MAIKY LOPES PAULO

DIVERSIDADE E ESTRUTURA GENÉTICA EM *Aechmea bambusoides* (BROMELIACEAE), ESPÉCIE AMEAÇADA DA REGIÃO SUDESTE DA MATA ATLÂNTICA BRASILEIRA

Dissertação apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Botânica, para obtenção do título de *Magister Scientiae*.

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APROVADA: 25 de fevereiro de 2019.

Jorge Abdala Dergam dos Santos

Pedro Bond Schwartsburd

Jéferson Nunes Fregonezi
(Orientador)

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RESUMO

PAULO, Maiky Lopes, M.Sc., Universidade Federal de Viçosa, fevereiro, 2019. **Diversidade e estrutura genética em *Aechmea bambusoides* (Bromeliaceae), espécie ameaçada da região sudeste da Mata Atlântica brasileira**. Orientador: Jéferson Nunes Fregonezi.

Com o objetivo de contribuir para a conservação, realizamos estudo de diversidade genética e estruturação de populações da espécie ameaçada de bromélia *Aechmea bambusoides*, endêmica a Mata Atlântica. Esta espécie epífita é uma ótima bioindicadora, por ter uma forma de vida muito dependente da qualidade de hábitat florestal. Foram realizadas coletas de dados de sete populações em seis localidades. As populações da espécie foram encontradas em áreas desflorestadas e fragmentadas próximas a cursos de rios. O material vegetal foi coletado para extração de DNA e realização de análises de diversidade genética através de marcadores microssatélites (SSR). Foram realizados testes de transferência de primers de SSR, descritos em outras Bromeliaceae, visando encontrar loci polimórficos na espécie alvo. Foram transferidos oito loci, usados nas análises estatísticas de diversidade genética e estruturação dentro e entre sete populações amostradas, com um total de 92 indivíduos. As populações da espécie apresentaram valores muito baixos de diversidade genética, tal como, baixo número de alelos, baixa diversidade gênica e baixa heterozigosidade observada e esperada, além de altos valores de coeficiente de endogamia encontrada nas populações. Esses resultados são muito evidentes e diferentes dos encontrados em outras bromélias estudadas anteriormente. Esses valores mostram que a espécie está altamente ameaçada, pelo baixo número de populações conhecidas, e também pela baixa diversidade genética. Possíveis explicações para esse fato é que a espécie sofreu grande erosão genética devido a fragmentação de hábitat, que levou a deriva genética e depressão endogâmica. Também contribui o fato de que esta espécie, assim como muitas epífitas e bromélias, poder apresentar forte emdocruzamento, ocasionado pela dificuldade de dispersão a longas distâncias, principalmente em florestas fragmentadas. O resultado de estruturação está de acordo com essa hipótese de dispersão limitada, pois a espécie apresentou alta estrutura genética. As populações apresentaram três grupos genéticos, os quais apresentaram tendência geográfica da distribuição. A espécie pode estar em processo de vórtex de extinção. São necessárias estratégias de manejo e conservação *in situ* e *ex situ* para diminuir os efeitos de perda de diversidade genética existente e garantir a viabilidade e sobrevivência da espécie a longo prazo. Concluímos que é fundamental a conectividade das populações para garantir o fluxo gênico. Pelos resultados obtidos sugerimos que é necessária a revisão do status de conservação de *Aechmea bambusoides*.

ABSTRACT

PAULO, Maiky Lopes, M.Sc., Universidade Federal de Viçosa, February, 2019. **Genetic diversity and genetic structure of *Aechmea bambusoides* (Bromeliaceae), a threatened species of southeast region of Brazilian Atlantic Forest**. Advisor: Jéferson Nunes Fregonezi.

In this study we investigated the genetic diversity and genetic structuring in populations of threatened bromeliad *Aechmea bambusoides*, endemic to the Atlantic Forest. This epiphyte species is an excellent bioindicator because it has a life form that is very dependent on the quality of forest habitat. Data were collected from seven populations in six locations, in deforested and fragmented areas near river courses. The plant material was collected for DNA extraction and analysis of genetic diversity through microsatellite markers (SSR). A series of SSR primer transfer tests were performed, described in other Bromeliaceae, to find polymorphic loci in the target species. Eight loci were transferred and used in the statistical analyzes of genetic diversity and structuring within and among seven sampled populations, with a total of 92 individuals. The populations of the species presented very low values of genetic diversity, such as low number of alleles, low gene diversity and low observed and expected heterozygosity, and high inbreeding coefficient values. These results are very different from those found in other bromeliads previously studied. These values show that the species is highly threatened by the low number of known populations and also by low genetic diversity. Probably the species suffered great genetic erosion due to habitat fragmentation, which led to genetic drift and inbreeding depression. This species, as well as many epiphytes and bromeliads, can present strong inbreeding caused by the difficulty of dispersion over long distances, especially in fragmented forests. The high genetic structuring found is in agrément with this hypothesis of limited dispersion. Three genetic groups were detected, which are related to geographic distribution. The species may be in process of extinction vortex. In situ and ex situ management and conservation strategies are needed to reduce the effects of loss of current genetic diversity and to ensure long-term viability and survival of the species. We conclude that populations connectivity is essential to guarantee the gene flow. Based on obtained results we suggest that it is necessary to review the conservation status of *Aechmea bambusoides*.

1 INTRODUÇÃO GERAL

A família Bromeliaceae Juss. é caracterizada por plantas herbáceas adaptadas a uma ampla variedade de ambientes. Apresentam grande variação morfológica entre espécies, com representantes que possuem hábitos epifíticos, rupestres e terrícolas, ocorrendo do nível do mar até acima de 5.000 m, em ambientes desérticos, florestas úmidas, sobre rochas quentes e em ambientes frios, suportando inclusive temperaturas negativas (SMITH; DOWNS, 1974; BENZING, 2000; GIVNISH, 2014). Em muitas espécies as folhas em forma de roseta formam um tanque (fitotelmata) que acumula água, e na maioria das espécies as folhas apresentam milhares de escamas peltadas, que realizam a absorção aérea de água e nutrientes (BENZING, 2000). Além disso, muitas bromélias apresentam diversos metabolismos fotossintéticos, como o metabolismo C3, C4 e CAM, que possibilitam a sobrevivência dessas plantas em diferentes condições de disponibilidade hídrica (BENZING, 2000; GIVNISH, 2014).

Bromélias desempenham importante função ecológica, servindo como fonte de alimento e habitat para pequenos animais que vivem associados aos seus tanques (PINTO et al., 1995; BENZING, 2000; PAULA: SILVA 2004). Na Mata Atlântica, por exemplo, representam 30% da fonte de recursos usados por beija-flores e morcegos (SAZIMA et al*.,* 1999). Além disso a retenção de água é importante para a manutenção da umidade nos ambientes, sendo que já foram encontrados 170.000 indivíduos de bromélias em um hectare de floresta, no qual foi estimado um armazenamento de até 50.000 litros de água (FISH, 1983).

Muitas espécies de bromélias vêm sendo utilizadas por povos nativos há muito tempo e atualmente mais de 90 espécies são utilizadas com diferentes finalidades (BENNET et al., 2002), por exemplo a espécie *Bromelia antiacantha* Bertol. (gravatá) tem uso medicinal (MANETTI et al., 2010). Porém a família é mais conhecida pela espécie comestível *Ananas comosus* (L.) Merril (abacaxi), a quarta fruta tropical mais importante comercialmente, após melancia, banana e manga (CHWEE: AHMAD, 2008). As bromélias são principalmente conhecidas pelas características ornamentais que apresentam, sendo o interesse pelo cultivo de bromélias para comercialização considerado recente, no início dos anos 1990 (BERED et al., 2008). O mercado crescente resultou em um aumento da produção de bromélias, mas também incentivou o extrativismo ilegal, assim sendo a retirada de exemplares da natureza e a destruição dos hábitats são atualmente a grande ameaça às espécies da família, principalmente aquelas que possuem longos ciclos de vida (ANDRADE & DEMATTÊ 1999; COFFANI-NUNES, 2004; PEREIRA et al., 2008).

Segundo o Angiosperm Phylogeny Group (APG 2019) a família possui 69 gêneros e 3403 espécies, sendo a segunda maior família de Angiospermas em número de espécies epífitas (GENTRY, 1993; BENZING, 2000). A recente e rápida radiação de Bromeliaceae, provavelmente a partir das Guianas, alcançou ampla distribuição na região neotropical, ocorrendo desde a América do Norte (estados de Virgínia, Texas e Califórnia) até a América do Sul (norte da Patagônia) (ZANELLA et al*.,* 2012; GIVNISH et al., 2011, 2014). Apenas a espécie *Pitcairnia feliciana* (A. Chev.) Harms & Mildbr, nativa do continente africano, ocorre fora da região neotropical (LUTHER 2006; GIVNISH et al., 2011). O Brasil apresenta 1.340 espécies (39% do total), distribuídas por todos os biomas, com prevalência no Cerrado e Mata Atlântica, sendo que 1.177 (87%) destas espécies são endêmicas (LEME, 1997; FLORA DO BRASIL 2020, em construção 2019). O gênero *Aechmea* Ruiz & Pav. é o mais diverso da subfamília Bromelioideae com 276 espécies (LUTHER, 2012), e apresenta uma ampla distribuição que vai do México e Antilhas até o Uruguai e norte da Argentina (SMITH; DOWNS, 1974; BENZING, 2000). O Brasil atualmente apresenta 187 espécies, cerca de 68% do gênero (Flora do Brasil 2020, em construção 2019), distribuídas por todos os biomas, sendo a Mata Atlântica seu centro de diversidade (SMITH, 1934).

A história evolutiva da família tem sido desvendada em diferentes trabalhos, como os de Givnish et al. (2004, 2007, 2011, 2014), Bouchenak-Khelladi et al. (2014), Evans et al. (2015) e Janssen e Bremer (2004), com estimativas de datação da origem e irradiação das espécies, apesar da limitação na datação desse grupo devido as suas baixas taxas de substituição e evolução molecular. Esse padrão é, geralmente, incomum entre as herbáceas, uma vez que estas apresentam maiores taxas, influenciadas pela história de vida, curto tempo de geração e altas taxas de cruzamento (SMITH; DONOGHUE 2008). Estes autores discutem que as baixas taxas de evolução nas bromélias podem se relacionar a sua estrutura populacional e sistema de acasalamento. Datações moleculares, tal como o método do relógio molecular relaxado, podem não revelar com fidelidade a história evolutiva desse grupo (DRUMMOND et al., 2006).

A partir de dados moleculares Givnish et al. (2011) indica que a família se originou a partir do ancestral no Escudo das Guianas, cerca de 100 milhões de anos atrás (Maa) e pode ter diversificado suas linhagens a partir do gênero *Brocchinia* das Guianas cerca de de 16-13 Maa. Devido a limitação das condições de fossilização, poucos registros são disponíveis para comprovar esse longo tempo entre a origem e irradiação de Bromeliaceae (GIVNISH et al. 2011). Um fóssil de *Protonanas*, semelhante a uma bromélia de uma família diferente, é datado de 100-110 Maa (LEME et al., 2005), que se aproxima da datação de origem de Bromeliaceae segundo Givnish et al. (2011). Porém o fóssil mais recente atribuível a família é de 36 Maa na Costa Rica (SMITH; TILL, 1998).

Givnish (2007, 2011, 2014) aponta que a irradiação de Bromeliaceae, cerca de 16-13 Maa, se deu de forma centrífuga apartir das Guianas, devido a uma série de eventos paleobiogeográficos, como soerguimento e desenvolvimento das cordilheiras dos Andes e Serra do Mar, que são os dois geossistemas que abrigam a maior diversidade de Bromeliaceae. Esses trabalhos apontam que as condições de nicho ecológico, tal como clima úmido propício ao desenvolvimento do epifitismo e condições bióticas como diversificação de polinizadores e dispersores foram importantes para irradiação da família. Os traços presentes nos grupos basais de Bromeliaceae, que por um modelo de evolução contigente, possibilitaram a rápida irradiação. A diversificação da subfamília Bromelioideae é a mais recente (ca. 10 Maa), a partir da separação de grupos basais de espécies de Puyoideae no sul dos Andes. Por seguinte, se deu a migração para o escudo brasileiro, com alta especiação, principalmente de epífitas a cerca de 5,5 Maa, devido a formação de clima úmido nas planícies e cordilheiras da Serra do Mar, que é o centro de diversidade das bromelioídeas.

