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Gene flow and diversification in a species complex of *Alcantarea* inselberg bromeliads

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Inselberg-adapted species of bromeliads (Bromeliaceae) have been suggested as model systems for understanding the evolutionary genetics of species complexes and radiations in terrestrial, island-like environments. Bromeliads are particularly suitable for addressing the potential roles of interspecific gene exchange during plant speciation and radiation. We have studied populations of five narrowly endemic *Alcantarea* species adapted to high-elevation inselbergs of the Atlantic Rainforest of Brazil with nuclear and plastid DNA markers, estimated outcrossing rates in the giant bromeliad *A. imperialis* using progeny arrays and carried out a pilot study on the use of next generation sequencing-based genotyping in this group. Our results suggest widespread and asymmetric interspecific gene flow in the studied species complex, which visibly affects patterns of genetic diversity in the phenotypically variable mixed outcrosser *A. imperialis*. Our data support the hypothesis that gene flow has contributed to the origin of phenotypic forms in the *A. imperialis s.l.* species complex. We discuss potential conflicts between our neutral marker data and previous taxonomic work and suggest how these might be resolved. We close with a brief outlook on the potential of genomic tools to uncover the hidden links between genotypes, phenotypes and niches in bromeliads and other plant radiations. *Botanical Journal of the Linnean Society*, 2016, **181**, 505–520

ADDITIONAL KEYWORDS: adaptive radiation – hybridization – microsatellites – outcrossing – plastid DNA – restriction site-associated DNA sequencing – speciation.

INTRODUCTION

Understanding the mechanisms underlying species diversification is of great importance for both evolutionary and conservation biology. This is particularly true for the process of speciation itself and for the evolutionary processes at work in complexes of radiating taxa with 'porous genomes', i.e. species that still experience occasional gene flow (Wu & Ting, 2004; Gavrilets & Vose, 2005; Arnold, 2006; Abbott *et al.*, 2013; Seehausen *et al.*, 2014). Evolutionary mechanisms operating at these evolutionary time scales are expected to have great impact on the strength and fate of reproductive barriers between diverging populations and species (Schluter, 2000; Coyne & Orr, 2004; Wu & Ting, 2004; Rieseberg & Willis, 2007; Lexer & Widmer, 2008). Thus, tractable complexes of multiple radiating species with porous genomes are welcome resources for understanding the mechanisms underlying the origin and

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maintenance of biological diversity (Gavrilets & Vose, 2005; Arnold, 2006; Feder, Egan & Nosil, 2012). Speciation genetic approaches for studying these mechanisms are, however, only starting to be extended to multi-species settings or entire species radiations (Lepais *et al.*, 2009; Feder *et al.*, 2012; Seehausen *et al.*, 2014).

There is now increasing awareness among botanists and zoologists alike that hybridization and gene flow between previously diverged populations and species of the same ploidy may positively impact on species diversification in various ways (Seehausen, 2004; Arnold, 2006; Rieseberg & Willis, 2007; Jiggins *et al.*, 2008; Abbott *et al.*, 2013). Due to the nature of the plant group that we study in this contribution, we focus on homoploid settings here; for hybridization involving polyploidy see, for example, the recent review by Soltis & Soltis (2009). Hybridization between taxa of the same ploidy may have an impact on diversification by contributing to the pool of standing genetic variation and hence to the 'genomic substrate' for speciation and radiation (Seehausen, 2004; Brawand *et al.*, 2014). More specifically, hybridization may contribute to diversification by classical 'mosaic genome' hybrid speciation (Rieseberg *et al.*, 2003), by providing introgressed genome segments with a direct role in species isolation (also coined 'hybrid trait speciation'; Jiggins *et al.*, 2008; Abbott *et al.*, 2010; The Heliconius Genome Consortium, 2012), or by promoting evolutionary processes related to the reinforcement and/or coupling of reproductive barriers (Abbott *et al.*, 2013). Given the great potential impact that hybridization and interspecific gene flow may have on biological diversity (above), including concerns about the potential breakdown of reproductive barriers and extinction (Frankham, Ballou & Briscoe, 2004; Hamilton & Miller, 2015), studying the consequences of inter-species gene flow in plants is a timely topic.

Bromeliaceae represent a textbook example of an adaptive radiation in which genetic interactions among species of the same ploidy are thought to have accompanied or facilitated diversification (Givnish *et al.*, 1997, 2014; Benzing, 2000; Barbará *et al.*, 2007; Palma-Silva *et al.*, 2011; Versieux *et al.*, 2012). It has long been known that many groups of bromeliads can easily be hybridized experimentally (Benzing, 2000) and evidence for hybridization between bromeliad species in nature has started to accumulate (Wendt *et al.*, 2001; Barbará *et al.*, 2007; Palma-Silva *et al.*, 2011). Although recent advances in molecular genetics have started to reveal the widespread occurrence, ecological settings and genetic determinants of incomplete reproductive barriers in this group (e.g. Barbará *et al.*, 2007; Palma-Silva *et al.*, 2011, 2015), much remains to be learned on the potential roles of interspecific gene flow during the adaptive radiation of this ecologically important and species-rich tropical family of flowering plants (Givnish, 2015). Here, we attempt to contribute a piece of the jigsaw in this endeavour by studying a relatively small, well-delimited and tractable radiation of terrestrial species, the genus *Alcantarea* (E.Morren ex Mez) Harms of Bromeliaceae subfamily Tillandsioidae.

Alcantarea comprises *c.* 40 species of terrestrial bromeliads adapted to life on sky-island inselbergs in the Atlantic Rainforest and savanna-like campos rupestre habitats of south-eastern Brazil (Grant, 1995; Versieux & Wanderley, 2015). Available chromosome counts for *Alcantarea* spp. indicate a high degree of homogeneity in chromosome numbers in the genus with $2n = 50$ for all species measured thus far (Ceita *et al.*, 2008; Gitaí *et al.*, 2014), and genetic marker genotypes in natural populations are consistent with diploidy (Barbará *et al.*, 2007, 2008; Versieux *et al.*, 2012). The rosettes of many *Alcantarea* spp. form water tanks (phytotelmata) that support a rich diversity of associated animal life (Benzing, 2000). This means that inselberg *Alcantarea* spp. are important ecological foundation species in their bare and rocky habitat islands, which are separated from the surrounding rainforest by steep ecological gradients (Porembski & Barthlott, 2000). *Alcantarea* forms a monophyletic group in molecular phylogenetic studies and is distinguished from its much larger sister genus *Vriesea* Lindl. by several phenotypic characters, including long and spiralescent petals, seeds with apical and basal appendages, and cryptically semi-inferior ovaries (Versieux *et al.*, 2012; Versieux & Wanderley, 2015). Genetic studies of speciation in *Alcantarea* have thus far focused primarily on rainforest inselberg taxa. This research has revealed surprisingly strong patterns of population differentiation reminiscent of island radiations (Barbará *et al.*, 2007), short-range gene dispersal consistent with a loss of dispersal power in island-like habitats (Barbará *et al.*, 2008), local population subdivision associated with sympatric colour morphs (Barbará *et al.*, 2007, 2008) and the apparent absence or rarity of asexual reproduction in high-elevation *Alcantarea* spp. (Barbará *et al.*, 2009). A less obvious finding of these earlier studies also was that phenotypically and ecologically divergent *Alcantarea* spp. appear to hybridize and experience interspecific gene flow in sympatry and parapatry (Barbará *et al.*, 2007, 2009).

