RESEARCH ARTICLE

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Incomplete lineage sorting and hybridization in the evolutionary history of closely related, endemic yellow-flowered *Aechmea* species of subgenus *Ortgiesia* (Bromeliaceae)[1]

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PREMISE OF THE STUDY: The yellow-flowered *Aechmea* subgenus *Ortgiesia* (yfAsO) (Bromeliaceae) is a group of seven morphologically similar bromeliads found mostly in the southern Brazilian Atlantic rainforest. The recent origin of this group probably contributes to its taxonomic complexity. The aims of this study were to investigate the levels of genetic diversity and structure at the population and species levels, to gain insight into the processes behind the diversification of the group, and to contribute to the establishment of species boundaries.

METHODS: We sequenced two noncoding regions of the chloroplast genome (*rpl32-trnL* and *rps16-trnK*) and the nuclear *phyC* gene in 204 and 153 individuals, respectively, representing the seven species of the group. Phylogeographical and population genetics approaches were used.

KEY RESULTS: Three of the seven yfAsO showed some degree of genetic differentiation among species. Divergence time for the group was dated to around 4 million years ago. Areas of conservation value were identified, and a scenario of multiple refugia in the southern Brazilian Atlantic rainforest during the Pleistocene climatic oscillations is suggested.

CONCLUSIONS: We hypothesized that incomplete lineage sorting and localized hybridization events are responsible for the low levels of genetic differentiation and the taxonomic complexity observed among and within the seven yfAsO species. Further studies on *Aechmea comata* and *Aechmea kertesziae* will be necessary to clarify the boundary between these two species. Most of the populations sampled showed high genetic diversity and/or unique haplotypes; they should be prioritized for conservation purposes.

KEY WORDS Brazilian Atlantic rainforest; Bromeliaceae; diversification; evolutionary history; genetic diversity; genetic structure; multiple refugia; phylogeography; Pleistocene

Speciation has fascinated evolutionary biologists since Darwin's time. Despite much research into the ecological and genetic mechanisms involved in speciation, many questions remain (Sobel et al., 2010). Geographical isolation and adaptation to new ecological conditions are among the main factors invoked to explain the appearance of new species (Lorenz-Lemke et al., 2010; Pavan et al., 2011; Hope et al., 2012; Fregonezi et al., 2012; Gehring et al., 2013; Wachowiak et al., 2013; Turchetto et al., 2014; Gao et al., 2015). These factors may lead to novel genetic combinations, thus giving rise to divergent populations and distinct genetic lineages (Seehausen, 2004). Studies focusing on the distribution of genetic diversity are of great importance to understand intra- and interspecific genetic differentiation. In particular, the investigation of closely related congeners can shed light on their evolutionary relationships and the processes involved in their diversification (Hewitt, 2001; López-Vinyallonga et al., 2015; Slovák et al., 2015; Feng et al., 2016).

Recently diversified related species frequently show complex genetic patterns. In plants, these can mostly be explained by

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incomplete lineage sorting and hybridization/introgression processes (Rieseberg, 1997; Bouillé and Bousquet, 2005; Abbott et al., 2013; Gaudeul et al., 2014; Segatto et al., 2014; Slovák et al., 2015). During speciation, both processes may result in shared genetic variation and, as a consequence, in nonmonophyletic species (de Queiroz, 2007). To pinpoint which of them is responsible for the complex genetic scenario seen is a challenging task (Joly et al., 2009; Seehausen et al., 2014), but the geographical pattern of genetic variation across species can provide useful insights. While incomplete lineage sorting leads to a spread-out pattern of shared genetic variation, hybridization/introgression is normally coincident with sympatric occurrence of species (Palme et al., 2004; Mims et al., 2010; Gaudeul et al., 2014; Segatto et al., 2014; Gao et al., 2015). Both incomplete lineage sorting and hybridization/introgression are often observed in taxa that are products of recent adaptive radiation, a process in which an evolutionary group experiences diversification into a variety of ecological niches in an extremely short amount of time (Gavrilets and Losos, 2009). Therefore, these taxa may have had no time to accumulate specific polymorphisms or alleles that could differentiate between them. Likewise, reproductive barriers preventing gene flow between diverging populations may be incomplete (Nosil et al., 2009; Mims et al., 2010).