Bromelioideae é frequentemente considerado monofilético, com baixo suporte devido aos grupos basais dos Andes que não estão bem resolvidos dentro da subfamília (GIVNISH et al. 2011). Alguns grupos do núcleo foram bem resolvidos, mas a maioria dos gêneros não tem delimitação certa, dificultado pela baixa taxa de evolução molecular (SMITH; DONOGHUE 2008) junto a grande variação morfológica. *Aechmea* apresenta baixa resolução e alto polifiletismo, com seis origens independentes indicadas por Givnish et al. (2011) e mais ainda por Sass e Specht (2010) e Evans et al. (2015). A espécie-alvo *Aechmea bambusoides* L.B.Sm. & Reitz pertence ao subgênero *Aechmea* (SMITH; DOWNS 1974), não apresenta um posicionamento filogenético bem definido dentro do gênero. Heller et al. (2015) a enquadram dentro do complexo *Aechmea fosteriana*-*Canistrum* subgen. *Cucullatanthus* junto a *Canistrum triangulare* L. B. Sm. & Reitz*,* predominado por epífitas obrigatórias. No trabalho de Evans et al. (2015), *A. bambusoides* aparece de forma polifilética, assim como outras espécies do subgênero *Aechmea* e *Canistrum* que parecem formar um complexo de espécies.

De acordo com a Lista Nacional Oficial de Espécies da Flora Ameaçadas de Extinção (MMA, 2014) e com o Livro Vermelho da Flora do Brasil (MARTINELLI; MORAES 2013) das 2.013 espécies listadas, 201 são bromélias. Estas espécies encontram-se nas seguintes categorias de ameaça: 30 classificadas como “vulneráveis”, 111 “ameaçadas” e 60 espécies no grau de “criticamente ameaçadas”, enquanto outras 21 são classificadas como próximas de ameaça. Dentre elas está *A. bambusoides* classificada como vulnerável no Livro Vermelho da Flora Brasileira (MARTINELLI; MORAES, 2013) e na Lista Nacional Oficial de Espécies da Flora Ameaçadas de Extinção (MMA, 2014). Também é citada na Lista Vermelha da Flora Ameaçada de Minas Gerais, como vulnerável (COPAM-MG, 2008).

Aechmea bambusoides (Figura 1) é uma espécie epífita, endêmica de Florestas Estacionais Semideciduais da região do Corredor de Biodiversidade da Serra do Mar, encontrada na porção média do vale do Rio Paraíba do Sul, domínio Mata Atlântica, nos estados de Minas Gerais e Rio de Janeiro, com uma distribuição restrita, e segundo Martinelli & Moraes (2013) ocorre em uma área estimada de 6.147,79 km^2. Porém, durante a execução do presente trabalho, verificamos que existem dois erros de ocorrência no mapa do Livro Vermelho, que foram confirmados por especialistas: a região de Piranga, no estado de Minas Gerais e Nova Friburgo, no estado do Rio de Janeiro. Com a exclusão destes registros incorretos, verificou-se uma área de ocorrência da espécie de 3.022 km^2.

Aechmea bambusoides não apresenta registros em Unidades de Conservação e está vulnerável ao processo de extinção devido a atividades agropecuárias, ao extrativismo e às queimadas dos períodos secos (MARTINELLI; MORAES, 2013; MMA, 2014). É uma espécie caracterizada pela inflorescência alongada, semelhante a bambus, de onde deriva o nome da espécie. Possui brácteas conspícuas de cor rosa vibrante, pétalas amarelas e sépalas verdes (Figura 1) (SMITH; REITZ 1964). Essas características fazem de *A. bambusoides* um alvo para o extrativismo ilegal para uso ornamental.

Os Corredores de Biodiversidade são regiões que compreendem um mosaico de grande diversidade e complexidade de usos da terra e áreas protegidas, definidas para o planejamento regional de conservação como áreas-chave (PINTO 2000). Dentre essas está presente o Corredor de Biodiversidade da Serra do Mar que apresenta blocos remanescentes de florestas da Serra do Mar, Serra da Mantiqueira e planícies adjacentes, que comportam uma extensa rede de áreas protegidas (AGUIAR et al., 2003).

Figura 1 – Espécie *Aechmea bambusoides* no habitat. População (a), detalhe da inflorescência (b) (Voucher: VIC 051041.

O Corredor de Biodiversidade da Serra do Mar é compreendido pela bacia hidrográfica do rio Paraíba do Sul, no Estado de São Paulo, parte da Serra da Mantiqueira em Minas Gerias e o estado do Rio de Janeiro, tendo como limite norte o Rio Paraíba do Sul. É considerada uma das regiões mais ricas da Mata Atlântica em espécies endêmicas e ameaçadas para diversos grupos (MANNE et al., 1999; BROWN; FREITAS, 2000; COSTA et al., 2000; ROCHA et al., 2000). Apesar da sua importância ecológica na conservação da biodiversidade, quase todos remanescentes de floresta desse corredor ecológico (98,69%) são menores que 100 ha (PINTO et al., 2006).

A Mata Atlântica (*lato sensu*) sofreu grande devastação após diversos ciclos econômicos desde a colonização do Brasil, e o percentual de remanescentes bem conservados (acima de 100 hectares) é de apenas 7% (SOS MATA ATLÂNTICA e INPE, 2017). Estes dados colocam a Mata Atlântica como segundo conjunto de ecossistemas mais ameaçados do mundo atrás das florestas de Madagascar (MMA, 2010). No Estado de Minas Gerais este bioma está reduzido a cerca de 4% de sua cobertura original, sendo necessários incentivos na criação de mais áreas de proteção (COSTA et al., 1998; VERSIEUX, WENDT, 2007).

O desmatamento resultante de atividades de cultivo, pastejo, extrativismo e urbanização é diretamente prejudicial pela derrubada e substituição da vegetação nativa, diminuindo os ambientes de florestas. De forma indireta, o prejuízo do desmatamento é a geração de efeito de borda nas florestas fragmentadas, com alteração da ecologia e estabilidade das condições, que prejudicam a manutenção de sua biodiversidade, como na migração de espécies, polinização, dispersão de sementes e estabelecimento de plântulas (VIANA; PINHEIRO 1998), além de aumentar a suscetibilidade ao fogo e facilitar o acesso para extrativismo (COCHRANE; LAURANCE, 2002; NASCIMENTO; LAURANCE, 2006; BROADBENT et al., 2008). A fragmentação leva a mudanças na estrutura genética das populações pela redução do fluxo gênico, endogamia e deriva genética (BROADBENT et al., 2008; LAURANCE; VASCONCELOS, 2009).

Em espécies epífitas, esses efeitos podem ser ainda mais prejudiciais pela derrubada dos forófitos que fornecem as condições para sobrevivência desses organismos extremamente dependentes (HAMRICK; GODT, 1996; TRAPNELL, et al., 2013). Dessa forma, epífitas são consideradas bons bioindicadores de ameaças e efeitos prejudiciais em ambientes florestais (BARTELS; CHEN, 2012; CASCANTE-MARÍN et al., 2014).

Em um cenário de mudanças ambientais as espécies sobrevivem basicamente devido a três mecanismos: plasticidade fenotípica, migração e adaptação a novas condições ao longo de muitas gerações. Quando esses mecanismos falham, a probabilidade de extinção é alta (SULTAN 2000; SPIELMAN et al. 2004; GIENAPP et al. 2008; KRAMER & HAVENS 2009). O que determina a eficiência desses mecanismos, bem como a sobrevivência das espécies, é a diversidade ou variabilidade genética, sobre a qual a seleção natural atua (FISHER, 1930; KRAMER; HAVENS 2009). Como a evolução ocorre pela seleção dos caracteres mais adaptados às condições ambientais exercidas pelas pressões seletivas, é de suma importância para a sobrevivência das espécies a maior disponibilidade de diferentes características (variações morfológicas, anatômicas e fisiológicas), principalmente em ambientes em rápida mudança, pois quanto maior for a variabilidade, maior será a chance de haver caracteres adequados para as novas condições (LINHART; GRANT, 1996; BALDUMAN et al., 1999; BOULDING; HAY, 2001; JUMP; PENUELAS, 2005; WILLI, 2006; KINNISON; HAIRSON, 2007; BARRETT; SCHLUTER, 2008; JUMP et al., 2009).

Em espécies raras e ameaçadas geralmente as populações são pequenas, isoladas umas das outras, podendo apresentar grandes flutuações no número de indivíduos entre gerações, e seu tamanho pode ainda variar consideravelmente dentro das espécies e geralmente apresentam menor variação genética (BARRETT; KOHN, 1991). A perda de hábitat, fragmentação e diminuição do tamanho de populações afetam o fluxo gênico e consequentemente agrava-se a perda de variabilidade genética (KARRON, 1987; HAMRICK; GODT, 1989; ELLSTRAND; ELAM, 1993; SPIELMAN et al., 2004). Segundo Huenneke (1991), os efeitos negativos da fragmentação do habitat podem ser mais pronunciados em espécies com populações recentemente declinantes, do que em espécies com populações naturalmente raras.

A perda de variabilidade leva a aumento da endogamia, raridade alélica, perda de heterozigosidade, eventos populacionais de gargalo de garrafa e isolamento populacional, podendo entrar em um processo denominado vórtex de extinção (GILPIN; SOULE 1986). Esse fenômeno aumenta a probabilidade de extinção da espécie, pela perda gradativa de valor adaptativo (LACY, 1997; FRANKHAM et al., 2002; SOLÉ-CAVA, 2012). Em estudos de meta-análise é mostrado que há correlação positiva entre perda de variabilidade genética e diminuição do *fitness* (valor adaptativo) das espécies (REED; FRANKHAM, 2003; SPIELMAN et al., 2004; LEIMU et al*.,* 2009).

Nas plantas a diversidade genética inclui variações e habilidades na reprodução, dispersão, estabelecimento, crescimento e competividade no ambiente, sendo fundamental no recrutamento de novos indivíduos para as populações (GRØNDAHL; BODIL, 2008; HUFFORD et al., 2008). Um exemplo no qual a diversidade genética foi fundamental para sobrevivência da espécie pela rápida evolução de caracteres é o de *Brassica rapa* (mostarda de campo, Brassicaceae), onde em algumas gerações ocorreu a seleção da floração precoce, como forma de seleção à seca (FRANKS et al., 2007).

Um exemplo de estudo de impactos genéticos na perda de hábitat foi realizado no sul da Austrália Ocidental com *Tetratheca paynterae* subsp. *paynterae* (Elaeocarpaceae), espécie endêmica de afloramentos de minério de ferro. Foi verificado limitado fluxo gênico, padrão de cruzamento entre vizinhos mais próximos, dispersão e colonização limitada pela falta de disponibilidade de hábitat. A viabilidade das plantas a longo prazo também foi afetada, pois a viabilidade estava relacionada a diversidade genética que era dependente da conservação de um tamanho mínimo das populações (BUTCHER et al., 2009).

A depressão endogâmica, que é o declínio da aptidão dos filhos quando os pais são geneticamente semelhantes (parentes), é uma das ameaças genéticas à persistência de populações mais comuns em plantas ameaçadas, e seus efeitos sobre a viabilidade de populações podem durar por várias gerações (EDMANDS; TIMMERMAN, 2003). Este fenômeno tem sido cada vez mais documentado, com evidências para o aumento do tempo médio de extinção de espécies ameaçadas (FENSTER; GALLOWAY, 2000; JOHANSEN-MORRIS; LATTA, 2006; O'GRADY et al., 2006; KINNISON; HAIRSTON, 2007; ETTERSON et al., 2007; LETTERS, 2008). Em populações com alta endogamia a viabilidade das populações é prejudicada pelo aumento da frequência de genes recessivos deletérios, efeitos fundadores e diminuição da heterozigosidade, o que pode levar a um menor vigor híbrido, aumentando o risco de extinção e limitando a evolução adaptativa (BARRETT; KOHN, 1991; ELLSTRAND; ELAM, 1993; LYNCH et al., 1995; YOUNG et al., 1996; REED; FRANKHAM, 2003; WILLI et al., 2007).