Here, we significantly expand this earlier genetic work of populations and speciation in *Alcantarea* spp. by adding more high-elevation inselberg species, more genetic markers from the nuclear and plastid genomes, including a pilot study on the use of next generation sequencing (NGS)-based genotyping-by-sequencing

in this group, and experimental estimates of outcrossing rates from progeny arrays. We use these data to address the potential roles of genetic species interactions in a comparatively tractable and well-delimited radiation of bromeliads situated in one of the most highly threatened biodiversity hotspots on Earth, the Atlantic Rainforest of Brazil (Ribeiro *et al.*, 2009). In particular, we address the following questions: (1) What is the extent of interspecific allele sharing and gene flow in the studied species complex of high-elevation inselberg *Alcantarea* spp.? (2) What might be the potential for hybridization to contribute to diversification in this group? (3) Are there any differences between the molecular genetic patterns seen here and previously recognized morphological species in this group and, if so, how could these differences be potentially reconciled?

MATERIAL AND METHODS

Study species

Alcantarea imperialis Harms, *A. brasiliana* (L.B > Sm.) J.R.Grant, *A. geniculata* (Wawra) J.R.Grant, *A. nevaresii* Leme and *A. martinellii* Versieux & Wand. are morphologically distinct species of Bromeliaceae subfamily Tillandsioideae. The five species are narrowly endemic to high-elevation inselbergs of the Serra dos Órgãos and Serra da Mantiqueira mountain ranges of the Brazilian Atlantic Rainforest (Martinelli, 1994; Grant, 1995; Versieux & Wanderley, 2015). In recent molecular phylogenetic work, these species fell in two clades close to the base of the tree, informally called the 'Serra dos Órgãos group', referring to their centre of occurrence in the Serra dos Orgãos mountain range (Versieux *et al.*, 2012). Little is known about ecological differences between these species, except for suspected variation in pollination syndromes (bats versus hummingbirds and moths; Versieux *et al.*, 2012). *Alcantarea imperialis*, also known as the 'giant bromeliad', is a polymorphic taxon with considerable morphological variability, for example in plant size and the coloration of rosettes and bracts (Barbará *et al.*, 2007, 2009; Versieux & Wanderley, 2015). *Alcantarea brasiliana*, sister to *A. imperialis* in the phylogenetic tree (Versieux *et al.*, 2012), is a taxon with a highly debated status (Smith, 1943; Versieux & Wanderley, 2015). Based on its mosaic-like morphological features, Versieux *et al.* (2012) hypothesized that this species might be a hybrid taxon between *A. imperialis* and *A. geniculata*. *Alcantarea geniculata* was clearly distinct from *A. imperialis* in the phylogenetic tree (Versieux *et al.*, 2012) and in molecular population genetic work (Barbará *et al.*, 2007, 2009), although interspecific gene flow was detected between it and *A. imperialis* in sympatry (Barbará *et al.*, 2007). *Alcantarea nevaresii* and the recently described *A. martinellii* (Versieux & Wanderley, 2009) are additional distinct taxa in this group, characterized by narrow inflorescences, much shorter rosettes and bright yellow petals. Together, these five species represent a close-to-complete sampling of the so-called 'Serra dos Órgãos' group of *Alcantarea* (Versieux *et al.*, 2012), with the exception of *A. regina* Harms. The latter taxon was included in previous population genetic work by our group (Barbará *et al.*, 2007, 2008, 2009). It was not included in the present study, because its geographical distribution is restricted almost entirely to lowland outcrops and only a single population is known from a distinct mountain system (Mantiqueira range) at higher elevations.

Plant Material

Natural populations of Atlantic Rainforest Alcantarea spp.

Seven populations of five inselberg species from the Serra dos Órgãos group of *Alcantarea* (Versieux *et al.*, 2012) were studied for nuclear and plastid DNA markers (see Fig. 1 and Table 1 for species and population names). These species are narrowly endemic to the focal study region in the Atlantic Rainforest of south-eastern Brazil (above). Thus, the collected populations were considered representative of the genetic diversity present in the gene pools of the species, with the obvious and well-known limitation of achieving complete phylogeographical coverage in tropical taxa from habitats that are difficult to access; many of these high-elevation inselberg rock outcrops are only accessible by abseiling or by helicopter. The sampled populations of *A. imperialis* and *A. geniculata* were previously described by Barbará *et al.* (2007), whereas those of the other three species were studied for the first time here (Table 1, Fig. 1). For *A. brasiliana*, we sampled the only known and documented extant population in its type locality. Sixty-seven plants were sampled for this population, to reach sufficient power to test the a priori hypothesis of a hybrid origin of this species (above). For *A. martinellii*, we sampled one of its two known, closely adjacent localities (Versieux & Wanderley, 2009, 2015). For *A. nevaresii*, we sampled one representative locality from its narrowly endemic range, which encompasses only few outcrops in the neighbouring municipalities of Nova Friburgo and Teresópolis, state of Rio de Janeiro (RJ), Brazil. We note that available molecular phylogenetic data for *Alcantarea* spp. (Versieux *et al.*, 2012) should be regarded as hypotheses to be refined by future work and that it would be highly desirable to add additional species

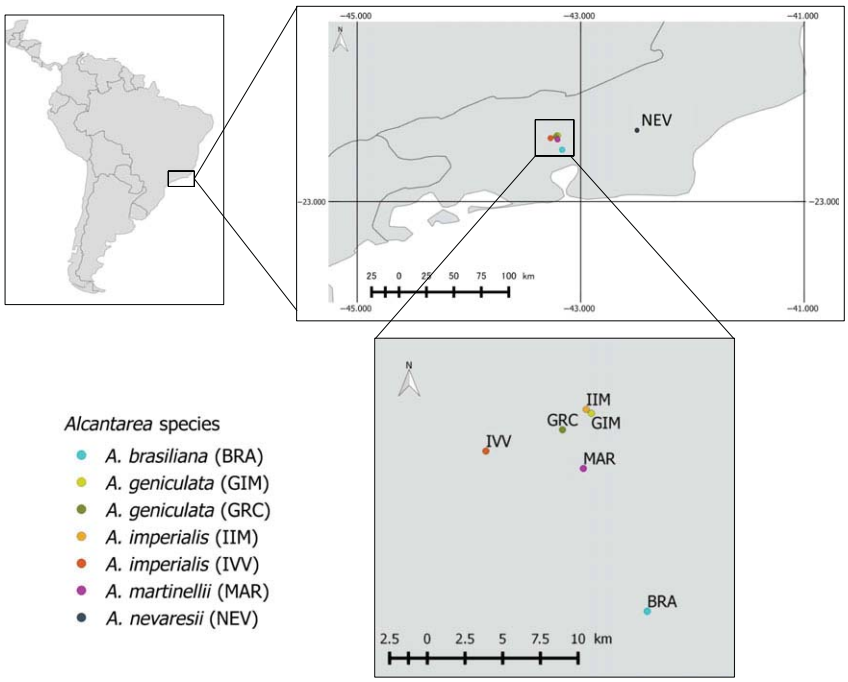

Figure 1. Geographical map of populations of five narrowly endemic high-elevation inselberg *Alcantarea* spp. sampled in the Atlantic Rainforest of Brazil. Population names follow Table 1 and population colour codes are identical to those used in Figures 2 and 5.