The genus *Aechmea* (Bromeliaceae) includes ~280 species with a geographical distribution ranging from Mexico to Uruguay (Smith and Downs, 1979; Luther, 2012). It has undergone a rapid diversification process, especially within the Brazilian Atlantic rainforest (Schulte et al., 2005, 2009; Silvestro et al., 2014; Goetze et al., 2016b). Phylogenetic studies have demonstrated that the genus is polyphyletic, presenting a serious taxonomic challenge (Faria et al., 2004; Horres et al., 2007; Schulte et al., 2005, 2009; Schulte and Zizka, 2008; Sass and Specht, 2010; Silvestro et al., 2014; Heller et al., 2015). As part of the core bromelioids clade, which originated around 5 million years ago (Ma), *Aechmea* is also an example of a group that has diversified relatively recently (Schulte et al., 2009; Givnish et al., 2011; Silvestro et al., 2014).

The subgenus *Ortgiesia* is one of the eight subgenera described within the genus *Aechmea* (Smith and Downs, 1979). In a previous study based on AFLP markers, we showed that these taxa are genetically close related, although their evolutionary relationships remain unresolved (Goetze et al., 2016b). Based on morphology, two main groups of species are observed within *Aechmea* subgenus *Ortgiesia*: one has yellow petals, the other has blue petals (Smith and Downs, 1979; M. Goetze, personal observation). The yellow-flowered *Aechmea* subgenus *Ortgiesia* (yfAsO) is a group of seven morphologically similar bromeliads, most of which are restricted to the southern Brazilian Atlantic rainforest (Smith and Downs, 1979; Goetze et al., 2016b). The morphological traits traditionally used to distinguish between these species are very polymorphic across the group and are characterized by continuous rather than discrete variation (Smith and Downs, 1979; Reitz, 1983; Goetze, 2010). Some of these species also present morphological plasticity, meaning that their distinct morphology depends on their habitat. Plasticity is particularly apparent in shaded vs. sunny environments (Reitz, 1983; Lenzi et al., 2006; Voltolini and Santos, 2011). Several yfAsO species can be found in sympatry, as well as sharing pollinators and flowering times (Smith and Downs, 1979; Reitz, 1983; Lenzi et al., 2006; Dorneles et al., 2011; Kamke et al., 2011; Goetze et al., 2016b). In our previous phylogenetic analysis, the *Aechmea* subgenus *Ortgiesia* showed shallow genetic differentiation, with low statistical support for the main clades recovered, a result of incomplete lineage sorting and possible hybridization events (Goetze et al., 2016b). All these features contribute to the taxonomic issues within this subgenus.

Here, we combined data from chloroplast (cp) DNA and nuclear genomes and applied phylogeographical and population genetics approaches to obtain insight into the processes driving the diversification and to contribute to the establishment of species boundaries in the yfAsO. We investigated patterns of genetic structure and diversity within the yfAsO at both population and species levels, across most of the group's geographical range. Our specific aims were (1) to examine whether morphologically identified species are genetically differentiated and to thus clarify species boundaries; (2) to identify hybridization events among yfAsO species and to shed light on their role in the diversification process and species delimitation; and (3) to quantify genetic diversity and its distribution among populations, with the goal of identifying areas of conservation value.

MATERIALS AND METHODS

Species description—The geographical range of the seven yfAsO species (Appendix S1, see Supplemental Data with the online version of this article) extends from the Brazilian state of São Paulo to Rio Grande do Sul (latitudes of 23° to 29° S), with the range of a single species extending into Argentina (Smith and Downs, 1979; Reitz, 1983; Goetze et al., 2016b; Fig. 1). Within this group, *Aechmea caudata* Lindm. is the most widespread species. It can be epiphytic, terrestrial, or saxicolous and occurs in both mountainous and coastal areas. *Aechmea blumenavii* Reitz is restricted to the northern area of the Brazilian state of Santa Catarina, occurring in the forest at elevations above 500 m a.s.l., mostly as an epiphyte, while *Aechmea kertesziae* Reitz grows on rocks or as a terrestrial and is found only in the coastal region of Santa Catarina state. *Aechmea comata* Baker and *Aechmea kleinii* Reitz are narrowly restricted species, the former occurring only in the coastal regions of Santa Catarina Island, the latter on the continent at elevations above 1000 m a.s.l., both in Santa Catarina state. *Aechmea comata* can be epiphytic, terrestrial or saxicolous, while *A. kleinii* grows mostly as an epiphyte. *Aechmea winkleri* Reitz is a disjunct species limited to two regions, which are around 480 km from one another (northern Santa Catarina state and the central region of the state of Rio Grande do Sul, Fig. 1). Growing mostly as an epiphyte, it is restricted to forested areas. *Aechmea calyculata* (E.Morren) Baker is the only species whose range extends to the western region of south Brazil and into Argentina. It has recently also been found in the northern central region of the Santa Catarina state. It grows mostly as an epiphyte in the forest. There is some geographical overlap among the yfAsO species, and several of them are found in sympatry (Smith and Downs, 1979; Reitz, 1983; Goetze et al., 2016b; Fig. 1).