Neste contexto de degradação de ecossistemas é urgente a necessidade de estudos de conservação da biodiversidade. A International Union for Conservation of Nature (IUCN) recomenda a conservação da variação genética como uma das principais ações (McNEELY et al., 1990) e é de amplo conhecimento que espécies são extintas pela atuação gradual de fatores genéticos anteriores (SPIELMAN et al., 2004). Assim a diversidade genética da espécie deve ser avaliada com cuidado nos programas de conservação e restauração ecológica, pois esta diversidade impacta não só a persistência e resiliência de populações, mas também é determinante para processos ecológicos nos ecossistemas (REUSCH et al., 2005; CRUTSINGER et al., 2006; WHITHAM et al., 2006; HUGHES et al., 2008; BAILEY et al., 2009).

A proteção da diversidade genética está incluída nos protocolos de conservação e o Brasil é signatário da Convenção sobre Diversidade Biológica, onde assumiu o compromisso de atender às metas de Estratégia Global para a Conservação de Plantas. Uma das metas é que até 2020, 75% das espécies de plantas ameaçadas estejam conservadas em coleções *ex situ*, e pelo menos 20% das espécies ameaçadas sejam utilizadas em programas de recuperação de hábitats (COSTA; BAJGIELMAN 2016).

Neste sentido a genética da conservação pode ser considerada uma disciplina de crise, pois se destina muitas vezes a identificar padrões de distribuição da diversidade genética em espécies e populações de distribuição restrita, endêmicas e ameaçadas, a fim de conservar a diversidade nas áreas de distribuição (FRANKHAM, 1995; MORITZ; FAITH, 1998; BUTCHER et al., 2009; SOLÉ-CAVA, 2012). A identificação de populações que apresentem maior variação alélica, presença de alelos exclusivos, e consequente potencial evolutivo, pode auxiliar na designação de áreas prioritárias para a conservação (MORITZ, 1994). Esses estudos também podem subsidiar o manejo das espécies e de suas áreas de ocorrência (FRANKHAM et al., 2002), bem como na captura da variação genética necessária a ser usada em programas de reintrodução de espécies e conservação *ex situ* (HOLTSFORD; HANCOCK, 1998).

Nas últimas três décadas houve aumento constante em trabalhos de genética da conservação (DeSALLE; AMATO, 2004; SOLÉ-CAVA, 2012). Esses estudos são fundamentais para o planejamento da restauração ecológica, estratégia-chave para a conservação das espécies (KRAMER; HAVENS, 2009), uma vez que bases convencionais de dados podem ser inadequadas para o planejamento, o uso de recursos naturais e em planos de conservação (PAIVA et al., 2016).

O nível de variação genética a ser detectado deve revelar diferenças individuais dentro e entre as populações, o que exige diferenciação em uma escala de tempo relativamente curta, quando comparada a estudos de filogenia molecular em níveis taxonômicos elevados, como gêneros ou famílias. Desta forma, são necessários marcadores moleculares que exibam altas taxas de mutação (HAMILTON, 2011; SOLÉ-CAVA, 2012).

Os microssatélites são adequados para este propósito pois possuem natureza co-dominate, exibem alto polimorfismo e altas taxas de mutação quando comparados a outros marcadores tradicionais (SCHLOTERER; TAUTZ, 1992; JARNE; LAGODA, 1996; SIA et al., 2000; SARTHOU et al., 2001; BOISSELIER-DUBAYLE et al., 2010; ZANELLA et al., 2012). São caracterizados por sequências formadas por um a nove pares de bases (motivos) repetidas *in tandem*, presentes em regiões codificadoras e não codificadoras do genoma de procariotos e eucariotos (FIELD; WILLS, 1996). Os motivos, na maioria das espécies, são dinucleotídicos e os mais usados em estudos de genética molecular são os di, tri e tetranucleotídicos (LI et al., 2002). Estes marcadores podem representar a diversidade do genoma quando são utilizados *loci* independentes (REED; FRANKHAM, 2003).

Microssatélites são largamente utilizados em estudos de genética de populações, genética da conservação e para indicar o adequado manejo dos recursos biológicos (CHISTIAKOV et al., 2006; MIJANGOS et al., 2014; BUPP et al., 2017; SPOLADORE et al., 2017). Kato et al. (2013) utilizaram marcadores de DNA plastidial e microssatélites nucleares para estimar a diversidade genética de *Pyrus calleryana* var. *dimorphophylla* (Rosaceae), uma espécie arbórea ameaçada e endêmica do distrito de Tóquio, no Japão central. Este estudo indicou que as estratégias de conservação adotadas não consideravam a estrutura genética encontrada nas populações. O manejo de populações naturais e mudas cultivadas poderiam causar alterações na diversidade ao longo da distribuição geográfica, e a identidade genética da espécie silvestre poderia ser perdida.

Na família Bromeliaceae a presença de *loci* conservados em diferentes espécies é relativamente comum por se tratar de um grupo de radiação recente (GIVNISH, 2011) com possibilidade alta de transferência e utilização em diferentes espécies (BARBARÁ et al., 2007; PALMA-SILVA et al., 2007; PAGGI et al., 2008; WÖRHMANN; WEISING, 2011; WÖRHMANN et al., 2012a, 2012b; ZANELLA et al., 2012; GOETZE et al., 2013). Este também é um fator importante pois permite comparações entre espécies da família, com investigações dos mecanismos envolvidos na divergência, especiação das populações, coesão e isolamento reprodutivo (NOOR; FEDER, 2006; BARBARÁ et al., 2007; PALMA-SILVA et al., 2011).

De acordo com Ruas (2018), foram realizados cerca de 45 trabalhos de genética de populações envolvendo espécies de Bromeliaceae, sendo este número considerado baixo em relação ao número de espécies da família. Em menor quantidade ainda são os trabalhos de genética da conservação envolvendo espécies ameaçadas. Esses trabalhos forneceram informações genéticas que podem ter implicações diretas na conservação. Paggi et al. (2013) sugerem que *Aechmea caudata* seja incluída na Lista Nacional Oficial de Espécies Ameaçadas devido a sua estrutura genética moderada. Barbará et al. (2009) encontraram grande diferenciação genética entre populações costeiras de *inselbergs* em quatro espécies relacionadas de *Alcantarea*. Segundo os autores, os bancos de sementes e estratégias de conservação *in situ* ou *ex situ* devem ser baseadas em um número suficiente de *inselbergs* para representar os grupos genéticos de cada espécie.

Um exemplo prático da importância de estudos de genética da conservação em bromélias foi o realizado por Hmeljevski et al. (2011) com *Dyckia ibiramensis*, uma reófita, rara, ameaçada e endêmica da Mata Atlântica do Estado de Santa Catarina. As populações habitavam uma área onde se pretendia construir uma usina hidrelétrica. A partir dos resultados obtidos, o órgão ambiental competente não aprovou a construção da usina neste local. Como conseqüência, a empresa responsável pela instalação sugeriu uma realocação da barragem, além da criação de uma área de conservação que inclui as subpopulações remanescentes da espécie.

Outro exemplo é o trabalho de Lavor et al. (2014) com *Vriesea minarum*, espécie endêmica e ameaçada do Quadrilátero Ferrífero de Minas Gerais, que empregou 10 *loci* de microssatélites em 12 populações naturais. Foi detectada baixa estruturação genética, indicando que a polinização e dispersão ainda garantem a conectividade entre as populações. Porém são necessárias estratégias de conservação *in situ*, *ex situ* e a manutenção de um banco de germosplasma, já que nesta região as atividades de mineração são intensas e ameaçam o ambiente natural desta e de outras espécies.

Por fim, a principal contribuição deste trabalho é o fornecimento de dados mais precisos para o planejamento de uso dos recursos naturais, de forma sustentável, junto a extratégias de conservação da espécie estudada, bem como do seu hábitat e biodiversidade associada.

2 OBJETIVOS

O presente trabalho tem como objetivo principal avaliar a distribuição da diversidade genética em populações de *Aechmea bambusoides* através de marcadores microssatélite, e tem como objetivos específicos:

- Transferir *loci* de microssatélites já descritos em outras espécies da família Bromeliaceae para as espécies-alvo por meio de testes com reações de PCR.

- Estimar índices de riqueza alélica, proporção de heterozigotos e coeficientes de endogamia para as populações de cada espécie e comparar com dados de outras espécies de Bromeliaceae endêmicas disponíveis na literatura.

- Avaliar o grau de estruturação genética de sete populações de *Aechmea bambusoides* em sua área de distribuição.

- Indicar possíveis populações ou locais de ocorrência das espécies que mereçam especial atenção por representarem a diversidade genética a ser preservada em futuras ações de conservação, seja na criação de áreas de preservação ou no fornecimento de matrizes para coleções *ex situ*.

A presente dissertação está organizada em capítulos que correspondem aos manuscritos a serem submetidos a periódicos da área. O artigo 1 é um *short communication* técnico sobre os experimentos de transferabilidade de loci de microssatélites descritos em outras espécies para a espécie-alvo. O artigo 2 aborda os resultados das análises genéticas populacionais utilizando os microssatélites transferidos, e as conclusões da dissertação.

Artigo 1

Transferability of eight nuclear microsatellite primers to *Aechmea bambusoides* (Bromeliaceae), a threatened species the Brazilian Atlantic Forest

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Abstract

Aechmea bambusoides is an endemic and threatened bromeliad species of Atlantic Forest, restricted to the Biodiversity Corridor of Serra do Mar, in Minas Gerais and Rio de Janeiro, Brazil. The access of diversity and genetic structure is very important to provide information for *in situ* and *ex situ* conservation. Studies in Bromeliaceae have supplied a rich source of primers for microsatellite (SSR) loci. Here, we tested 21 nuclear SSRs primers for one population of *Aechmea bambusoides* to search for polymorphisms and evaluated the utility of loci developed in other Bromeliaceae species. Eight loci showed successful amplifications, and two of them were monomorphic. The number of alleles are lower than those found in other studies, as well as the observed heterozygosity values. FIS values in this population were extremely high even when compared to the highest values available in other surveys. Habitat fragmentation and decrease in random matting could explanain the results found. The successfully amplified loci found in this study could be used in the future to investigate the genetic variability of this endangered species of the Brazilian Atlantic Forest, and these results will help future studies on conservation genetics.

Keywords: SSR, cross-amplification, threatened species, conservation genetics.

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Introduction

Aechmea bambusoides L.B.Sm. & Reitz (Bromeliaceae) (Fig. 1), is an endemic bromeliad species from Atlantic Forest, restricted to the Biodiversity Corridor of Serra do Mar, at Minas Gerais and Rio de Janeiro States, Brazil (Martinelli and Moraes 2013; Brazilian Flora 2020 under construction 2019). This epiphyte species threatened due to increasing deforestation activities and urban growth, which have caused the decline in its area of occupancy and quality of habitat, added to the climatic changes that may be affecting its propagation (Paulo and Paula 2018). Currently this species is classified as vulnerable in the Brazilian Flora Red Book (Martinelli and Moraes 2013), Official National List of Endangered Flora Species (MMA 2014) and Red List of Flora Threatened in Minas Gerais (Copam / MG 2008). However, to define this threat status, genetic diversity studies were not considered.

In order to have a conservation plan for this endangered species, it is necessary to know the genetic structure of the populations of *A. bambusoides*. For this purpose, microsatellite markers (SSRs) are suitable and widely used in the Bromeliaceae family, as it is a group of recent radiation (Givnish 2011) with many conserved loci, with high transferability between different species (Barbará et al. 2007; Palma-Silva et al. 2007; Paggi et al. 2008; Wörhmann and Weising 2011; Zanella et al. 2012; Goetze et al. 2013; Neri et al. 2015). Aiming at the conservation of *Aechmea bambusoides* through future genetic studies, our objective in this work is to optimize for this species a set of primers of microsatellite loci already described in other bromeliad species.

Materials and methods

A total of 17 individuals were collected at Cantagalo locality, in Carmo municipality, Rio de Janeiro State. For extraction of genomic DNA from fresh leaves we employed an adapted protocol based on CTAB extraction, described by Doyle and Doyle (1987).

Twenty-one SSR loci previously described were tested: *Aechmea caudata* Lindm. (Goetze et al. 2013 – loci: Ac01, Ac11, Ac25 and Ac55), *Ananas comosus* (L.) Merr. (Wöhrmann and Weising 2011 – loci: Acom12.12, Acom71.3, Acom78.4, Acom82.8 and Acom91.2), *Dyckia distachya* Hassler (Zanella et al. 2012 – loci: Dd03, Dd07, Dd10, Dd20), *Orthophytum ophiuroides* Louzada & Wand (Aoki-Gonçalves et al. 2014 – loci: Op77), *Pitcairnia albiflos* Herb. (Paggi et al. 2008 – loci: PaD07), *Vriesea simplex* (Vell) (Neri et al. 2005 – loci: Vs1, Vs2, Vs8 and Vs9), and *Vriesea gigantea* (Palma-Silva et al. 2007 – VgC01 and VgF02).