Table 1. Genetic diversity in populations of high-elevation *Alcantarea* spp. sampled in the Atlantic Rainforest of Brazil, including species affiliations, population names, geographical coordinates, number of chromosomes sampled (N) and the following genetic diversity parameters for nuclear microsatellite loci: variance in allele size (Var), allelic richness (A_s) and expected (H_E) and observed heterozygosity (H_O)

Species	Population	Coordinates	N	Var	A_s	H_E	H_O
A. brasiliana	BRA	22°32′5.46″S, 43°9′58.86″W	134	29.060	2.467	0.340	0.240*
A. geniculata	GIM	22°24′24.06″S, 43°12′8.58″W	30	51.650	3.337	0.490	0.372*
A. geniculata	GRC	22°25′2.64″S, 43°13′15.72″W	34	24.551	2.677	0.370	0.325
A. imperialis	IIM	22°24′24.06″S, 43°12′8.58″W	24	42.609	3.110	0.424	0.337
A. imperialis	IVV	22°25′52.2″S, 43°16′13.68″W	30	43.068	3.843	0.536	0.468
A. martinellii	MAR	22°25′58.88″S, 43°14′36.78″W	20	8.684	1.774	0.271	0.356
A. nevaresii	NEV	22°22′10.56″S, 42°29′46.44″W	46	20.280	1.848	0.217	0.206

*Highly significant departures from HWE at the 0.001 level.

[e.g. *A. regina* and *A. farneyi* (Martinelli & A.F.Costa) J.R.Grant] to the genetic dataset as they are discovered, described and sampled at the population level. Information on herbarium vouchers and access to all populations used in the present study is available from the authors on request (see also Barbará *et al.*, 2007; Versieux *et al.*, 2012).

Progeny arrays of Alcantarea imperialis

To estimate outcrossing rates in *A. imperialis*, the most important focal taxon of this study, we collected open pollinated progeny from one locality, Macaé-de-Cima, near Nova Friburgo (RJ), Brazil. This locality was described in detail by Barbará *et al.* (2007). Knowing outcrossing rates in *A. imperialis* was of interest in the context of asymmetric patterns of migration seen in our multi-species data (below). Unfortunately, not many plants flowered in the year of sample collection. Nevertheless, we were able to collect sufficient seeds for six maternal families and the resulting seedlings were cultivated at Jardim Botânico do Rio de Janeiro (JBRJ), Brazil. Upon

tissue collection, on average 27 plants per family were available for genetic analysis. For completeness, we note that the Macaé-de-Cima population of *A. imperialis* comprises two different colour morphs that differ in the coloration of rosettes and bracts. Thus, this population reflects some of the phenotypic variability typical for this species. Partial assortative mating among colour morphs (Barbará *et al.*, 2008) suggests that estimates of outcrossing rates gathered in this population are conservative.

DNA isolation and genotyping

Genomic DNA of all plants was extracted from silica-dried leaf tissue using the DNeasy Plant Mini Kit (Qiagen) following the manufacturer's instructions. Following extraction and purification, the genomic DNA was diluted to 5 ng μL^{-1}, based on quantification using a NanoDrop Instruments spectrophotometer.

Nuclear microsatellites

The microsatellite marker data gathered for natural populations in the present study greatly extend the dataset of Barbará *et al.* (2007), adding three species and more markers to the previous study of *A. imperialis* and *A. geniculata*. The following nine marker loci were used to genotype the population samples: Ai4.10, Ai4.3, VgG02 and VgF02 (Palma-Silva *et al.*, 2007); Pit8 (Sarthou *et al.*, 2003); and CT5, E6b, E6 and P2P19 (Boneh, Kuperus & Van Tienderen, 2003). Polymerase chain reaction (PCR) primers were labelled with the fluorophores PET, NED, VIC and FAM (Applied Biosystems). Seven of the markers (excluding VgG02 and VgF02) were used for genetic analysis of progeny arrays in *A. imperialis*. All markers were PCR-amplified with standard PCR protocols as in Barbará *et al.* (2007) and resulting DNA fragments were sized against a Liz-500 size standard with the help of an ABI 3130 Genetic Analyzer (Applied Biosystems) and associated analysis software.

Plastid microsatellites and Sanger sequences

For all *Alcantarea* population samples, the plastid microsatellite markers VgCP3 and VgCP4 (Palma-Silva *et al.*, 2009) were amplified in 10-μL PCRs containing 10 ng of DNA template, 1× Bioline Taq buffer, 1.5 mM Bioline $MgCl_2$, 100 μM dNTPs, 5 pmol forward primer with M13 tail (Schuelke, 2000), 10 pmol reverse primer, 2 pmol fluorophore-labelled universal M13 primer (Schuelke, 2000) and 0.5 units Taq polymerase. The PCR cycling programme followed Palma-Silva *et al.* (2009) and DNA fragment analysis was carried out as described for nuclear microsatellites (above).

Plastid DNA sequencing for single nucleotide polymorphism (SNP) discovery was carried out for fewer samples per population: six individuals from population BRA of *A. brasiliana*, three individuals from GIM (sequences were completely monomorphic) and four individuals from each of the populations GRC, IIM, IVV, MAR and NEV. Two plastid DNA regions were sequenced: *pet/Bex2 – Dex2* (Ebert & Peakall, 2009) and the *ndhA* intron (Shaw *et al.*, 2007). Amplicon sequencing was carried out as described by Lexer *et al.* (2014), making use of the service provider Macrogen Europe, and sequences were aligned, concatenated and analysed in BioEdit (Hall, 1999).

Restriction site-associated DNA (RAD) sequencing

Apparent contrasts between genetic patterns for neutral markers observed here and morphology-based species delimitation for the same taxa in previous studies (discussed below) motivated us to explore alternative sources of genetic markers with the potential to bridge patterns seen for neutral markers on the one hand and taxonomically informative phenotypic traits on the other. Thus, we gathered first pilot data on the feasibility of NGS-based genotyping-by-sequencing in these bromeliads. Our method of choice was RAD sequencing (RAD-seq; Hohenlohe *et al.*, 2010). Our rationale was to check whether classical, single-enzyme RAD-seq (Amores *et al.*, 2011) is potentially useful for studying patterns of genomic diversity and delimiting *Alcantarea* spp. or whether alternative genotyping-by-sequencing protocols need to be explored (e.g. Parchman *et al.*, 2012; Peterson *et al.*, 2012).

We constructed RAD-seq libraries for two individuals of *A. imperialis* and two individuals of *A. geniculata* and eight individuals from four species of two other angiosperm families studied in our laboratory, Restionaceae (monocots) and Salicaceae (eudicots). RAD-seq was carried out following the protocols of Amores *et al.* (2011) for all samples in the same experiment, employing the same procedures used previously by our group (Lexer *et al.*, 2013; Stölting *et al.*, 2013). The libraries were checked for quality, quantity and molecular size range on a 2100 Bioanalyzer (Agilent Technologies). Then, all samples were pooled and sequenced on a single lane on the Illumina HiSeq 2000 platform at the University of Lausanne Genomic Technologies Facility, Switzerland, using 100-bp single-end sequencing chemistry.