Within the yellow-flowered *Aechmea* subgenus *Ortgiesia*, the main morphological traits used to distinguish the species rely on inflorescence and flower characters. *Aechmea caudata* and *A. winkleri* are species with compound inflorescences. These two taxa are mainly distinguished by the size of the flowers, which are smaller in *A. winkleri*. *Aechmea caudata* occurs in both inland and coastal regions, whereas *A. winkleri* is found only in inland areas. The remaining yellow-flowered species possess simple inflorescences and can be separated in two groups, one with dense and other with lax inflorescences. In the group with lax inflorescences, we find

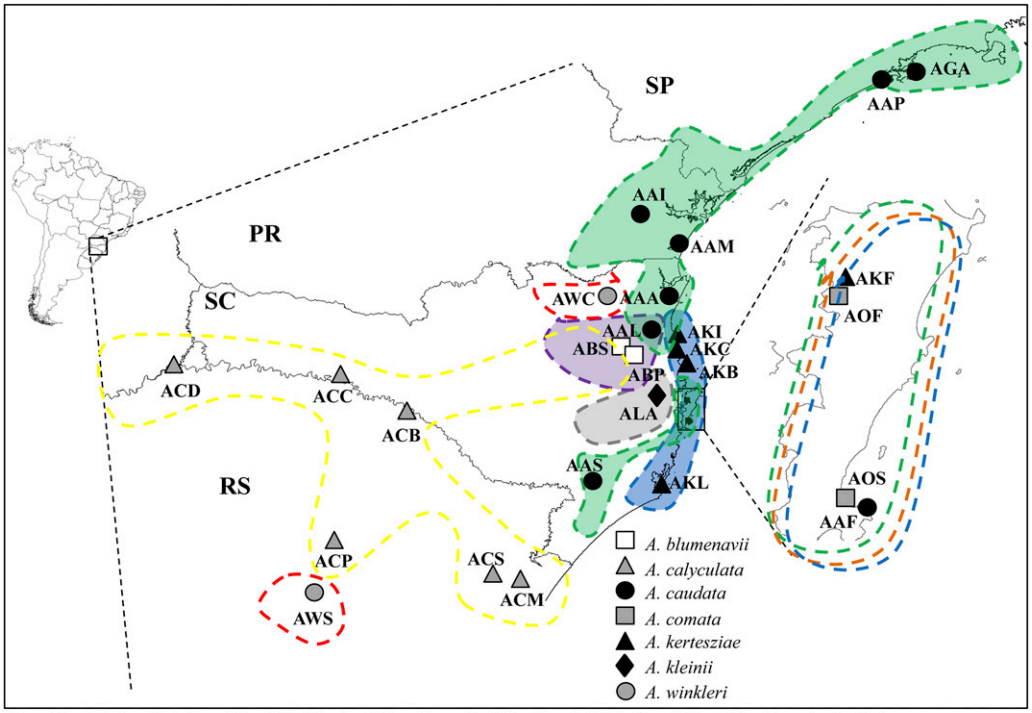

FIGURE 1 Map showing the sampled populations of the yellow-flowered *Aechmea* subgenus *Ortgiesia* species and their described geographical distribution according to verified herbaria records: purple, *A. blumenavii*; yellow, *A. calyculata*; green, *A. caudata*; orange, *A. comata*; blue, *A. kertesziae*; gray, *A. kleinii*; and red, *A. winkleri*. Population codes correspond to those in Table 1.