Three primers were used for each SSR locus in PCR reactions: a forward SSR-specific primer with the M13 tail at its 5' end, a reverse locus-specific primer, and a universal M13 (5' CACGACGTTGTAAAACGAC 3') primer labeled with three fluorescent dyes (6-FAM, HEX and TAMRA, provided by Macrogen Inc., Seoul, South Korea). The microsatellite fragments were amplified in a reaction volume of 10 μL containing:10 ng of DNA template, 1X GoTaq buffer (Promega), 2 mM $MgCl_2$ (Promega), 100 μM dNTPs, 5 pmol forward primer, 10 pmol reverse primer, 1 pmol universal M13 primer and 0.5 U of GoTaq polymerase (Promega). The amplifications were performed in a Veriti thermocycler (Applied Biosystems)

For sixteen loci (Ac01, Ac11, Ac25, Ac55, Dd03, Dd07, Dd10, Dd20, VgC01, VgF02, Op77, PaD07, Vs1, Vs2, V8 and Vs9), the "touchdown" program was used following Palma-Silva (2007): 95ºC for 3 min, then 10 cycles of 94ºC for 30 s, 58ºC decreasing to 48ºC at 1ºC per cycle for 30 s, 72ºC for 30 s followed by 30 cycles of 94ºC for 30 s, 48ºC for 30 s, 72ºC for 30 s, followed by a final extension of 30 min at 72ºC before cooling down to 4ºC.

For five SSR loci (Acom12.12, Acom71.3, Acom78.4, Acom82.8 and Acom91.2) the program used following Wohrmann et al. (2011) was: 94ºC for 6 min, then 11 cycles of

94ºC for 45 s, 65ºC decreasing to 54ºC at 1ºC per cycle for 30 s, 72ºC for 45 s followed by 19 cycles of 94ºC for 45 s, 54ºC for 30 s, 72ºC for 30 s, followed by a final extension of 8 min at 72ºC before cooling down to 4ºC.

Microsatellite alleles were resolved on an ABI 3130XL capillary analyser (Applied Biosystems) by Macrogen Inc. (Seoul, South Korea) and were sized using Peak Scanner Software v.1.0 (Applied Biosystems). The estimates of allelic diversity and number of alleles per locus were calculated in Fstat v. 2.9.3.2 (Goudet 1995, 2001). The inbreeding coefficient (Fis) was estimated by Genepop v. 4.7.0 (Rousset 2008). Arlequin 3.5 software (Excoffier and Lischer, 2010) was used estimate values of observed heterozygosity (Ho), expected heterozygosity (He) and to perform the test for departure from Hardy–Weinberg equilibrium (HWE).

Results and discussion

Of the 21 primers tested in the present study, 8 showed successful amplifications: Ac01, Acom12.12, Dd10, Dd20, Op77, PaD07, VgC01 and Vs2. Loci Dd10 and Vs2 were monomorphic in the sampled population. The other six loci were polymorphic, with an average allele number of 2.5 (2-4). The average observed heterozygosity (Ho) was 0.035, and the average expected heterozygosity (He) was 0.299. Fis values were extremely high, ranged from 0.7400 (locus Ac01) to 1.0 (loci Acom12.12, Op77, and PaD07). Deviations from HWE were found in five loci, where levels of observed heterozygosity were much lower than expected (Table 1).

The number of alleles for the transferred loci in *A. bambusoides* population are lower than those found in other studies, such as Dd20 (3 alleles in this study, compared to 7 alleles found by Zanella et al. 2012), as well as two loci that showed the presence of only one allele (Dd10 and Vs2). For the Vs2 loci, for example, Neri et al. (2015) found 8 alleles. The only locus that showed similar amounts of alleles with other studies was the Ac01 (4 alleles were also found in *Aechmea caudata*, Goetze et al. 2013). In addition, the observed heterozygosity values (ranging from zero to 0.07692) were very low when compared to other species, such as *Vriesea scalaris* (Ho = 0.195, Neri et al. 2017), *Aechmea coelestis* (Ho = 0.440, Lavor et al. 2014), *Bromelia antiacantha* (Ho = 0.437, Zanella et al. 2012) and *Alcantarea glaziouana* (Ho = 0.404, Barbará et al. 2007). Likewise, Fis values in this population were extremely high (0.7400 - 1,000) even when compared to the highest values available in other surveys, such as *Vriesea scalaris* (Fis = 0.797, Neri et al. 2017). Among population genetics studies in Bromeliaceae, only one species, *Guzmania monostachia*, showed similar values to those found in this study, with lowest Ho = 0.060, and Fis values ranging from 0.739 to 1,000 (Cascante-Marín et al. 2014). These authors explain these findings as a consequence of a possible population decline, followed by increased rates of inbreeding.

This scenario also seems to explain the causes that led the population of *A. bambusoides* to display these genetic patterns. The species is a threatened epiphytic bromeliad, inhabiting trees in the forests environments along streams and rivers, which were intensively explored and fragmented along the Atlantic Forest of southeastern Brazil. Habitat fragmentation and decrease in random matting could be one explanation for the it results. Moreover, the mixed cross-matting/self-compatibility reproductive system, reported in other species of epiphytic bromeliads (Cascante-Marín et al., 2014), could also lead to the large increase of homozygote individuals observed in this population.

The percentage of transferred loci for *A. bambusoides* can be considered reasonable, since 8 (38%) of 21 primers pairs provided positive amplifications. In Bromeliaceae family the presence of conserved loci in different species is relatively common because it is a group of recent radiation (Givnish 2011) with high possibility of transferability and use in different species (Barbará et al., 2007). This is an important aspect as it permits amplifications of same loci and comparisons between Bromeliaceae species. In this way, it will be possible to investigate the genetic variability of this endangered species of the Brazilian Atlantic Forest, and these results will help future studies on conservation genetics.

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Figures

Figure. 1 Specimens of *Aechmea bambusoides* in its habitat. Individuals Population (a), detail of the inflorescence (b). (Voucher: VIC 051041).

Tables

Table 1. Description and characteristics of the eigth primers transferred for one population of *Aechmea bambusoides*.

Source Species	Locus	Reference	Size PB	T_a (°C)	A	He	Ho	Fis[a]
Aechmea caudata	Ac01	Goetze et al. (2013)	263–275	TD58-48	4	0.2672	0.0714	0.7400*
Ananas comosus	Acom12.12	Wohrmann et al. (2012)	204	TD65-54	2	0.4923	0.0000	1.000*
Dyckia distachya	Dd10	Zanella et al. (2012)	248–254	TD58-48	1	-	-	-
Dyckia distachya	Dd20	Zanella et al. (2012)	227–253	TD58-48	3	0.5221	0.0625	0.8837*
Orthophytum ophiuroides	Op77	Aoki-Gonçalves et al. (2014)	151–168	TD58-48	2	0.2898	0.0000	1.000*
Pitcairnia albiflos	PaD07	Paggi et al. (2008)	233–239	TD58-48	2	0.1476	0.0000	1.000*
Vriesea gigantea	VgC01	Palma-Silva et al. (2007)	208–218	TD58-48	2	0.0762	0.0762	0.0000ns
Vriesea simplex	Vs2	Neri et al. (2015)	239–258	TD58-48	1	-	-	-

T_a: annealing temperature (TD=touchdown cycles), A: number of alleles, Ho: observed heterozygosity, He: expected heterozygosity, Fis: inbreeding coefficient. [a]Departures from Hardy–Weinberg equilibrium are indicated by asterisks ($p < 0.05$), ns: not significant. Loci Dd10 and Vs2 were monomorphic, values of He, Ho and Fis were not calculated.

Artigo 2

Low genetic diversity and high inbreeding rates in fragmented populations of the endemic and endangered *Aechmea bambusoides* (Bromeliaceae) in Brazilian Atlantic Forest: implications for conservation

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Study premise: Atlantic Forest is the biodiversity center of Bromeliaceae, in which many species are threatened by habitat loss due to forest fragmentation. *Aechmea bambusoides* is a vulnerable species occurring in a highly restricted area, with isolated populations occurring in small forest fragments. For the conservation of endangered species, studies of genetic diversity are fundamental to subsidize conservation strategies. Knowledge of the distribution of genetic diversity and structuring within and among populations makes possible to identify factors that are acting to reduce the viability of the species, as well as which populations deserve conservation priority. Thus, we aim to carry out studies and survey the genetic diversity and structuring of *Aechmea bambusoides* to indicate actions necessary for the conservation of this endangered species.

Methods: For statistical analysis of diversity and genetic structuring, eight polymorphic loci of microsatellite were used, transferred and genotyped from 92 individuals from seven populations. The diversity and alleles values (A, Rs, Gd) were calculated using the Fstat v. 2.9.3.2. The estimation of the heterozygosities (He and Ho) was performed

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in Arlequin 3.5 and for calculating the Inbreeding Coefficient (Fis) was used Genepop v. 4.7.0. Genetic differentiation among populations was estimated through AMOVA and structural coefficient (Fst) using Arlequin software. Genetic structuring was estimated in Structure software v. 2.3.4, using the Evano method for definition of the best number of structured groups (K).

Results: The species showed low genetic diversity, populations had few alleles (11-17), very low Ho (0.028 - 0.133) in relation to He (0.299 - 0.517) and low Gd (0.073 - 0.253) and Rs (mean = 1.253). The Fis was very high ranging from 0.705 to 0.949. The Fst was 0.27, a moderate-high value. The best value of K = 3, and the populations showed a geographical distribution of the genetic diversity of these three groups.

Conclusions: *Aechmea bambusoides* presents low genetic diversity, compared to other Bromeliaceae members. The species was intensely affected by habitat loss and forest fragmentation. The fragmentation led to loss of alleles and contributed to the increase of inbreeding. The high genetic structuring reveals limited gene flow. The genetic structuring of *Aechmea bambusoides* stresses the importance of habitat connectivity conservation. Thus, we conclude that it is necessary conservation actions such as in situ and ex situ conservation of the species. From the data presented it is necessary to review the threat status of the species.

Key words: bromeliad; microsatellite; SSR, conservation genetics, epiphyte, threat status.

Introduction

The knowledge of genetic diversity and genetic structure of a given species is very important to understand their biological-ecological patterns and their distribution dynamics (Ford, 1964; Pimentel, 1968; Futuyma, 1986; Grøndahl and Bodil, 2008; Hughes et al., 2008). Genetic diversity and its distribution within and between populations can support decision-making needed to conservation strategies (Moritz, 1994; Holtsford and Hancock, 1998; Frankham et al., 2002). In the case of threatened species, the maintenance of natural genetic diversity should be one of the first actions for conservation (McNeely et al., 1990), since it is widely known that some populations can be extinguished by the gradual action of previous genetic factors (Spielman et al. 2004).

The Serra do Mar Biodiversity Corridor (SMBC) region, belonging to the Atlantic Forest Biosphere Reserve approved by UNESCO, is considered one of the richest regions of the Atlantic Forest regarding endemic and endangered species (Manne et al., 1999; Brown & Freitas 2000; Costa et al., 2000; Rocha et al., 2000). Despite the ecological importance of this region, almost all remnants of Atlantic Forest (98.69%) are less than 100 ha (Pinto et al., 2006). This fragmentation has direct evolutionary effects by the loss of habitat and leads to changes in the genetic structure of populations through reduction of gene flow, inbreeding and genetic drift (Broadbent et al., 2008; Laurance and Vasconcelos, 2009).

Bromeliaceae family is highly representative in Atlantic Forest, and plays important ecological functions, as a habitat for several organisms (Pinto et al., 1995; Benzing, 2000; Paula and Silva, 2004), as food source for several species, representing about 30% feeding of hummingbirds and bats (Sazima, 1999) and especially as water reservoir in their phytothemata, an important role for humidity maintenance in forest environments (Fish, 1983).