Statistical analysis

Nuclear DNA diversity, structure and allele sharing

Genetic diversity for nuclear microsatellite marker loci was estimated via the expected and observed

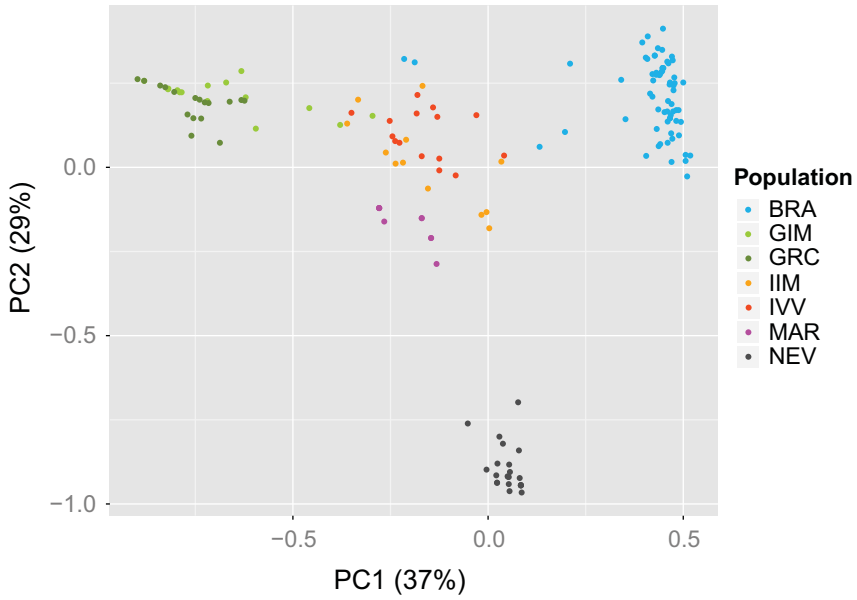

Figure 2. Principle coordinates analysis (PCoA) of variation at nine nuclear microsatellite markers studied in populations of five narrowly endemic high-elevation inselberg *Alcantarea* spp. sampled in the Atlantic Rainforest of Brazil. The per cent of variance explained is indicated along each axis. Colour codes for populations are identical to those used in Figures 1 and 5.

heterozygosity (H_E and H_O, respectively), allelic richness and the variance in allele size, using the MSA and FSTAT software programs (Goudet, 1995; Dieringer & Schlotterer, 2003). We tested for departures from Hardy–Weinberg equilibrium (HWE; differences between H_E and H_O) using exact tests in GENEPOP (Rousset, 2008) and adjusted significance levels for multiple testing using the sequential Bonferroni method. Genetic divergence (F_{ST}) between pairs of populations was estimated with FSTAT and significance levels were determined with 10 000 permutations.

A model-free and a model-based approach were chosen to study genetic structure and allele sharing between populations and species. Firstly, principal coordinates analysis (PCoA) in GenAlEx (Peakall & Smouse, 2012) was used to depict genetic relationships. Secondly, Bayesian ancestry analysis with STRUCTURE (Pritchard, Stephens & Donnelly, 2000) was used for model-based clustering, based on an admixture model assuming independent allele frequencies and an initial alpha of 1.0. Markov chain Monte Carlo (MCMC) simulations were run with a burn-in period of 500 000 followed by 1000 000 iterations and chain convergence was checked by graphical examination of traces. The most likely number of different gene pools or genetic clusters (K) present in the data was computed following the method of Evanno, Regnaut & Goudet (2005), using the estimated Ln probability of the data as additional guidance. The ancestry proportions of individual plants from these K clusters were visualized in the form of bar plots.

Coalescent-based estimates of interspecific gene flow

Theta ($4N_e\mu$, with N_e = effective population size and μ = mutation rate) and the effective number of migrants (N_em) between pairs of populations were estimated using the coalescent theory and maximum-likelihood-based approach implemented in MIGRATE (Beerli & Felsenstein, 1999). Pair-wise estimation was preferred over simultaneous analysis of multiple populations based on the rationales described by Beerli (2004) and Barbará *et al.* (2007). The migration rate was allowed to be asymmetric. Thus, to facilitate graphical presentation of the results, adjacent sampling localities of *A. imperialis* and *A. geniculata* were combined into a single population for each species, as justified by their homogeneous appearance in both PCoA (Fig. 2) and STRUCTURE (Fig. 3) and by their moderate F_{ST} (Supporting information, Table S1). The MCMC simulations used to explore the parameter space included 10 000 iterations and chain heating. Effective population sizes N_e and migration rates were computed for all populations and species from theta values, using a stepwise mutation model approximated via Brownian motion and assuming a microsatellite mutation rate of 10^{-4} per gamete and generation (Zhang & Hewitt, 2003).

Plastid DNA diversity and haplotype sharing

Plastid DNA haplotypes were defined based on length variants at the two studied plastid microsatellites and SNPs and were indels identified by Sanger sequencing (Supporting Information, Table S3). Haplotype diversity was analysed by comparing identities and counts of haplotypes across populations and species. In addition, we constructed a haplotype network using the median-joining method implemented in the NETWORK software (www.fluxus-

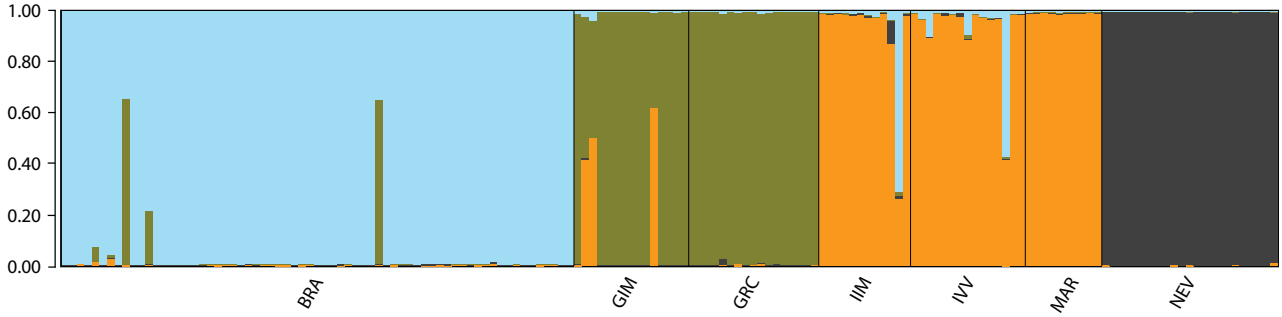

Figure 3. Bayesian ancestry proportions Q (y-axis) for all plants (x-axis) from all sampled populations of five high-elevation inselberg *Alcantarea* spp., estimated for the best model ($K = 4$ gene pools) identified based on Evanno *et al.* (2005) and model probabilities. The colours of the four gene pools allow easy cross-referencing with the population colour codes used in Figures 1, 2 and 5.

engineering.com), following the user manual. The positions of haplotypes within the network were used to infer likely ancestral and derived haplotypes, following the rationale of Posada & Crandall (2001).

Mating system analysis in Alcantarea imperialis

Inferences on plant mating systems can readily be made based on multi-locus data for open pollinated progeny arrays derived from individual maternal plants (Ritland, 2002). In the present study, we were primarily interested in estimating outcrossing rates in *A. imperialis*, as a confirmation of very low inbreeding coefficients (F_{IS}) previously estimated for populations of this species (Barbará *et al.*, 2007). Thus, multi-locus (t_m) and single-locus outcrossing rates (t_s) were estimated for open pollinated progeny arrays from an exemplary, large population of this species (population IMC studied by Barbará *et al.*, 2007), using the MLTR software.

Pilot study on RAD-seq in Alcantarea spp.

After joint Illumina sequencing of individually barcoded RAD-seq libraries from multiple samples, sequence reads were processed as described by Stölting *et al.* (2013). Briefly, reads were separated by individual and sequencing barcodes were removed using custom scripts. Subsequently, reads not containing the expected enzymatic (*Pst*I) restriction site were identified and discarded. Then, the *Stacks* Software (Catchen *et al.*, 2013) was used to explore the number of loci present in the data by assembling the loci de novo with the ustack software tool (arguments: -p 4 -t fastq -i 1 -d -r -m 3).