A. blumenavii and *A. kertesziae*, which are distinguished by the anthesis beginning in the middle (*A. kertesziae*) or at the base of the inflorescence (*A. blumenavii*). In addition, *A. kertesziae* is restricted to the coast, whereas *A. blumenavii* is found in inland areas. However, in some populations, *A. kertesziae* individuals possess an inflorescence that is a mixture of dense and lax; it can also be found with basal ramifications in the inflorescence, which can place it in the compound inflorescence group. In the group with dense inflorescences, we observe *A. calyculata*, *A. comata*, and *A. kleinii*. *Aechmea calyculata* is distinct from the other two species by presenting yellow sepals. It also shows smaller floral bracts when compared with *A. comata*. *Aechmea kleinii* possess a shorter inflorescence when compared with *A. comata* and is found only at altitude (above 1000 m a.s.l.), while *A. comata* occurs at sea level (Smith and Downs, 1979; Reitz, 1983; Goetze et al., 2016b).

Of the seven species investigated here, *A. kertesziae* and *A. kleinii* are currently listed as endangered species by the Brazilian government. *Aechmea winkleri* is critically endangered (MMA, 2014). *Aechmea kleinii* is also listed as endangered on the IUCN Red List of Threatened Species (Moraes, 2014).

Sampling and DNA isolation—A total of 204 individuals were collected from 26 populations (Fig. 1) covering most of the geographical range of all seven yellow-flowered species of the *Aechmea* subgenus *Ortgiesia*. To avoid misidentifications, samples were collected only from reproductive individuals. Information on sampling localities and on the number of individuals from each population used in chloroplast or nuclear DNA sequencing is summarized in Table 1. To avoid repeated sampling of the same individual, we collected samples at least 10 m apart. Young leaves were collected and stored in silica gel for drying. The cetyltrimethylammonium bromide (CTAB) protocol (Doyle and Doyle, 1990) was employed to extract total genomic DNA. Herbarium vouchers of each population and species sampled are indicated in Appendix 1.

Amplification and sequencing—Nine cpDNA regions (*psbA-trnH*, *trnTa-trnLb*, *trnLFc-trnLFf*, *rpL16-rpL16*, *trnD-trnT*, *petG-trnP*, *trnL* intron, *rpl32-trnL*, and *rps16-trnK*) were initially screened in representative samples from each species and population. Two regions, *rpl32-trnL* and *rps16-trnK*, were found to be polymorphic; they were subsequently amplified and sequenced for all individuals using primers described by Shaw et al. (2007). Additionally, the nuclear gene *phytocrome C* (*phyC*) was amplified and sequenced for a subset of 176 individuals from all sampled populations using primers described by Louzada et al. (2014). All PCR reactions were carried out as described by Goetze et al. (2016a). PCR products were sequenced in both directions using the BigDye Kit (Applied Biosystems, Foster City, California, USA) at Macrogen (Seoul, Korea). All sequences have been deposited in GenBank under the following accession numbers: KT318281–KT318323 and KY556898–KY557058 (*rpl32-trnL*), KT318324–KT318366 and KY557173–KY557333 (*rps16-trnK*), and KT351013–KT351049, KT351051–KT351052, and KY557059–KY557172 (*phyC*).

Sequence alignment—All sequences were checked using the program Chromas 2.32 (http://technelysium.com.au/) and aligned using MUSCLE (Edgar, 2004), which is implemented in the program MEGA version 5.10 (Tamura et al., 2011). Mononucleotide repeat length variations in cpDNA and *phyC* were excluded due to ambiguous alignment. Indels of more than 1 bp were coded as a single

TABLE 1. Details of the yellow-flowered *Aechmea* subgenus *Ortgiesia* species used in the study, geographical coordinates, elevation, sample sizes, and haplotypes found.