Aechmea Ruiz & Pav. is the most diverse genus in Bromelioideae subfamily with 276 species (Luther, 2012), and presents a wide distribution, from Mexico and Antilles to Uruguay and northern Argentina (Smith and Downs, 1974; Benzing, 2000). Brazil currently harbours 187 species - 68% of the genus (Brazilian Flora 2020 under construction, 2019), distributed across all biomes, with the Atlantic Forest being its center of diversity (Smith, 1934). According to Givnish et al. (2011, 2014) epiphytes Bromeliodeae as well *Aechmea*, present very recent radiation (ca. 5.5 Maa) due the formation of Serra do Mar mountain ranges and adjacentes plans, specific niches and humidification of these regions.

Aechmea bambusoides L.B.Sm. & Reitz is an epiphytic bromeliad, endemic to SMBC and is considered threatened, classified as "vulnerable" in Brazilian Flora Red List (Martinelli & Moraes, 2013, MMA, 2014) as well as in Minas Gerais State Red List (Copam-MG, 2008). This species does not have records in Conservation Units and is threatened by the great fragmentation of forests due to agricultural activities, extractivism and fires in dry seasons. According to Brazilian Flora Red List the ocorrency extension (EOO) of *A. bambusoides* is estimated for 6.147,79 km^2 (Martinelli and Moraes, 2013).

Epiphytes can be considered indicators of forest conservation, where species diversity is related to the quality and availability of these functional groups (Bartels and Chen, 2012; Cascante-Marín et al., 2014) and with atmospheric and substrate conditions (Benzing, 1998, 2000). There is a relation and occurrence of epiphytes with demographic conditions, forest conditions age, microhabitat quality and with the conditions of the forophytes: their diversity, height, trunk diameter, bark type, as well as species-preference of trees (Johansson, 1974; Benzing, 1978; Sugden, 1981; Hietz and Hietz-Seifert, 1995; Hietz, 1999; Wolf, 2005; Flores-Palacios and García-Franco, 2006). Another factor that determines the diversity of epiphytes, as well as the number of individuals in the populations, is the seed and pollen dispersion ability, which is directly related to colonization and long-term survival in the environment (Nadkarni, 2000; Cascante-Marín et al., 2009).

Habitat fragmentation and the intrinsic barriers of the closed forest environment are important factors for the restriction of gene flow in epiphytes, leading to loss of genetic diversity in populations (Hamrick and Godt, 1996; Trapnell et al., 2013). In addition, limited seed dispersal leads to formation of populations with closely related individuals (Cascante-Marín et al., 2014). As many bromeliads are typically self-compatible, increased inbreeding is expected, which causes more genetic effects, such as inbreeding depression, which may result in an extinction vortex (Gilpin and Soule, 1986; Ellstrand and Elam, 1993; Matallana et al., 2010).

Loss of genetic diversity in plant populations should be carefully evaluated in ecological conservation and restoration programs because the genetic diversity of a species is also determinant for key ecological processes in ecosystems and this information can help to conserve the biodiversity of their distribuition range (Reusch et al., 2005; Crutsinger et al., 2006; Whitham et al., 2006; Hughes et al., 2008; Bailey et al.,2009). The designation of priority areas for conservation may be more precise through identification of those populations that have a high allelic variation or evolutionary potencial (Moritz, 1994; Frankham et al., 2002). Knowledge of genetic diversity is central to species conservation programs as it facilitates the capture of the genetic variation needed to be reintroduced in situ and preserved ex situ (Holtsford and Hancock, 1998).

Studies focusing on distribution patterns of genetic diversity in plant populations of the Atlantic Forest are scarce, especially with Bromeliaceae family, in view of their great diversity (Ruas, 2018). Despite recent studies supplied valuable information (Loh et al., 2015; Goetze et al., 2017; Neri et al., 2017; Sheu et al., 2017; Meireles and Manos, 2018), the genetic diversity of endangered species in fragmented forest environments such as the Atlantic Forest is an unexplored but very important area of research for conservation biology issues.

In view of this critical scenario of species being driven to extinction, we have the following objectives for the threatened *Aechmea bambusoides* in this study: (1) to carry out the survey and evaluation of the distribution of genetic diversity, as well as the degree of structure population of this species using microsatellite markers; (2) identify areas of conservation value; (3) provide data to support future conservation actions for the species.

Materials and methods

Study species

Aechmea bambusoides is an epiphyte that occurs preferentially in trees along rivers and tributaries, in valleys along the border region of Minas Gerais and Rio de Janeiro states. This region, called the middle portion of the Paraíba Valley, belongs to the SMBC, a mosaic of protected and fragmented areas, and a total extension of 126,000 km² in the southeast of Brazil (STCP, 2011). However, *A. bambusoides* was found and restricted to small area of 3,022 km² in SMBC (Fig 2).

Through field observation, it was possible to identify that populations have settled in fragmented regions of semideciduous forests. Often the individuals were found in isolated trees, near to highways and edges of watercouses that have been transformed into pastures, in the private farms (see photos, in Appendix S2-S6). Preliminary observations indicate that clonal reproduction is the main form of propagation in *A. bambusoides*. Seed reproduction seems to be very low, with few seedlings and isolated individuals, despite the high germination rate and large number of seeds that show their fleshy fruits (Paulo and Paula, 2018), which are dispersed by birds and frequently pollinated by hummingbirds, attracted by their colored inflorescences (Fig. 1).

Population sampling

To find the populations, previously known records from virtual herbaria were accessed, as well as specialists were consulted for searching viable habitats: forests near watercourses, especially valleys. All localities records available in herbaria with precise location were found, except for one recently found during the final period of this work, in Itamarati, MG (*D.E.F. Barbosa & G.A. Basilio 558* - CESJ 71983). Other registered locations in herbaria without georeferencing were searched. The type locality (*A. Seidel 526* - US 2427460) indicated to occur between Muriaé, MG and Ponte Nova, MG cities is likely to occur closer to the Muriaé because it is a typical valley region smaller than 400 m altitude, as well as the records (*R. Menescal, R. Bello, EMC Leme* - RB00109217) and (*R. Menescal, R. Bello, EMC Leme* - RB01207243). In the locality of the Simplício Hydroelectric Plant (*Biopreservação Consultoria e Empreendimentos - Furnas* - RB00655190) no individual was found due to the difficulty of access to the site, but this is a region close to the collections of Carmo, RJ. The record (*Heron 286* - RB00600760) indicated for occurrence in Pirapetinga, MG was not found, and by a scanning error it was plotted on the map of the Red Book (Martinelli and Morais, 2013) as a record belonging to Piranga, MG region. Another erroneously record was plotted in this same map, at Nova Friburgo, RJ, and such errors were confirmed by specialists (Martinelli, Paula 2018, personal communication), and interfered in the estimation of the EOO of *A. bambusoides*.

The populations were georeferenced with global positioning system (GPS) and we provided the geographic coordinates of the populations in Appendix 1, because with the exact location of populations, regulatory agencies can monitor these areas and ascertain compliance with the law of Areas of Permanent Protection (APPs). The map of sampled populations (Fig. 2) was designed using DIVA-GIS software v. 5.4 (Hijmans et al. 2001).

Samples of fresh leaves were collected in 2017, from August to December, during flowering and fruiting season. Individuals from different trees (forophytes) were primarily collected (Appendix S1); when they occurred in the same tree, the sampling was done in different and distant branches, to minimize clonal sampling. We aimed to sample at least 15 individuals per population, but in three localities it was not possible due to low number of individuals found. The leaves were packed with silica gel for dehydration. In total, 92 individuals from seven populations were collected from six locations in the states of Minas Gerais and Rio de Janeiro (Tab. 2). Vouchers were made from inflorescences and leaves collected in each population, and were deposited in the Herbarium of the Federal University of Viçosa - Herbarium VIC (Appendix 1).

DNA extraction and microsatellite analysis

Genomic DNA was extracted based on CTAB method described by Doyle and Doyle (1987) with modifications. Eight microsatellite loci described for different Bromeliaceae species were previously transferred to *Aechmea bambusoides* (Paulo et al., unpublished manuscript) and were used in the present study for population genetic analysis. The source species and the respective loci were: *Aechmea caudata* Lindm. (locus Ac01; Goetze et al., 2013); *Ananas comosus* (L.) Merr. (locus Acom12.12; Wöhrmann and Weising, 2011); *Dyckia distachya* Hassler (loci Dd10 and Dd20; Zanella et al., 2012); *Orthophytum ophiuroides* Louzada & Wand (locus Op77; Aoki-Gonçalves et al., 2014); *Pitcairnia albiflos* Herb (locus PaD07; Paggi et al., 2008); *Vriesea gigantea* Gaudich. (locus VgC01; Palma-Silva et al., 2007) and *Vriesea simplex* (Vell) (locus Vs2; Neri et al., 2015).

DNA fragments were obtained by PCR for each locus for all 92 individuals, following the protocols and touchdown cycling program described by Palma-Silva (2007). For locus Acom12.12 was used the touchdown cycling program described by Wöhrmann and Weising (2011). Three primers were used for each locus in separate PCR reactions: a forward SSR-specific primer with the M13 tail at its 5' end, a reverse locus-specific primer, and a universal M13 (5' CACGACGTTGTAAAACGAC 3') primer labeled with three fluorescent dyes (6-FAM, HEX and TAMRA, provided by Macrogen Inc., Seoul, South Korea). The microsatellite fragments were amplified in a reaction volume of 10 µL containing:10 ng of DNA template, 1X GoTaq buffer (Promega), 2 mM MgCl2 (Promega), 100 µM dNTPs, 5 pmol forward primer, 10 pmol reverse primer, 1 pmol universal M13 primer and 0.5 U of GoTaq polymerase (Promega). The amplifications were performed in Veriti thermocycler (Applied Biosystems). Microsatellite alleles were resolved on an ABI 3130XL capillary analyser (Applied Biosystems) by Macrogen Inc. (Seoul, South Korea) and were sized using Peak Scanner Software v.1.0 (Applied Biosystems). The estimates of allelic diversity and number of alleles per locus were calculated in Fstat v. 2.9.3.2 (Goudet 1995, 2001). The inbreeding coefficient (Fis, which represents reduction of heterozygosity of a given population due to the inexistence of random crosses within the population) was estimated by Genepop v. 4.7.0 (Rousset, 2008). Arlequin 3.5 software (Excoffier and Lischer, 2010) was used to estimate values of observed heterozygosity (Ho), expected heterozygosity (He) and to perform the test for departure from Hardy–Weinberg equilibrium (HWE). Correlation between genetic and geographic distances between populations (genetic isolation by distance) was tested using Mantel test with Genepop (Rousset, 2008), using pairwise distance matrices among populations.

Genetic differentiation among populations was estimated through the analysis of molecular variance - AMOVA (Excoffier et al., 1992), and the Fst and Rst indices were calculated in the Arlequin program. The Rst index is analogous to Fst, but more appropriate for microsatellite markers since it assumes evolutionary models such as stepwise mutation model, which more adequately explains changes in allele size (Slatkin, 1995).

Genetic structuring (number of genetically close groups within species) was estimated using the Structure software v. 2.3.4 (Pritchard et al., 2000), which calculates the probability of each individual belonging to a particular genetic group, without previous information regarding the population of origin of that individual. The definition of the best number of structured groups (K) was done by the method described by Evano et al., (2005) in the software Structure Harvester 0.6.92 (Earl and VonHoldt, 2012). The program Clumpp v. 1.1.2 (Jakobsson and Rosenberg, 2007) was used to summarize the calculated replicates of each K, and the graphical representation of the results was performed in the program Distruct v. 1.1 (Rosenberg, 2004).

Results

Eight loci used were polymorphic, with an average of 3 alleles per locus, ranging from 2 (loci Ac12.12, Op77 and VgC01) to 5 (loci Ac01); the average allelic richness (Rs) was 1.347 and the mean of the gene diversity (Gd) was 0.2103. Observed (Ho) and expected (He) heterozygosity varied at loci from 0.0126/0.09859 to 0.01515/0.61723, respectively. Almost all loci deviated from the Hardy-Weinberg equilibrium, with exception of the locus VgC01, that was not significant (P-value> 0.05). The inbreeding coefficients (Fis) ranged from 0.79518 (locus Vs2) to 0.97048 (locus Acom 12.12) with an average value of 0.884. (Table 1).