To explore the potential use of RAD-seq for studying patterns of divergence and allele sharing in *Alcantarea* spp., we called single nucleotide variants (SNVs) in the two studied individuals of *A. geniculata* and the single studied individual of *A. imperialis* and subsequently filtered all bi-allelic variants in VCFtools v0.1.12b. We used a set of stringent parameter settings, including a minimum base calling quality of 20 and minimum sequencing depths of six reads per locus and two reads per allele. With the resulting high-quality variants, we estimated the percentages of shared alleles among individual plants. Note that all three plants originate from the same narrow sampling region shown in Figure 1. One plant of *A. geniculata* originated from population GRC, also studied with other markers in the present study. The remaining plants of *A. geniculata* and *A. imperialis* were from nearby populations GRR and IMC, previously studied by Barbará *et al.* (2007). None of these three populations has previously shown signs of recent genome admixture (Barbará *et al.*, 2007, 2009). Thus, the studied individuals are useful for exploring patterns of allele sharing within and between species in a simple setting.

RESULTS

Patterns of nuclear genetic diversity and allele sharing

Genetic diversity (Table 1) in the sampled populations (Fig. 1) was comparable to previous estimates for *Alcantarea* spp. based on fewer markers (Barbará *et al.*, 2007). Among the five species studied here, diversity (e.g. H_E, allelic richness and variance in allele size; Table 1) was greatest in populations IIM and IVV of *A. imperialis* and population GIM of *A. geniculata*, for which interspecific gene flow with *A. imperialis* in sympatry was previously demonstrated (Barbará *et al.*, 2007). Two departures from HWE were detected in our study, in populations BRA of *A. brasiliana* and GIM of *A. geniculata*

(Table 1). Interspecific F_{ST} estimates were high, generally ranging from 0.4 to 0.7 (Supporting Information, Table S1), thus attesting to the pronounced interspecific differentiation among these five morphologically delimited taxa of the Serra dos Órgãos species complex of *Alcantarea*.

The PCoA (Fig. 2) and Bayesian ancestry analysis with STRUCTURE (Fig. 3) revealed consistent and characteristic patterns of differentiation and allele sharing among the studied taxa.The first two PCoA axes accounted for 37 and 29% of the variation in the genetic data, respectively, and clearly separated populations of *A. brasiliana* (BRA) and *A. nevaresii* (NEV) from those of *A. geniculata* (GIM and GRC). Perhaps the most conspicuous feature of the PCoA, however, was the positioning of *A. imperialis* in principle coordinates space: this species occupied a central position, with several individuals exhibiting affinities with *A. brasiliana* and *A. geniculata*, respectively (Fig. 2). The mixed genomic composition (= hybrid status) of these plants was confirmed by Bayesian ancestry analysis with STRUCTURE (Fig. 3). As populations of these narrow endemics were sampled in close spatial proximity (on adjacent inselbergs in the Atlantic Rainforest near Petropolis, RJ, Brazil; Fig. 1), these patterns indicate allele sharing due to genetic contact between these three members of the *Alcantarea* species complex, *A. imperialis*, *A. brasiliana* and *A. geniculata* (Figs 2, 3). *Alcantarea martinellii* (MAR) was weakly differentiated from *A. imperialis* in the PCoA and STRUCTURE (Figs 2, 3), although genetic divergence between populations of the two species was significant (Supporting Information, Table S1).

Extent and direction of interspecific gene flow

Coalescent-based analysis (Beerli & Felsenstein, 1999; Beerli, 2004) yielded informative patterns regarding the direction of gene flow between species (Fig. 4; Supporting Information, Table S2). Low and asymmetric migration rates (N_em) were found for all

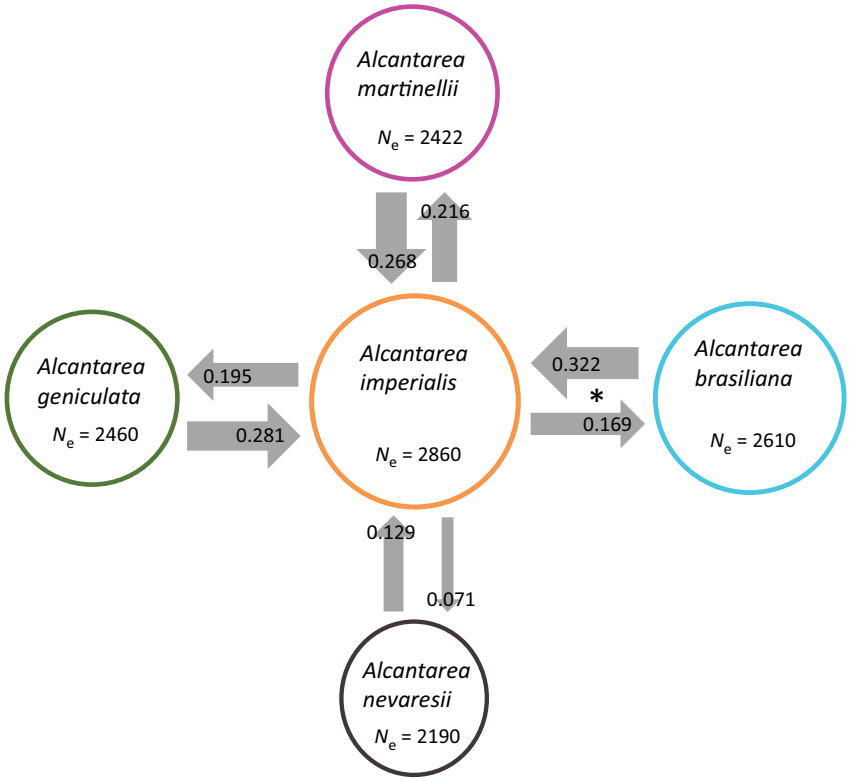

Figure 4. Effective population sizes (N_e) and effective numbers of migrants (N_em) between species estimated by the coalescent- and maximum-likelihood-based approach in MIGRATE. The sizes of circles indicate N_e for each species, and sizes of arrows indicate migration rates of *Alcantarea imperialis* with each of the remaining species. Populations were arranged spatially in such a way as to resemble their positioning in principle coordinates space (Fig. 2) as closely as possible. Species are indicated by colours that permit easy cross-referencing with the population colour codes used in Figures 1, 2 and 5. The asterisk indicates statistically significant asymmetric migration between *A. brasiliana* and *A. imperialis*. For a complete listing of N_e and N_em estimates for all pairwise comparisons, see Supporting Information (Table S2).

species, with a tendency for asymmetric migration towards *A. imperialis* (Fig. 4). The difference was statistically significant for *A. imperialis* and *A. brasiliana* (Fig. 4; 95% confidence intervals did not overlap). Little migration was found between *A. nevaresii* and the remaining species, but an asymmetric pattern with *A. imperialis* was also confirmed for this taxon (Fig. 4). Thus, coalescent-based analysis of migration rates revealed the probable processes responsible for the patterns of allele sharing revealed by PCoA and STRUCTURE.

Patterns of plastid DNA diversity and haplotype sharing

In contrast to the nuclear marker results, little haplotype sharing was observed for maternally inherited plastid DNA (Table 2, Fig. 5), indicating that interspecific gene flow (Fig. 4) occurred via pollen rather than seeds. In fact, only a single instance of interspecific plastid DNA haplotype sharing was observed, namely between *A. imperialis* and *A. martinellii* (Table 2). The other plastid haplotypes were fixed within species, with the exception of one polymorphism residing between different inselberg populations of *A. imperialis* (Table 2).

In the plastid DNA network (Fig. 5), the haplotypes of *A. imperialis* and *A. geniculata* were located in a central position, consistent with them being ancestral (Posada & Crandall, 2001), within the limits to inference imposed by the notoriously slow rates of plastid DNA evolution seen in bromeliads (Barfuss *et al.*, 2005). The haplotypes found in *A. brasiliana*, *A. nevaresii* and *A. martinellii*, by contrast, were located in more marginal positions, separated from the haplotypes of *A. imperialis* and *A. geniculata* in the core of the network by three or more mutation steps. These haplotypes were thus inferred to be derived (Fig. 5).