						Sample size		Haplotypes	
Species	Population	Code	Latitude S	Latitude W	Elevation (m a.s.l.)	cpDNA	*phyC*	cpDNA	*phyC*
A. blumenavii	Blumenau/ SC	ABP	27°03′	49°05′	285	9	5	1, 2	1, 2
	Blumenau/ SC	ABS	27°00′	49°06′	308	9	6	2, 3	1, 3-6
A. calyculata	Derrubadas/ RS	ACD	27°08′	53°52′	141	8	8	4	4, 7
	Concórdia/ SC	ACC	27°17′	52°07′	396	8	8	4, 5	4, 7
	Barracão/ RS	ACB	27°37′	51°31′	758	2	3	4	4, 7
	Putinga/ RS	ACP	29°04′	52°13′	207	8	8	4	4, 7
	São F. de Paula/ RS	ACS	29°27′	50°33′	580	8	6	4	4, 5, 8–10
	Maquiné/ RS	ACM	29°30′	50°14′	104	9	6	4	7, 11, 12
A. caudata	Guarujá/ SP	AAG	23°59′	46°11′	31	7	6	6	13
	Praia Grande/ SP	AAP	24°01′	46°03′	30	4	4	6	6, 13, 14
	Piraquara/ PR	AAI	25°31′	49°02′	1021	5	2	7, 8	15-17
	Matinhos/ PR	AAM	25°47′	48°31′	8	5	5	6, 7, 9	18-22
	Araquari/ SC	AAA	26°22′	48°43′	8	11	7	6, 10, 11	15, 19, 20, 22–29
	Ilhota/ SC	AAL	26°48′	48°55′	569	7	7	12–15	7, 14, 19, 21, 30–33
	Florianópolis/ SC	AAF	27°45′	48°29′	84	7	8	6	23, 34, 35
	Lauro Müller/ SC	AAS	28°23′	49°31′	804	8	7	12, 15, 16	15, 36–40
A. comata	Florianópolis/ SC	AOF	27°31′	48°30′	95	8	7	17–19	41-43
	Florianópolis/ SC	AOS	27°45′	48°30′	12	9	4	8, 20, 21	43, 44
A. kertesziae	Itajaí/ SC	AKI	26°55′	48°38′	19	15	12	12, 22	2, 4, 45–49
	Camboriu/ SC	AKC	27°00′	48°34′	5	6	3	8, 12, 23	4, 7
	Bombinhas/ SC	AKB	27°08′	48°29′	19	8	2	8, 12, 24	2, 9, 12
	Florianópolis/ SC	AKF	27°31′	48°30′	139	8	3	8, 25, 26	4, 5
	Laguna/ SC	AKL	28°30′	48°45′	11	15	10	27–31	7, 43, 44, 50, 51
A. kleinii	Antônio Carlos/ SC	ALA	27°27′	48°52′	637	3	2	34	5, 56
A. winkleri	Corupá/ SC	AWC	26°23′	49°20′	425	8	6	8, 12, 32, 33	19, 29, 52, 53
	Santa C. do Sul/ RS	AWS	29°41′	52°26′	125	9	8	12	54, 55
Total						204	153	34	56

Notes: Brazilian Federal States—SP, São Paulo; PR, Paraná; SC, Santa Catarina; RS, Rio Grande do Sul.

mutational event. The two cpDNA sequences (*rpl32-trnL* and *rps16-trnK*) were concatenated for all analyses. Haplotypes in sequences of *phyC* with heterozygous nucleotide positions were resolved using the Bayesian algorithm of PHASE version 2.1 (Stephens et al., 2001; Stephens and Donnelly, 2003), as implemented in the program DnaSP 5.10.01 (Librado and Rozas, 2009). The analysis was run with the default values for 10,000 iterations. All of the following analyses were performed separately for the cpDNA and the phased *phyC* sequences.

Genetic diversity and population structure analyses—To characterize genetic diversity, we estimated haplotype (h) and nucleotide (π) diversities (Nei, 1987), GC content, and number of variable sites for each species and data set using the software Arlequin version 3.5.2.2 (Excoffier and Lischer, 2010). The software DnaSP was used to identify the haplotypes, and the evolutionary relationships among them were estimated with Network 5 (available at http://www.fluxus-engineering.com) using the median-joining (MJ) method (Bandelt et al., 1999). To remove unnecessary links and median vectors observed for *phyC*, we postprocessed the MJ network with a maximum parsimony algorithm (Polzin and Daneshmand, 2003) implemented in Network. An analysis of molecular variance (AMOVA) was conducted to assess the genetic differentiation among species and populations, using Arlequin with 10,000 permutations. Pairwise comparisons of Φ_{ST} among populations and species were estimated with the program Arlequin using 10,000 permutations.

BAPS version 6 (Corander et al., 2008) was employed to analyze the population genetic structure by clustering sampled individuals into groups. This analysis was carried out as described by Goetze et al. (2016a). Ten iterations of each K from 1 to 9 were conducted.