Populations sampled presented an average of 14.43 alelles, ranging from 11 to 17 in AP4 e CAN populations, respectively. The allelic richness and gene diversity were low in all populations, with Rs ranged from 1.11 (AP4) to 1.323 (ITU) and Gd ranged from 0.073 (AP4) to 0.253 (ITU). Private alleles were very low, distributed in four populations: MUR (1), AP2 (1), CAN (2) and PAL (1), and three populations did not present private alleles: AP4, ITU and LAR. All populations departed significantly from Hardy-Weinberg equilibrium, in which the observed heterozygosity values were much lower than the expected values: Ho ranged from 0.0285 to 0.133 and He ranged from 0.299 to 0.517. The coefficient of inbreeding ranged from 0.705 to 0.949, in LAR and ITU, respectively. (Table 2), presented a mean Fis of 0.860, considered very high inbreeding coeficient.

Mantel test was not significant, showing no correlation between genetic distance and geographic distance, thus not presenting isolation by distance between populations. AMOVA identified a partition of variances within and between populations of 73.88 and 26.12, respectively. Genetic differentiation among the seven populations was high, with Fst = 0.270 (Table 3).

Structure results identified three genetic groups as the most likely number, K = 3. These genetic groups detecting by Structure were present in all populations, but some of them have high attribution percentage to one of the defined groups. Genetic Group 1 (Green color in Figs. 2 and 3) were more represented in MUR (about 67% of assignment probability), PAL (about 52%) and ITU (50%) populations; Genetic Group 2 (Blue color in Figs. 2 and 3) had a predominance of occurrence in AP2 (41%) and AP4 (65%) populations; Genetic Group 3 (Red color in Figs. 2 and 3) was more frequent in CAN (46%) and LAR (44%) populations. The percentages of genetic composition of each population, assigned by Structure software, are shown in pie charts of each population in Fig. 2. The proportions of each individual and each population to the genetic groups were shown in Fig. 3.

Discussion

The genetic diversity values, expressed in number of alleles, private alleles and allelic richness, found for loci and populations were generally low when compared with those obtained in other Bromeliaceae species (Tab. 4). Observed and expected heterozygosity was extremely low, highlighting that the observed values were much smaller than expected. According to Lavor et al. (2014) this may be a general pattern for the family, since other species have low values of heterozygosity (Zanella et al. 2011; Lavor et al. 2014; Neri et al. 2017; Ruas, Universidade Federal do Rio Grande do Sul, in prep). However, these species did not present extremely low values as *A. bambusoides*, and it is important to point out that most of these studies did not include endangered species (Tab. 4). An exception is the study of Cascante-Marín et al. (2014) with *Guzmania monostachia*, which presented similar values. It is an epiphyte bromeliad that inhabits Central and South America, is considered threatened in Brazil, and has been studied in fragmented forests in Costa Rica (Martinelli and Moraes 2013; Cascante-Marín et al. 2014).

Low heterozygosity and excess of homozygotes in *A. bambusoides* can be interpreted as result of autogamy, due to mixed mating systems that this species probably presents. As in many organisms and terrestrial plants it is remarkable that mating systems influence the genetic diversity of populations (Wright 1943; Loveless & Hamrick 1984, Slatkin 1985; Hamrick and Godt 1996). Parental mating systems are common in bromeliads, especially epiphytes (Benzing 2000; Cascante-Marín et al., 2006; Matallana et al., 2010).

Genetic diversity presented in *A. bambusoides* were much lower than those found in other Bromeliaceae (Tab. 4), mainly in forest formation of Atlantic Forest, such as number of alleles, allelic diversity, allelic richness and private alleles (Abondanza 2012, Goetze et al., 2016, Neri et al., 2017, Soares et al, 2018; Costa, Universidade Federal do Rio Grande do Sul, in prep). These results suggest that *A. bambusoides* underwent a loss of alleles, probably by population decline caused by forest fragmentation (Barton & Charlesworth 1984; Eckert 1996; Tremblay & Ackerman 2001; Cascante-Marín et al., 2014), which must have led the species to genetic drift since it is more intense in small populations and can lead to allele fixation (Hedrick and Miller 1992). In *A. bambusoides*, inbreeding may have contributed even more to loss and fixation of alleles at various loci, thus greatly reducing their genetic diversity (Slatkin and Excoffier 2012). The high inbreeding rate can also be explained by the limited seed dispersion. As reported by Cascante-Marín et al. (2014) in epiphytic bromeliads, individuals from the same tree or nearby trees are half-siblings or full-siblings.

Another possible explanation for low genetic diversity is the recent speciation of *A. bambusoides* (Evans et al., 2015; Heller et al., 2015), as well as many *Aechmea* epiphytes from the Serra do Mar and adjacent plains, about 5.5 Maa (Givnish et al., 2007, 2011). The species may have arisen naturally with low genetic diversity, from a few individuals in speciation, and may still be in process of expanding their populations and genetic diversity (Barrett and Kohn 1991; Ellstrand and Elam 1993; Young et al., 1996; Willi et al., 2006). Added to this, the species for which *A. bambusoides* diverged, might have an inbreeding system, and predominate clonal reproduction, contributing to low values of genetic diversity. AMOVA results showed that 26.12% of the genetic variation was found among populations, suggesting low gene flow (Lenormand 2002), and thus presents a moderate-high Fst value (0.27), which reflects a significant genetic structure when compared to other *Aechmea* species from the Atlantic Forest (Tab. 3). Fragmented populations and genetic drift may have intensified this genetic structuring (Loveless and Hamrick 1984).

Results obtained by Structure also suggest population differentiation, and indicate a geographical distribution of genetic diversity related to latitude, hydrography and altimetry in the occurrence region of *A. bambusides*. MUR, PAL and ITU (Group 1) are located northermost, while LAR population (Group 3) is located in a central position of the distribution. The populations belonging to these groups tended to be distributed along the tributaries of the Paraíba do Sul River Basin. In contrast, populations belonging to Group 2 (mainly AP2 and AP4) occur southernmost, along tributaries near Paraíba do Sul River. (Fig. 3).

Populations belonging to Group 2 presented the lowest values of allelic richness, heterozygosity and allelic diversity, as well higher inbreeding coefficient (Tab. 2). One possible explanation is that these populations are isolated from the others, due to a series of mountains near the high mountain ranges of the Serra do Mar in the state of Rio de Janeiro (See map in Fig. 2). Regarding LAR, it seems to be one of the populations of the central region that provides some connectivity between the northern and southern populations, and although it presents most of the group 3 genotype group, also presents a considerable proportion of Group 1 and 2.

High structure in Bromeliaceae were found in species occurring in Atlantic Forest inselbergs: *Alcantarea imperialis* (Carriere) Harms. (Barbaria et al., 2007), *Alcantarea glaziouana* (Leme) J.R. Grant (Barbará et al., 2009), *Alcantarea regina* (Vell.) Harms (Barbará et al., 2009) and *Encholirium horridum* L.B.Sm (Hmeljevski et al. 2017), in highland forests of the Caatinga: *Encholirium spectabile* Mart. ex Schult.f. (Gonçalves-Oliveira et al., 2017) and cangas in the Pantanal (*Dyckia excelsa* Leme). These environments are highly restricted, occuring in isolated islands and, therefore, present high genetic structuring (Porembski, 1997; Barbará et al., 2009).

In forest environments, few studies have indicated high structuring in bromeliad populations (Tab. 3). One of these was published by Palma-Silva (2009) for *Vriesea gigantea* (Fst= 0.211). Paggi et al. (2010) reported that *V. gigantea* seeds are dispersed over short distances, and most of the seeds are released near the mother plant, which has already been observed in several species of bromeliads (Matlack, 1987; García-Franco & Rico-Gray, 1988; Mondragón & Calvo-Irabien, 2006). Limited seed dispersal among populations may be one of the explanations for the high structure of *A. bambusoides.* As described in the literature for *Aechmea* genus and Bromelioideae subfamily, *A. bambusoides* is dispersed by birds. (Fischer & Araujo, 1995; Benzing 2000, Lenzi, Matos & Orth, 2006, Paulo and Paula 2018). Forest fragmentation may be hindering the dispersion of the seed among populations (or forest fragments), despite the great flying capacity of birds (Willis 1979; Carvalho and Vaconcelos 1999; Cosson et al., 1999, Andresen 2003). Thus, the gene flow via seed may be limited to each forest fragment. Similarly, forest fragmentation seems to limit pollination, even though many bromeliads are self-pollinated, the lack of trees that can serve as stepping stones: tick, old-growth and branched (Fuchs and Hamrick 2011; Ottewell et al., 2009; Fischer and Lindenmayer 2002) for very frequent pollinators in bromeliads, such as hummingbirds and bees (Canela and Sazima 2003; Schmid et al., 2011) may limit gene flow and population connectivity (Menz et al., 2011).

The low observed number of individuals recruited via seed indicates that the fragmentation is affecting the survival and viability of seedlings. This is due to the lack of forest continuity that provides less warm, shaded and humid environment for seed germination and seedling growth (Lüttge, 2008; Pereira et al., 2009). Thus, it may be that the species is more endangered due to the lower habitat quality than low genetic diversity. Yet, further research with population viability and genetic analyzes is needed to draw conclusions about this.

Implications for conservation

Considering that the genetic diversity found in *A. bambusoides* was much lower than expected for bromeliads, this threatened species needs special attention in conservation plans. The results obtained in this study (low values of allele numbers, allelic richness, decreased heterozygosity and increased inbreeding) suggest that this species faces an extinction vortex scenario (Gilpin & Soule 1986). In this process the species can reach inbreeding depression, loss of hybrid vigor and fixation of deleterious alleles (Ellstrand and Elam 1993). These factors generate loss of fitness, which leads to difficulties in the populations viability (Lacy 1997; Frankham et al., 2002; Solé-Cava 2012). Thus, in view of the possibility of the species being more impaired over time, actions are needed that seek to diminish the detrimental effects on their survival.

In relation to the difficulty of *A. bambusoides* recruiting new individuals, it is necessary studies and tests of reproductive management of this species, with monitoring of the success in the viability of seeds, seedlings, young plants and population size. The management can also be through the crossing of individuals from different populations, in order to find more heterozygous individuals for diminish the effects of genetic variability and enable the increase of the viability of the individuals. These individuals can be kept in ex situ collections and inserted into populations.

Since that the species produces a high quantity of seeds with high germination rates (Paulo and Paula, 2018), we suggest the formation and development of a germplasm bank of all possible individuals and populations, in order to guarantee material for future studies and conservation programs. As well as the formation of ex situ collection from all populations sampled. Thus, we will send a project of conservation of this species to UPCB (Bromeliaceae Research and Conservation Unit) at Federal University of Viçosa, since this unit already carries out this type of work and has ex situ collections of several endangered species in Brazil.

In relation to the populations, we consider that the LAR population deserves conservation priority, although all of them present low genetic diversity, being necessary the capture seeds of these populations to guarantee the maximum genetic pool of the species. LAR showed higher observed heterozygosity, and higher numbers of alleles and gene diversity. Moreover, this population presented a balanced presence of genetic groups attributed to the south and north of the distribution, in relation to the other populations. This reinforces the importance of connectivity between populations in the transition from gene flow (Fischer and Lindenmayer, 2002). Preservation of habitat quality, as well as host trees for connectivity of epiphyte populations, may be the main point for conservation of this species (Fuchs and Hamrick, 2011; Ottewell et al., 2009). In this way a greater effort can be invested in the preservation of the remaining forophytes and forest environments, thus guaranteeing the distribution of genetic diversity in the populations, as well as making possible its increase.

The Law number 12.727/12 (Brazil, 2012) determines riparian environments as Permanent Protection Areas (APPs) and establishes minimum distances for the preservation of these environments according to the width of the watercourses. For example, in a river up to 10 m wide, 30 m of all vegetation to the edge of this should be preserved. The populations MUR, AP2, CAN, ITU and LAR were found within the limits of these areas. Besides that, it was observed that these areas were highly deforested by agricultural activities. If the law on the preservation of riparian enviroments was obeyed it is possible that *A. bambusoides* was more distributed and would not be assessed as threatened.

Despite this, it is still possible that populations can maintain their size, with the preservation of APPs. Especially if the recovery of these areas is encouraged, the species may increase its distribution with more individuals and populations. For this, we suggest the recovery using easily agriculturally and native species, through an agroforestry system (AFS) that can benefit farmers owners. Specimens presenting vigorous trunks and branches such as some identified as sheltering *A. bambusoides* should be used, for example *Mangifera indica* L. (hose) (Anacardiaceae) and *Guarea guidonia* (L.) Sleumer (Meliaceae).