Table 2. Relative frequencies of plastid DNA haplotypes found in populations of high-altitude *Alcantarea* spp. based on plastid microsatellite and sequence data

Species	Population	H1	H2	H3	H4	H5
A. brasiliana	BRAS	1.0	–	–	–	–
A. geniculata	GIM	–	1.0	–	–	–
A. geniculata	GRC	–	1.0	–	–	–
A. imperialis	IIM	–	–	1.0	–	–
A. imperialis	IVV	–	–	–	1.0	–
A. martinellii	MAR	–	–	–	1.0	–
A. nevaresii	NEV	–	–	–	–	1.0

For population information see Table 1, for haplotype definitions see Supporting Information (Table S3) and for sample sizes for plastid microsatellite genotyping and Sanger sequencing see text.

Mating system analysis in *Alcantarea imperialis*

The consistent tendency of asymmetric interspecific gene flow in the direction of *A. imperialis* (above; Fig. 4) motivated us to examine outcrossing rates in this species more closely. Multi-locus outcrossing rates t_m in the examined population of *A. imperialis* were intermediate to high (mean = 0.741, range = 0.556–0.984; Table 3). Single-locus outcrossing rates t_s were sometimes higher than multi-locus estimates t_m, as expected when using a relatively large number of marker loci with low allelic diversities (Ritland, 2002). The high outcrossing rates measured here (t_m effectively up to 98%) are consistent with the widespread absence of significant inbreeding coefficients (F_{IS}) previously noted for this species (Barbará *et al.*, 2007). The apparent propensity of *A. imperialis* to outcross is of interest in the context of our discussion of asymmetric interspecific gene flow towards this species (below).

Pilot study on RAD-seq in *Alcantarea* spp.

Three of four RAD-seq libraries constructed for *Alcantarea* spp. in this pilot project were successfully sequenced on the Illumina HiSeq. One library for *A. imperialis* failed to pass pre-sequencing quality control on the Bioanalyzer, apparently due to issues with DNA extract quality. The remaining three sequencing libraries yielded between 1.5 and 2.4 million sequence reads containing the expected enzymatic (*Pst*I) restriction site (Table 4). These reads assembled de novo into *c.* 72 000–96 000 RAD stacks or genetic loci sequenced from the respective *Alcantarea* genomes (Table 4). After accounting for differences in total read numbers for each individual, these numbers are well in line with *de novo* assembly success in other monocot and eudicot taxa (Table 4) with comparable ranges in genome size (http://data.kew.org/cvalues/; Gitaí *et al.*, 2014). The results are of interest in the light of our discussion of potential future avenues to bridge information gained by taxonomically informative morphological traits and neutral markers in the Neotropical radiation of Bromeliaceae.

A total of 7481 high-quality SNVs were called from RAD-seq data for the studied individuals. The average depth of coverage per individual was 25.6× (range: 21.3–29.5×). Out of all markers, 6716 (89.8%) were shared across individuals of *A. geniculata* and *A. imperialis* and proportions of missing data per individual were low (3.4% on average). This suggests that RAD-seq will be a useful tool for studying geno-

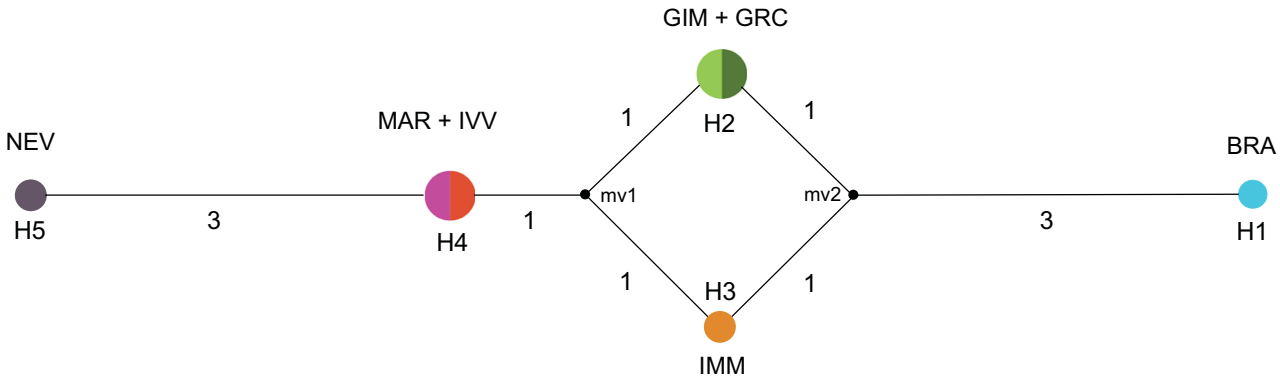

Figure 5. Median-joining network of plastid DNA haplotypes found in populations of five high-elevation inselberg *Alcantarea* spp. The haplotypes are indicated by filled circles, the size of each circle being proportional to their frequencies. The numbers of mutations required to explain transitions among haplotypes are indicated along the lines of the network, and median vectors are labelled mv1 and mv2. Population names follow Table 1, and population colour codes are identical to those used in Figures 1 and 2. For haplotype definitions, see Supporting Information (Table S3).

Table 3. Multi-locus (t_m) and single-locus outcrossing rates (t_s) and their standard deviations (SD) estimated from six progeny arrays (sample sizes in parentheses) of a population of *Alcantarea imperialis*, using a mixed mating model following Ritland (2002)

Family	t_m (SD)	t_s (SD)
Fam1 (56)	0.673 (0.084)	0.748 (0.084)
Fam2 (15)	0.632 (0.010)	0.796 (0.087)
Fam3 (50)	0.656 (0.009)	0.800 (0.032)
Fam4 (6)	0.984 (0.007)	0.883 (0.021)
Fam5 (17)	0.943 (0.029)	0.874 (0.033)
Fam6 (20)	0.556 (0.021)	0.791 (0.100)

mic diversity within and between *Alcantarea* spp. The percentage of shared alleles was much greater for the intraspecific comparison within *A. geniculata*, compared with interspecific comparisons involving *A. geniculata* and *A. imperialis* (Fig. 6), demonstrating the potential of RAD-seq for species delimitation in this group.

DISCUSSION

Extent and direction of interspecific gene flow in inselberg *Alcantarea*

Knowing the extent and direction of interspecific gene flow among recently radiated taxa is a prerequisite for assessing the potential role(s) that hybridization may play in speciation and radiation (Seehausen, 2004; Gavrilets & Vose, 2005; Arnold, 2006; Rieseberg & Willis, 2007; Lexer & Widmer, 2008; Abbott *et al.*, 2013; Brawand *et al.*, 2014). Testing for among-species gene flow is also crucial in the context of species delimitation, which is widely regarded as central for defining biologically meaningful units for conservation (Frankham *et al.*, 2004). Here, we have explored these issues for an ecologically important and taxonomically challenging group of bromeliads in the genus *Alcantarea* adapted to Neotropical inselberg sky islands (Grant, 1995; Benzing, 2000; Versieux, 2009; Versieux *et al.*, 2012), building on earlier population genetic work on this group based on fewer species and genetic markers (Barbará *et al.*, 2007, 2009). The results of our study indicate taxonomically widespread but geographically localized allele sharing for neutral markers sampled from nuclear genomes (Figs 2, 3). These patterns, revealed by model-free multivariate statistics (PCoA; Fig. 2) and model-based analysis of genetic ancestry and admixture (Fig. 3), are highly suggestive of recent gene flow in this group of high-elevation inselberg bromeliads. Although shared ancestral polymorphism may in principle affect genetic parameters estimated based on differences in allele frequencies, it is unlikely to be the explanation for the patterns we observed, as these populations and species are highly divergent (Supporting Information, Table S1) and microsatellites are known for their high mutation rates and rapid evolution (Zhang & Hewitt, 2003).