Demographic analyses and divergence time—The species demographic history was assessed by Tajima's D (Tajima, 1989) and Fu's F_s (Fu, 1997) neutrality tests, which were carried out using cpDNA and *phyC* data separately in Arlequin. Statistical significance was determined based on 10,000 simulations. Additionally, changes in population size over time were estimated with a Bayesian skyline plot (BSP) analysis (Drummond et al., 2005), using cpDNA data for species presenting more than three haplotypes (see Results) and *phyC* sequences for all species. The analyses were carried out using BEAST version 1.7.5 (Drummond et al., 2012) with the following priors: lognormal relaxed clock (uncorrelated) with a substitution rate previously estimated for cpDNA and *phyC* in subfamily Bromelioideae (cpDNA: $7.64 \times 10^{-4} \pm 4.5 \times 10^{-6}$, *phyC*: $1.69 \times 10^{-3} \pm 7.59 \times 10^{-6}$; D. Silvestro, University of Gothenburg, personal communication). The HKY nucleotide substitution model was used for the majority of the analyses conducted on the cpDNA (except for *A. kertesziae*, where the GTR + G model was used). For the analyses of *phyC*, the model used was HKY + I for the majority of the species (with the exception of *A. caudata*, *A. comata*, and *A. kleinii,* where the HKY model was used). Substitution models were selected based on the Akaike information criterion (AIC) implemented in the program jModelTest 0.1.1 (Posada, 2008). Markov chains were run for 10,000,000 to 100,000,000 steps, sampling every 1000 steps, depending on the species and markers considered. The BSP computation and convergence checks were performed in the program Tracer 1.5 (available at http://beast.bio.ed.ac.uk/Tracer). The effective sample

size (ESS) > 200 was used as a threshold (Drummond and Rambaut, 2007).

The time of cpDNA haplotype divergence was estimated using a Bayesian approach implemented in the software BEAST, using *Bromelia antiacantha* Bertol. (GenBank accession numbers: KY557334 for *rpl32-trnL* and KY557335 for *rps16-trnK*) as an outgroup. Priors used included the Yule speciation model, the HKY nucleotide substitution model, and the lognormal relaxed clock (uncorrelated). The same substitution rate for cpDNA was used as in the BSP analysis. Markov chains were run for 50,000,000 steps, sampling every 1000 steps. The results were viewed in Tracer to check for convergence to a stationary distribution and for an effective sample size (ESS) >200 (Drummond and Rambaut, 2007). TreeAnnotator 1.7.5, part of the BEAST package, was used to summarize the trees, and the statistical support for all branches was measured in Bayesian posterior probabilities (PP). The software FigTree 1.3.1 was used to draw the tree (http://tree.bio.ed.ac.uk/software/figtree/).

RESULTS

Genetic variation—The sequencing of the intergenic spacers *rpl32-trnL* and *rps16-trnK* generated fragments of 826 and 821 bp long, respectively. The final data set of concatenated cpDNA spacers, after removing mononucleotide repeats and editing indels longer than 1 bp, totaled 1584 bp with a GC content of 27.5%. A total of 42 polymorphic sites were observed (20 transitions, 14 transversions, and 11 indels). Thirty-four different haplotypes were found in the 204 individuals of the seven species analyzed. The sequencing of *phyC* generated a fragment of 922 bp. Genotypes for 153 of 176 individuals were resolved by PHASE with probabilities ≥0.9 and thus retained for analysis, resulting in a final data set comprising a total of 306 alleles with a GC content of 48.12%. Thirty polymorphic sites were found (29 transitions and 1 transversion), resulting in 56 distinct haplotypes.

Nucleotide and haplotype diversity levels of both genomes are presented in Table 2. The highest cpDNA haplotype and nucleotide diversity values were found in *A. kertesziae*, *A. comata*, and *A. caudata*. Although *A. comata* is restricted to an island, its diversity values were similar to those of the other two, more widespread, species. For *phyC*, *A. caudata* and *A. kertesziae* showed the highest haplotype and nucleotide diversities (Table 2). Two or more haplotypes were present in the majority of the 26 populations analyzed here, with the exception of 10 populations, where only a single cpDNA haplotype was observed, and one population was found to be monomorphic for a single *phyC* haplotype (Table 1). The largest number of cpDNA haplotypes of all populations sampled were found in AKL from *A. kertesziae* (five haplotypes), followed by AAL and AWC (with four haplotypes each), from *A. caudata* and *A. winkleri*, respectively. The most diverse populations for *phyC* were AAA (*A. caudata*) and AAL (*A. caudata*), with 11 and eight haplotypes, respectively (Table 1).