Through the analysis and results presented in this work, we verified the need to review the conservation status of *A. bambusoides*. Currently, it is classified as “vulnerable” according to the Brazilian Red List (Martinelli and Moraes, 2013; MMA, 2014) in the classification VU B1ab(iii) according to IUCN criteria (Baillie et al., 2004). In criterion B1, the extent of occurrence (EOO) was overestimated to 6,147.79 km² due to two erroneous records (see section MATERIALS AND METHODS). According to the present survey, the corrected occurrence is an EOO of 3,022 km² (Fig. 2).

Even if there is an extrapolation to a polygon of larger area that covers other possible populations, this species is suitable for the endangered status (EN) for criterion B (1 - EOO <5,000 km²). In this criterion, the specifications “a” (severe fragmentation) and “b” ((iii) - decline of area, extension or habitat quality) should remain and "b" ((v) - decline in the number of mature individuals) should be added, due to the threat of cutting forophytes, particularly in private properties and along road edges (see Appedix S2-S6). Considering the low number of individuals and the threatening of forophytes decline, the species is also adequate in the EN classification in criterion C (number of mature individuals <2,500). For example, the existence of 2,500 mature individuals demands the existence of 25 populations with 100 individuals, which is an unlikely value based on field observations. In C criterion the specification (2 - continuous decline of mature individuals – “a” (i) - mature individuals in each subpopulation <250) is adequate for the species. Finally, the status suggested by us for *A. bambusoides* is “endangered” EN B1ab(iii,v);C2a(i).

Conclusions

Aechmea bambusoides presents low genetic diversity, compared to those found in other Bromeliaceae members. The species was intensely affected by habitat loss and forest fragmentation in the valley region of Paraíba do Sul River Basin, Serra do Mar Biodiversity Corridor. The fragmentation led to loss of alleles and contributed to the increase of inbreeding. The high genetic structuring reveals limited gene flow. Based on these results, we conclude that it is necessary in situ and ex situ conservation of the species, with management, in order to preserve the remaining genetic diversity. The genetic structuring of *Aechmea bambusoides*, and their distribution along water courses stresses the importance of habitat connectivity conservation, in order to ensure maintenance of the genetic diversity of geographically restricted species. The law of preservation of riparian environments (APPs) has not been obeyed, and is affecting the size of the populations of the species. Incentive actions are needed to recover these areas in order to promote a population increase of this bromeliad, and we suggest the development of AFS systems. The results demonstrated that the main threats to the species and their occurrence were more restricted than previously reported, so the species conservation status needs to be reviewed, which the endangered category is most indicated.

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Author contributions

M. L. P. Collected field and laboratory data, analyzed data and wrote the manuscript.

C. C. P. Indicated the location of most populations and confirmed the identification of the samples.

J. N. F. Idealized, led and guided the research. He collected data in the field, guided the collection of laboratory data, performed data analysis and wrote the manuscript.

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APPENDIX 1. Coordinates, Municipality and Voucher number of seven populations.

Population (abbrev.)	Municipality, Estate	Coordinates	Voucher number
Muriaé (MUR)	Muriaé, Minas Gerais	21°06'36.4"S 42°24'31.2"W	VIC 051037
ALPA2 (AP2)	Carmo, Rio de Janeiro	21°53'10.7"S 42°38'15.0"W	VIC 051039
Cantagalo (CAN)	Carmo, Rio de Janeiro	21°51'34.0"S 42°35'09.2"W	VIC 051040
ALPA4 (AP4)	Além Paraíba, Minas Gerais	21°44'54.9"S 42°43'50.0"W	VIC 051041
Ituí (ITU)	São João Nepomuceno, Minas Gerais	21°31'07.6"S 42°54'28.4"W	VIC 051042
Palma (PAL)	Miracema, Rio de Janeiro	21°23'11.8"S 42°14'37.4"W	VIC 051043
Laranjal (LAR)	Leopoldina, Minas Gerais	21°23'35.9"S 42°29'31.1"W	VIC 051044

FIGURES

Figure. 1. Specimens of *Aechmea bambusoides* in habitat. Population (a), detail of the inflorescence (b). (Voucher: VIC 051041).

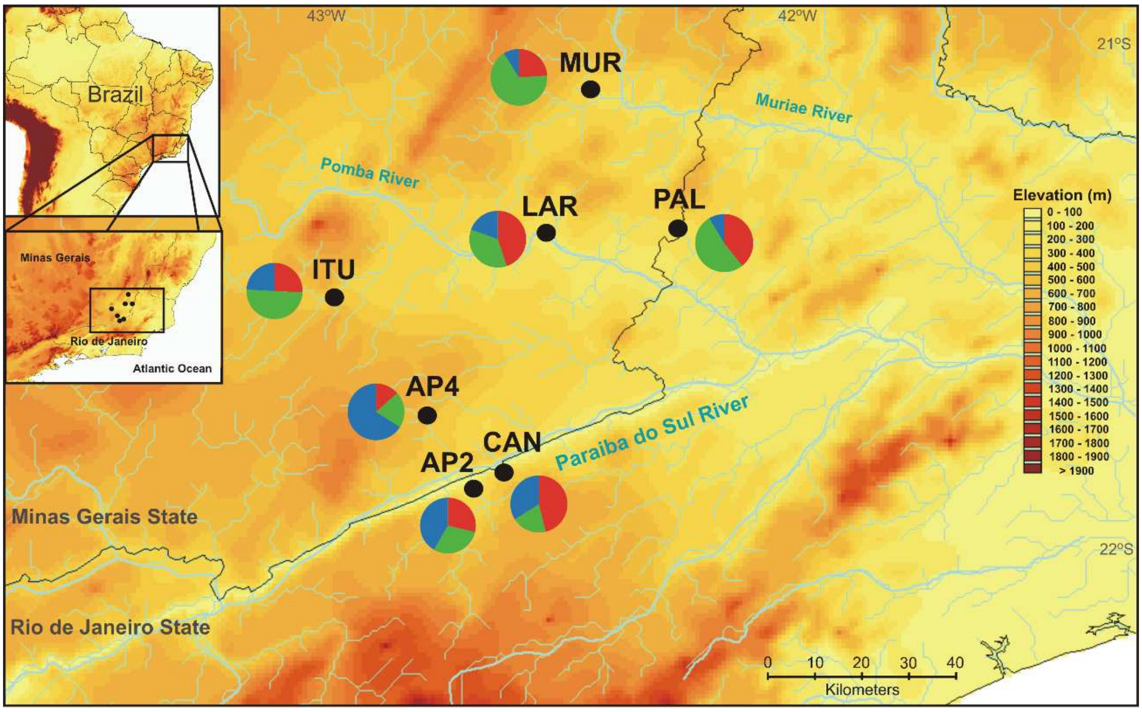


Figure 2. Map of the *Aechmea bambusoides* populations sampled in the Serra do Mar Biodiversity Corridor region (EOO = 3,022 km²) and used in the present study. The pie charts show the probability of assignment for each genetic group defined by Structure in each population. Colors correspond to genetic groups presented in Fig. 3. For population abbreviations, see Table 2.

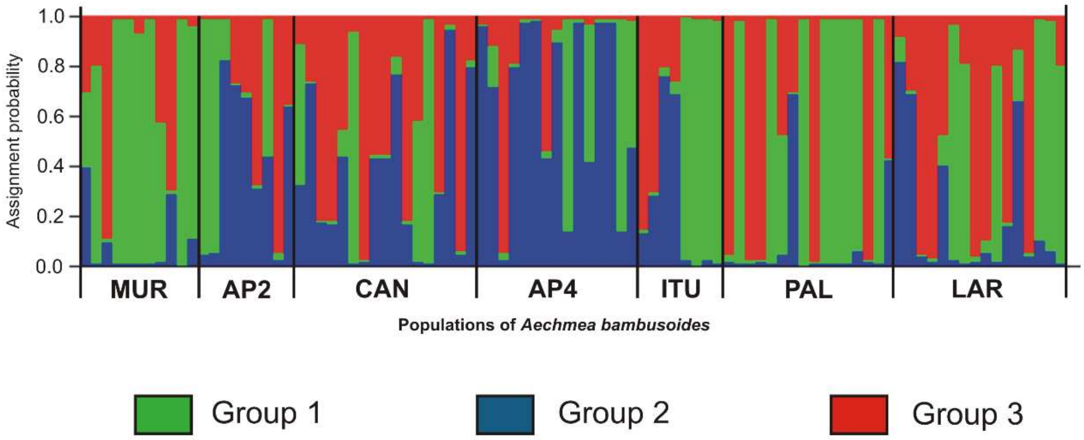


Figure. 3. Bayesian analysis generated by the STRUCTURE program of 92 individuals from seven *Aechmea bambusoides* populations. This figure shows the assignment probability of each individuals and populations in the three clusters (K=3) defined by Evano method; for population abbreviations, see Table 2.

TABLES

Table. 1. Genetic diversity values for the eight microsatellite loci in seven populations of *Aechmea bambusoides*.

Locus	A	Rs	Gd	Ho	He	Fst	Fis
Ac01	5	1.452	0.4555	0.03896	0.45242	0.355516	0.91440**
Dd10	4	1.087	0.0874	0.01266	0.08651	– 000320	0.85448**
Dd20	3	1.617	0.6213	0.04651	0.61723	0.068903	0.92505**
Acom12.12	2	1.502	0.5065	0.01493	0.50196	0.149972	0.97048**
Op77	2	1.481	0.4864	0.03922	0.48146	0.270218	0.91929**
VgC01	2	1.015	0.0152	0.01515	0.01515	– 0.019231	0.00000ns
Pad07	3	1.526	0.5292	0.09859	0.52572	0.485958	0.81355**
Vs2	3	1.093	0.0942	0.01923	0.09317	0.848273	0.79518*
Mean	3	1.347	0.2103	0.03566	0.3467	0.27	0.884633

Notes: A = number of alleles; Rs = allelic richness; Gd = gene diversity; Ho = observed heterozygosity; He = expected heterozygosity; Fst = genetic differentiation between populations; Fis = inbreeding coefficient. ns = not significant (p.0.05) departure from Hardy-Weinberg equilibrium. * P <0.01. ** P <0.001.

Table. 2. Localities and populations sampled in the states of Minas Gerais and Rio de Janeiro, number of samples per population, approximate altitude and genetic diversity values in *Aechmea bambusoides* populations.

Locality. Municipality, State	Population	Elevation (m)	N	A	Apr	Rs	Gd	Ho	He	Fis
Rio Muriaé, Mun. Muriaé, MG	MUR	220	11	15	1	1.273	0.21088	0.08636	0.43658	0.812
Rio Paquequer, Carmo, RJ	AP2	195	9	15	1	1.259	0.17811	0.05357	0.41448	0.881
Rio Paraíba do Sul, Carmo, RJ	CAN	234	17	17	2	1.225	0.14996	0.03514	0.29936	0.887
Rod. Santos Dumont, Além Paraíba, MG	AP4	260	15	11	0	1.11	0.07324	0.03846	0.43993	0.916
Rio Novo (Ituí), São João Nepomuceno, MG	ITU	300	8	13	0	1.323	0.25357	0.02857	0.51739	0.949
Rod. RJ-200, Miracema, RJ	PAL	170	16	16	1	1.307	0.24424	0.05449	0.40976	0.872
Rio Pomba (Laranjal), Leopoldina, MG	LAR	165	16	14	0	1.274	0.21102	0.13333	0.43859	0.705
	Mean	220	13	13.14	0.714	1.253	0.189	0.0614	0.422	0.860

Notes: N = number of samples; A = number of alleles; Apr = private alleles; Rs = allelic richness; Gd = gene diversity; Ho = observed heterozygosity; He = expected heterozygosity; Fst = genetic differentiation between populations; Fis = inbreeding coefficient; All population departured from Hardy-Weinberg equilibrium.

Table 3. FST values in Bromeliaceae species of the Atlantic Forest.

Species	FST (mean)	Reference
Aechmea calyculata	0.231	Goetze et al., 2016
Vriesea gigantea	0.211	Palma-Silva et al., 2009
Aechmea coelestes	0,168	Abondanza, 2012
Vriese reitzii	0.123	Soares et al., 2018
Aechmea kertezeae	0.110	Goetze et al., 2018
Aechmea winkleri	0.032	Goetze et al., 2015
A. bambusoides	**0.27**	**This study**

Table 4. Values of genetic diversity in Bromeliaceae species.