Coalescent-based estimates of migration rates (Beerli & Felsenstein, 1999; Beerli, 2004) suggest that interspecific gene exchange has an asymmetric component (Fig. 4). *Alcantarea imperialis*, the most polymorphic and phenotypically variable of all the studied taxa (Barbará *et al.*, 2008; Versieux, 2009; Versieux *et al.*, 2012), exhibited a consistent tendency to act as a recipient rather than a donor of

Table 4. Potential utility of RAD-seq in *Alcantarea imperialis* and *A. geniculata* (Bromeliaceae), with two species of the monocot family Restionaceae (Poales) and two species of the eudicot family Salicaceae (Malpighiales) included in the same experiment for cross-validation purposes

Family	Species	Internal sample ID	Total no. of reads obtained	No. of reads with restriction site	No. of RAD stacks recovered
Bromeliaceae	*Alcantarea geniculata*	Ag_GRC-31_1295	2 917 224	2 245 215	81 313
Bromeliaceae	*Alcantarea geniculata*	Ag_GRR_1293	2 150 977	1 565 267	71 672
Bromeliaceae	*Alcantarea imperialis*	Ai_IMC_1294	3 516 517	2 437 069	95 700
Restionaceae	*Hypodiscus aristatus*	Ha_B19_1274	4 328 623	1 274 825	37 334
Restionaceae	*Hypodiscus aristatus*	Ha_B19_1275	5 174 724	2 248 871	112 159
Restionaceae	*Restio triticeus*	Rt_T18_1272	1 919 540	759 949	39 816
Restionaceae	*Restio triticeus*	Rt_T18_1273	10 873 986	7 842 064	236 092
Salicaceae	*Populus alba*	Pa_i173_1284	7 289 382	5 010 599	143 095
Salicaceae	*Populus alba*	Pa_i182_1285	20 588 601	16 885 188	334 481
Salicaceae	*Populus tremula*	Pt_i191_1286	12 222 258	9 845 915	208 298
Salicaceae	*Populus tremula*	Pt_i202_1287	13 585 321	10 671 447	231 755

Shown are the total number of Illumina sequence reads obtained, number of reads including the expected *Pst*I restriction site and number of RAD stacks or loci recovered with the STACKS software (Catchen *et al.*, 2013). One sequencing library of *A. imperialis* did not pass quality control and was thus not sequenced. For details see text.

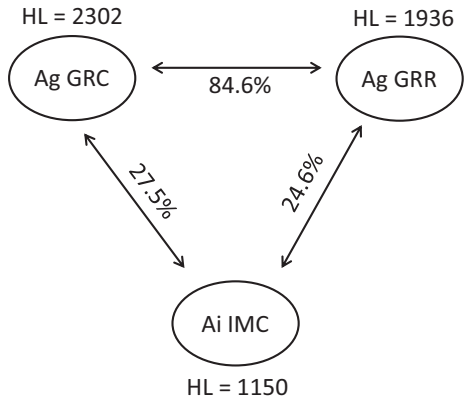

Figure 6. Schematic representation of RAD-seq allele sharing between individuals from different populations of *Alcantarea geniculata* (Ag GRC and Ag GRR) and *A. imperialis* (Ai IMC), respectively. Percentages of shared alleles are indicated along the arrows connecting individuals. Numbers near ellipses represent total numbers of heterozygous loci (HL) detected in each individual.

genetic material (Fig. 4). This may explain its central position in genetic PCoA space (Fig. 2) and it points to the potential role(s) of hybridization during diversification of this species complex, also referred to as *A. imperialis s.l.* (Versieux *et al.*, 2012; discussed below).

Our results for nuclear genetic markers are in contrast to those obtained for plastid DNA (Table 2). Most plastid DNA haplotypes found in *Alcantarea* were fixed within local populations and species and only a single case of interspecific plastid DNA haplotype sharing was found (between *A. imperialis* and *A. martinellii*; Table 2). These results contrast strongly with a recent study of plastid DNA haplotype sharing in a species complex of another bromeliad genus, *Pitcairnia* L'Hér. (Palma-Silva *et al.*, 2011), in which plastid DNA haplotypes were shared extensively among related species, to the extent that geographical subdivisions were better predictors of plastid DNA variation than the taxonomically recognized species themselves (Palma-Silva *et al.*, 2011). In fact, this pattern (widespread plastid DNA sharing in the face of largely intact integrity of nuclear genomes) appears to be widespread in plants (Petit & Excoffier, 2009; Palma-Silva *et al.*, 2011). We do currently not know why inselberg *Alcantarea* spp. behave so differently in this respect, but we can offer a potential explanation: data on fine-scale spatial genetic structure show that high-elevation *Alcantarea* spp. exhibit short-range gene dispersal (in the range 10–30 m), consistent with their plumose (rather immobile when wet) seeds leading to reduced seed dispersal power in isolated habitat islands (Barbará *et al.*, 2008; Paggi *et al.*, 2010). As plastid DNA is generally inherited via the maternal line, short-range seed dispersal in *Alcantarea* spp. may be sufficient to explain strong fixation of plastid DNA haplotypes in local populations and species. Consequently, our results suggest an important role for animal-based pollen dispersal by bats (and possibly hummingbirds; Versieux *et al.*, 2012) in generating the observed patterns of gene flow in inselberg *Alcantarea* spp.

Potential role of hybridization in bromeliad diversification

There are several different, widely recognized routes by which interspecific gene flow between species of the same ploidy can contribute to species diversification (see Introduction; Seehausen, 2004; Arnold, 2006; Rieseberg & Willis, 2007; Jiggins *et al.*, 2008; Abbott *et al.*, 2013). One widely discussed route involves classical or 'mosaic genome' homoploid hybrid speciation, as previously demonstrated for wild, annual sunflowers (*Helianthus* L.; Rieseberg *et al.*, 2003; Rieseberg & Willis, 2007). In this mode of 'speciation-with-gene-flow', hybrids receive balanced genetic contributions from each parental taxon, including the genomic variation required to colonize novel habitats and become reproductively isolated from their parents (Rieseberg *et al.*, 2003). A possible hybrid origin has recently been hypothesized for *A. brasiliana*, one of the inselberg *Alcantarea* spp. studied here, based on mosaic-like patterns of phenotypic traits and DNA sequence polymorphisms observed in phylogenetic work (Versieux *et al.*, 2012). Based on our present molecular genetic data for more than 60 individuals from the only truly well-documented population of this taxon (Figs 2, 3), we can safely reject this hypothesis; *A. brasiliana* does not exhibit the balanced genomic composition expected for stabilized 'mosaic genome' hybrid species (Fig. 3; Rieseberg *et al.*, 2003; Jiggins *et al.*, 2008).

