e Downs RJ. 1977. *Tillandsioideae* (Bromeliaceae). *Flora Neotropica Monograph* 14: 663–1492.
- Smith LB e Downs RJ. 1979. *Bromelioideae* (Bromeliaceae). *Flora Neotropica Monograph* 14: 1493–2142.
- Smith LB e Till W. 1998. Bromeliaceae. In: Kubitzki K. (eds) Flowering Plants · Monocotyledons. The Families and Genera of Vascular Plants vol 4. Springer, Berlin, Heidelberg.
- Soltis SP, Soltis ED, Bennett MD e Leitch IJ. 2003. Evolution of genome size in the angiosperms. *American Journal of Botany.* 90(11):1596–1603.
- Soltis SP e Soltis ED. 2000. The role of genetic and genomic attributes in the success of polyploids. *PNAS.* 97(13):7051-7057.
- Soltis SP e Soltis ED. 2009. The role of hybridization in plant speciation. *The Annual Review of Plant Biology.* 60:561-88.
- Strehl T. 2004. Novas espécies de Bromeliaceae do Rio Grande do Sul, Brasil. *Vidalia.* 2(2):19-25.

- Strehl T e Beheregaray RCP. 2006. Morfologia de sementes do gênero *Dyckia*, subfamília *Pitcairnioideae* (Bromeliaceae). *Botânica.* 57:103-120.
- Stone JL. 1995. Pollen donation patterns in a tropical distylous shrub (*Psychotria Suerrensis*; Rubiaceae). *American Journal of Botany.* 82(11):1390-1398.
- Sybenga J. 1992. Cytogenetics in plant breeding. SpringerVerlag, Berlin.
- Tacuatiá LO, Eggers L, Kaltchuk-Santos E e Souza-Chies TT. 2012. Population genetic structure of *Sisyrinchium micranthum* Cav. (Iridaceae) in Itapuã State Park, Southern Brazil. *Genetics and Molecular Biology.* 35(1):99-105.
- Versieux LM e Wendt T. 2006. Checklist of Bromeliaceae of Minas Gerais, Brazil, with notes on taxonomy and endemism. *Selbyana.* 27(2):107-146.
- Versieux LM e Wendt T. 2007. Bromeliaceae diversity and conservation in Minas Gerais state, Brazil. *Conservations Genetics.* 16:2989-3009.
- Versieux LM. 2008. Checklist and one new species of Bromeliaceae from Pico do Itambé, Minas Gerais, Brazil. *Botanical Journal of the Linnean Society.* 158(4):709–715.
- Vosgueritchian SB e Buzato S. 2006. Sexual reproduction of *Dyckia tuberosa* (Vell.) Beer (Bromeliaceae, Pitcairnioideae) and plant-animal interaction. *Brazilian Journal of Botany.* 29:433–442.
- Wendel JE, Schnabel A e Seelanan T. 1995. Bidirectional interlocus concerted evolution following allopolyploid speciation in cotton (Gossypium). Proceedings of the National Academy of Sciences USA 92:280-2.
- Wendt T, Canela MBF, Faria APG e Rios RI. 2001. Reproductive biology and natural hybridization between two endemic species of *Pitcairnia* (Bromeliaceae). *American Journal of Botany.* 88:1760–1767.
- Wendt T, Canela MBF, Klein DE e Rios RI. 2002. Selfing facilitates reproductive isolation among three sympatric species of *Pitcairnia* (Bromeliaceae). *Plant Systematics and Evolution.* 232:201–212.
- Wendt T, Coser TS, Matallana G e Guilherme FAG. 2008. An apparent lack of prezygotic reproductive isolation among 42 sympatric species of Bromeliaceae in southeastern Brazil. *Plant Systematic and Evolution.* 275:31-41.
- Wesselingh RA e Arnold ML. 2000. Pollinator behaviour and the evolution of LouiaiN iris hybrid zones. *Journal of Evolutionary Biology.* 13(2):171-180.
- Winkler S. 1980. Ursachen der Verbreitungsmuster einiger Bromeliaceae in Rio Grande do Sul (Südbrasilien). *Flora.* 170:371–393.
- Zanella CM, Janke A, Palma-Silva C, Kaltchuk-Santos E, Pinheiro FG, Paggi GM, Soares LE, Goetze M, Büttow MV e Bered F. 2012. Genetics, evolution and conservation of Bromeliaceae. *Genetics and Molecular Biology.* 35:1020–1026.
- Zanella CM, Palma-Silva C, Goetze M e Bered F. 2016. Hybridization between two sister species of Bromeliaceae: *Vriesea carinata* and *V. incurvata*. *Botanical Journal of the Linnean Society.* 181:491–504.