Species	Ecosystem	NP	NL	A	Ap	Rs	Ho	He	Fis	Reference
Aechmea winkleri	Atlantic Forest	5	7	na	na	3.27 – 3.57	0.506 – 0.604	0.552 – 0.614	–0.011 – 0.094	Goetze et al., 2015
Aechmea calyculata	Atlantic Forest	6	12	42 – 80	2 – 14	3.25 – 4.23	0.389 – 0.638	0.504 – 0.623	–0.039 – 0.181	Goetze et al., 2016
Aechmea kertesziae	Sand coastal – AF	5	9	57 – 73	3 – 9	5.68 – 7.22	0.550 – 0.691	0.661 – 0.747	0.075 – 0.221	Goetze et al., 2018
Bromelia antiacantha	Sand coastal – AF	8	5	na	na	1.51 – 2.13	0.193 – 0.437	0.363 – 0.661	0.145 – 0.623	Zanella et al., 2011
Alcantarea geniculata	Inselbergs – AF	4	8	na	na	2.13 – 2.66	0.330 – 0.408	0.341 – 0.455	0.094a	Barbará et al., 2007
Alcantarea imperiales	Inselbergs – AF	4	8	na	na	2.2 – 2.75	0.304 – 0.400	0.327 – 0.452	0.099a	Barbará et al., 2007
Alcantarea glaziouana	Inselbergs – AF	5	8	na	na	1.21 – 2.62	0.094 – 0.404	0.085 – 0.557	–0.125 – 0.397	Barbará et al., 2009
Alcantarea regina	Inselbergs – AF	2	8	na	na	3.478 – 3.514	0.453 – 0.505	0.412 – 0.505	–0.104 – 0.001	Barbará et al., 2009
Encholirium horridum	Inselbergs – AF	11	8	10 – 95	0 – 17	1.24 – 11.05	na	0.010 – 0.833	–0.009 – 0.429	Hmeljevsk et al., 2017
Encholirium spectabile	Inselbergs – CA	20	8	19 – 55	na	2.20 – 4.08	0.397 – 0.768	0.565 – 0.805	0.024 – 0.410	Gonçalves–Oliveira et al., 2017
Pitcairnia geyskesii	Inselbergs – AR	14	7	na	na	1.5 – 5.17	0.152 – 0.522	0.166 – 0.657	–0.180 – 0.285	Boisselier–Dubayle et al., 2010
Pitcairnia albiflos	Inselbergs – AF	5	15	na	na	2.43 – 3.69	0.317 – 0.522	0.334 – 0.528	0.010 – 0.260	Palma–Silva et al., 2011
Pitcairnia staminea	Inselbergs – AF	4	15	na	na	2.81 – 4.47	0.206 – 0.499	0.345 – 0.560	0.087 – 0.548	Palma–Silva et al.,, 2011
Vriesea gigantea	Atlantic Forest	13	10	na	0 –11	1.18 – 3.49	0.039 – 0.614	0.065 – 0.727	0.053 – 0.489	Palma–Silva et al., 2009
Vriesea minarum	Iron rock outcrops – AF	12	10	3 – 7	1 – 4	2.44 – 2.685	0.297 – 0.475	0.533 – 0.620	0.225 – 0.572	Lavor et al., 2014
Vriesea simplex	Atlantic Forest	5	15	111 – 170	6 – 32	5.71 – 7.80	0.538 – 0.584	0.616 – 0.732	0.102 – 0.215	Neri et al., 2017
Vriesea scalaris	Atlantic Forest	5	15	29 – 55	4 – 19	1.88 – 2.73	0.084 – 0.195	0.221 – 0.385	0.145 – 0.797	Neri et al., 2017
Vriesea reitzii	Atlantic Forest	6	7	28 – 43	0 – 6	3.59 – 5.30	0.360 – 0.499	0.452 – 0.629	0.019 – 0.310	Soares et al., 2018
Bromelia hieronymi	Pantanal	3	5	15 – 20	0 – 6	2.99 – 3.55	0.397 – 0.948	0.483 – 0.614	– 0.552 – 0,179	Godoy et al., 2018
Pitcairnia azouryi	Inselbergs – AF	5	9	na	na	2.952 – 3.391	0.438 – 0.527	0.44 – 0.552	0.047 – 0.208	Manhães, 2015
Tillandsia aeranthos	Pampas – AF	13	7	na	na	3.349 – 6.678	0.576 – 0.880	0.616 – 0.870	0.068 – 0.259	Aoki–Gonçalves, 2018
Aechmea coelestis	Atlantic Forest	8	7	29 – 83	na	3.489 – 7.571	0.140 – 0.556	0.497 – 0.832	0.117 – 0.709	Abondanza, 2012
Guzmania monostachia	Tropical forest (Costa Rica)	18	6	na	na	na	0.000 – 0.060	0.218 – 0.471	0.739 – 1.000	Cascante–Marín et al., 2014
Dyckia excelsa	Iron rock outcrops – PA	7	8	10 – 16	0 – 5	1 – 1.87	0.000 – 0.238	0.000 – 0.241	0.126 – 0.625	Ruas, in prep
Aechmea caudata	Atlantic Forest	3	10	17 – 29	0 – 2	1.53 – 1.94	0.293 – 0.348	0.293 – 0.454	– 0.033 – 0,226	Costa, in prep
Aechmea comata	Atlantic Forest	4	10	54 – 66	3 – 9	2.37 – 2.68	0.621 – 0.692	0.611 – 0.713	– 0.041 – 0,079	Costa, in prep
Aechmea bambusoides	**Atlantic Forest**	**7**	**8**	**11 – 17**	**0 – 2**	**1.11 – 1.323**	**0.028 – 0.013**	**0.299 – 0.517**	**0.705 – 0.949**	**This study**

Notes: AF = Atlantic Forest; CA = caatinga; AR = amazon rainforest; PA = pantanal; NP = number of populations; NL = number of loci; A = number of alleles; Apr = private alleles; Rs = allelic richness; Ho = observed heterozygosity; He = expected heterozygosity; Fis = inbreeding coefficient; a = average value; na = note available.

SUPPORTING INFORMATION

APPENDIX S1. Number of forophytes sampled per population of *Aechmea bambusoides*.

Population	Number of forophytes
Muriaé	6
ALPA2	3
Cantagalo	7
ALPA4	11
Ituí	8
Palma	8
Laranjal	8
All population	51

APPENDIX S2.

Forophyte of *Aechmea bambusoides*, showing the transformed environment in pasture área near water course (Muriaé population).

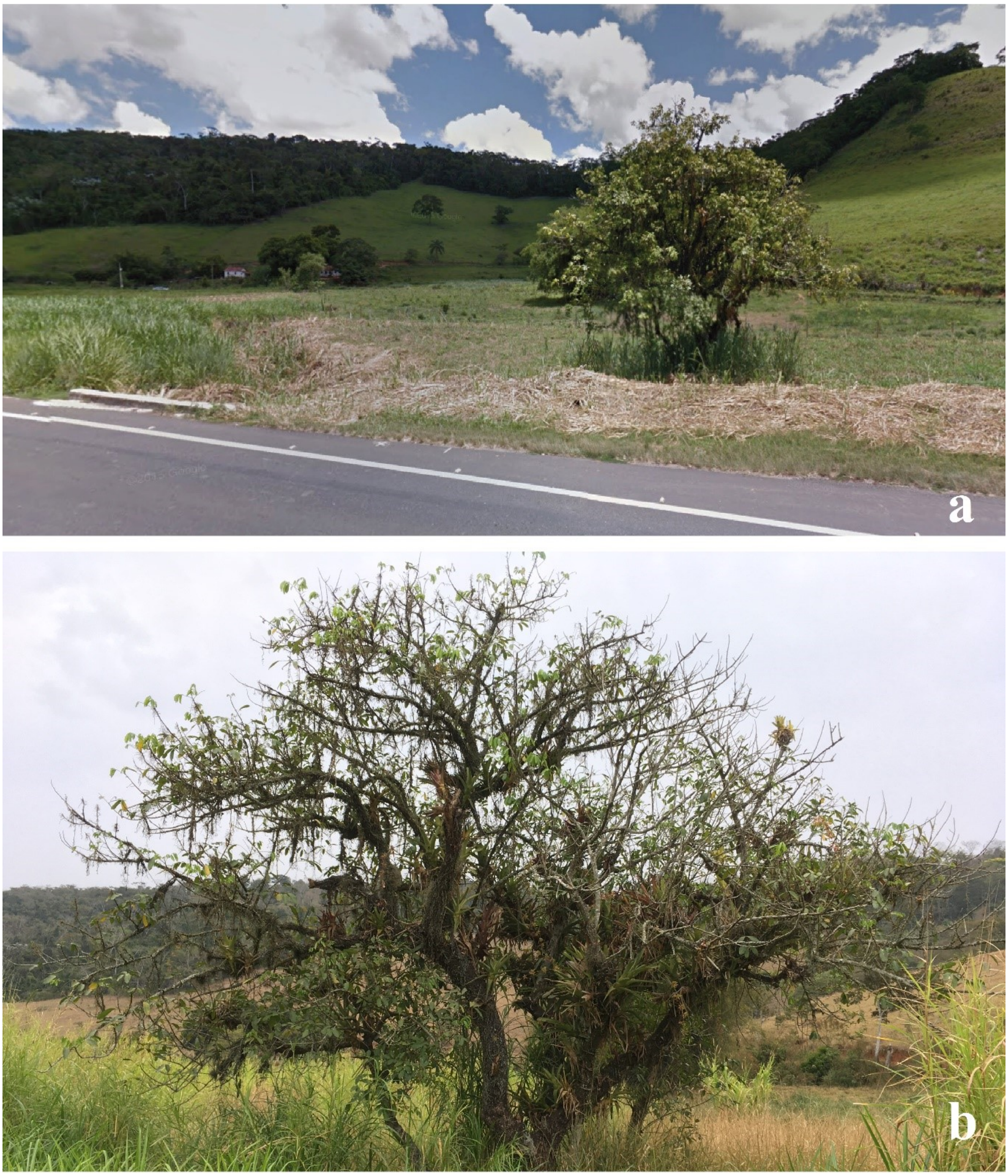
APPENDIX S3.

Occurrence area of *Aechmea bambusoides* individuals, showing isolated trees near watercourse (population ALPA2).

APPENDIX S4.

Forophyte *Guarea guidonia* housing individuals of *Aechmea bambusoides* that form many grouped rosettes (Cantagalo population).

APPENDIX S5.



Isolated forophyte of *Aechmea bambusoides*, near the highway. Detail for highly visible habitat fragmentation in (a); in (b) the same forophyte with few leaves (ALPA4 population).

APPENDIX S6.

Isolated forophyte of *Aechmea bambusoides*, detail of the tree near the highway and rural road (Palma population).

3 CONCLUSÕES GERAIS E PERSPECTIVAS

Com os resultados obtidos nessa dissertação concluímos que a espécie estudada *Aechmea bambusoides* está altamente ameaçada devido a perda de diversidade genética, causada por perda de hábitat e fragmentação florestal. Devido a isso são necessárias estratégias e ações para conservação da espécie, assim esperamos que esse trabalho possa servir de estimulo. Que a leis de proteção de APPs como ambientes ripários sejam obedecidas, devido a importância desses ambientes na conectividade de populações, e em vista de que *A. bambusoides* não ser encontrada em unidades de conservação.

Sendo esse um dos primeiros estudos com *Aechmea bambusoides*, os nossos dados contribuirão para outras pesquisas com a espécie. Esperamos que sejam realizados estudos e programas de conservação *ex situ* e *in situ* buscando manejar e capturar a diversidade genética da espécie mais necessária a ser preservada. Estamos encaminhando os resultados deste trabalho, bem como as suas implicações, para a UPCB (Unidade de Pesquisa e Conservação de Bromeliaceae), como uma proposta de projeto de conservação da espécie. Dessa forma, os indivíduos de diferentes populações poderão ser manejados e germinados apartir de sementes, afim de gerar matrizes de indivíduos com maior variabilidade genética.

Com os resultados deste trabalho propomos uma nova classificação de status de ameaça para Aechmea bambusoides, através de indicações baseadas nos critérios da IUCN. Após a publicação dos manuscritos em periódicos da área, iremos encaminhas os dados para os órgãos competentes reverem o status da espécie de forma a atender à nossa indicação de classificação como “endangered”.

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