A second route by which hybridization may contribute to diversification has received much less attention thus far: 'hybrid trait speciation', i.e. speciation triggered by the transfer of genetic material among previously diverged taxa (Jiggins *et al.*, 2008; The Heliconius Genome Consortium, 2012). In this mode of speciation-with-gene-flow, also known in plants (Abbott *et al.*, 2010), interspecific introgression of genetic ancestry blocks provides the raw material required for adaptation of the recipient population to a new habitat or niche, thus triggering reproductive isolation and speciation (Jiggins *et al.*, 2008). This mode of speciation involving hybridization is difficult to detect, and thus easy to overlook, because its genomic footprint is much more subtle than that of 'mosaic genome' hybrid speciation (Jiggins *et al.*, 2008; Mavárez & Linares, 2008; Abbott *et al.*, 2010) and because it may resemble other manifestations of introgressive evolution (Hamilton & Miller, 2015). Our genetic data are consistent with the hypothesis that this mode of speciation-with-gene-flow is operating in the *A. imperialis s.l.* complex, an argument we develop below.

Our multivariate (Fig. 2), Bayesian-based (Fig. 3) and coalescent-based (Fig. 4) analyses of nuclear genetic data all indicate that *A. imperialis* has received genetic material from several other closely related *Alcantarea* spp., including *A. geniculata*, *A. brasiliana*, *A. martinellii* and possibly *A. nevaresii* (Fig. 4), by interspecific gene flow. Note that these interspecific migration (N_em) estimates are of similar magnitude as intraspecific migration rates expected for inselberg *Alcantarea* taxa (Barbará *et al.*, 2007). We regard it unlikely that the observed patterns are solely due to shared recent ancestry, as overall genetic divergence (F_{ST}) between all studied taxa was significant and high (ranging from 0.4 to 0.7; Supporting Information, Table S1). Consistent with *A. imperialis* being a recipient of immigrants (Fig. 4), our analysis of progeny arrays (Table 3) indicates intermediate to high outcrossing rates for *A. imperialis*, consistent with previous results on the breeding system of this species obtained from hand pollination experiments (Martinelli, 1994) and population-level inbreeding coefficients F_{IS} (Barbará *et al.*, 2007, 2008). It is also noteworthy that the plastid DNA haplotypes found in *A. imperialis* were placed in internal (= putatively ancestral) positions in the haplotype network (Fig. 5), whereas those of other taxa such as *A. brasiliana* and *A. nevaresii* were located in marginal (= derived) positions (Fig. 5; Posada & Crandall, 2001). Taken together, our nuclear and organellar data are broadly consistent with a scenario in which the highly outcrossing *A. imperialis s.s.* has received nuclear genetic material by gene flow from related taxa across extended time scales, thus leading to the increased genetic diversity present in this taxon (Table 1; Barbará *et al.*, 2007), which is also visible from the extended PCoA space occupied by *A. imperialis* and affiliated genotypes (Fig. 2). The increased genetic diversity seen in *A. imperialis* also represents a plausible explanation for the high level of phenotypic variation present in this taxon (Barbará *et al.*, 2007; Versieux *et al.*, 2012; Versieux & Wanderley, 2015). We suspect that the distinct new species and forms that continue to be discovered in this species complex of inselberg bromeliads (Versieux & Wanderley, 2009; Versieux *et al.*, 2012) represent an evolutionary legacy of this variation *sensu* Jiggins *et al.* (2008). This represents a testable hypothesis for future work.

Neutral markers versus traditional taxonomy: mind the gap?

At first sight, our results obtained with neutral markers appear to be at odds with previous findings from traditional taxonomy, as distinct species and forms continue to be described and synonymized in the *A. imperialis s.l.* species complex, for example

A. martinellii (Versieux & Wanderley, 2009) and *A. brasiliana* (Versieux & Wanderley, 2015). According to our molecular genetic data, all of the studied species experienced gene flow with *A. imperialis* in the recent past, perhaps with the exception of *A. nevaresii* (Figs 2–4). One could also be polemic and state that the most likely reason for *A. nevaresii* appearing better separated from *A. imperialis* than the remaining taxa is because it was sampled at a greater distance (> 60 km) from the remaining populations (Fig. 1). How can the seemingly contrasting results from molecular genetics and traditional taxonomy be reconciled?

To appreciate the differences between biosystematic patterns obtained by neutral markers and taxonomically informative phenotypic traits, it helps to keep in mind that the genomes of radiating species of the same ploidy have a strong tendency to remain porous for extended periods of time (Wu & Ting, 2004; Gavrilets & Vose, 2005; Lexer & Widmer, 2008). This certainly is the case for other groups of bromeliads (Benzing, 2000; Barbará *et al.*, 2007; Palma-Silva *et al.*, 2011). During that time, gene flow and meiotic recombination between the genomes of divergent taxa will constantly or repeatedly uncouple neutral marker loci from the genes or genetic elements that control the morphological (e.g. floral) differences between them. It is thus natural that neutral marker studies will often provide a different picture of population and species relationships than traditional taxonomy (Lexer *et al.*, 2009). We argue that these differences do not speak for or against the use of one or the other type of data in biosystematic studies of bromeliads or other taxa. Rather, the differences can point us to the actual mechanisms responsible for the origin of the morphological diversity we see. It is our hope that future studies may utilize these concepts and approaches on a larger scale, integrating more populations and species into the systematic and genetic database and consistently refining hypotheses on phylogenetic relationships and drivers of divergence in *Alcantarea* spp. and other groups of bromeliads.

Outlook: towards resolving the conflict

Perhaps one of the most exciting aspects of the post-genomic era for evolutionary and conservation biology is the rapid pace with which new technologies allow us to push the limits of DNA-based inference at potentially any taxonomic level. Current genome re-sequencing and genotyping-by-sequencing approaches facilitate assays of tens of thousands to millions of DNA polymorphisms in a single experiment or project (Hohenlohe *et al.*, 2010; Elshire *et al.*, 2011; The Heliconius Genome Consortium, 2012; Brawand *et al.*, 2014). With genome and transcriptome assemblies underway for Bromeliaceae (Zhang, Liu & Ming, 2014), it is clear that bromeliad evolutionary and conservation biology will benefit greatly from these new developments. Our first pilot experiment on RAD-seq in *Alcantarea* already indicates that tens of thousands of DNA regions can easily be assayed in bromeliads (Table 4) and that the thousands of polymorphisms recovered by RAD-seq will be useful for species delimitation in this group (Fig. 6). As an alternative to RAD-seq, we are currently compiling sequence capture 'baits' for the targeted re-sequencing of many genes and genome regions in bromeliads.

The scaling-up of population and conservation genomics with the help of new technologies holds the promise (in bromeliads and many other plant taxa) of revealing and tracking genomic patterns of variation and ancestry at an unprecedented depth, including conserved coding regions, regulatory elements and adjacent sequence regions expected to 'hitchhike' along with the mutations causing differences in phenotypes or niches (Lexer *et al.*, 2013, 2014; Evans *et al.*, 2014; Stölting *et al.*, 2015). Ultimately, joint analysis of genomic information with sufficient quantitative data on phenomes and niches should allow us to establish the currently hidden links among the genotypes and phenotypes that matter to the survival and persistence of wild species. This will be especially important in species-rich radiations under severe threat of habitat loss and extinction, such as many groups and species in Bromeliaceae.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Table S1. Genetic divergence (F_{ST}) between pairs of populations of *Alcantarea* 'inselberg' bromeliads.

Table S2. Estimates of effective population sizes (N_e) and migration rates (N_em) for all studied *Alcantarea* species, including maximum likelihood estimates (MLE) and 0.025 and 0.975 percentiles.

Table S3. Definition of plastid DNA haplotypes based on two single nucleotide polymorphisms (SNPs) in region *petBex2–Dex2* (Ebert & Peakall, 2009), one SNP and one indel in region *ndh*A intron (Shaw *et al.*, 2007), and fragment sizes at the two plastid microsatellite markers VgCP3 and VgCP4 (Palma-Silva *et al.*, 2009).