Haplotype relationships and distribution—Two of 34 cpDNA haplotypes were shared between several species: H8 was shared by *A. caudata*, *A. comata*, *A. kertesziae*, and *A. winkleri*, while H12 was shared by *A. caudata*, *A. kertesziae*, and *A. winkleri* (Tables 1, 2; Fig. 2A). Although the analyzed species were not reciprocally monophyletic, all remaining haplotypes were restricted to a single species. The most frequent haplotype, H4, was found in 42 individuals, all of them *A. calyculata*. Haplotypes 23 and 34, which belong to *A. kertesziae* and *A. kleinii*, respectively, are more divergent lineages; they are connected to H8 by seven and five mutational steps, respectively. A group of eight haplotypes from *A. comata* and *A. kertesziae* (H17–H19, and H27–H31 respectively) was also found to be more distantly related to the remaining haplotypes. Haplotype 20, of *A. comata*, was found to connect *A. caudata* haplotypes from inland and coastal populations, possibly indicating an ancient hybridization event with chloroplast capture (Table 1, Fig. 2A).

The relationship among the 56 *phyC* haplotypes identified here is shown in Fig. 2B. Eleven haplotypes were shared among different species (Hn2, Hn4–Hn7, Hn9, Hn12, Hn19, Hn29, and Hn43–Hn44). A shallow genetic differentiation can be seen between haplotypes, as most of them are connected to each other by a single mutational step. The most frequent haplotypes were Hn4, Hn7, and Hn43 (Fig. 2B).

Species and population differentiation—AMOVA analyses revealed that interspecific differences account for 34.84% of the genetic variation in cpDNA. The corresponding value for the nuclear

TABLE 2. Sample sizes, haplotypes observed and genetic diversity for the seven yellow-flowered *Aechmea* subgenus *Ortgiesia* species based on cpDNA and *phyC*.

Species	*N*	AC	NH	Haplotypes	*h* (SD)	π (SD)
cpDNA						
A. blumenavii	18	—	3	H1, H2, H3	0.5686 (0.0964)	0.001103 (0.000754)
A. calyculata	43	—	2	H4, H5	0.0465 (0.0439)	0.000030 (0.000083)
A. caudata	54	—	11	H6 to H16	0.8190 (0.0431)	0.001992 (0.001166)
A. comata	17	—	6	H8, H17 to H21	0.8529 (0.0473)	0.003120 (0.001791)
A. kertesziae	52	—	12	H8, H12, H22 to H31	0.8710 (0.0284)	0.003351 (0.001827)
A. kleinii	3	—	1	H34	0.0000	0.000000
A. winkleri	17	—	4	H8, H12, H32, H33	0.4191 (0.1413)	0.000463 (0.000407)
phyC						
A. blumenavii	11	22	6	Hn1 to Hn6	0.6797 (0.0952)	0.002484 (0.001579)
A. calyculata	39	78	8	Hn4, Hn5, Hn7 to Hn12	0.6597 (0.0453)	0.001086 (0.000820)
A. caudata	46	92	30	Hn6, Hn7, Hn13 to Hn40	0.9419 (0.0120)	0.003878 (0.002202)
A. comata	11	22	4	Hn41 to Hn44	0.5714 (0.1059)	0.000789 (0.000682)
A. kertesziae	30	60	15	Hn2, Hn4, Hn5, Hn7, Hn9, Hn12, Hn43 to H51	0.9017 (0.0172)	0.002925 (0.001749)
A. kleinii	2	4	2	Hn5, Hn56	0.6667 (0.2041)	0.002892 (0.002303)
A. winkleri	14	28	6	Hn19, Hn29, Hn52 to Hn55	0.7593 (0.0588)	0.003710 (0.002177)

Notes: N, sample size; AC, allele copies; NH, number of haplotypes; *h*, haplotype diversity; π, nucleotide diversity; SD, standard deviation.

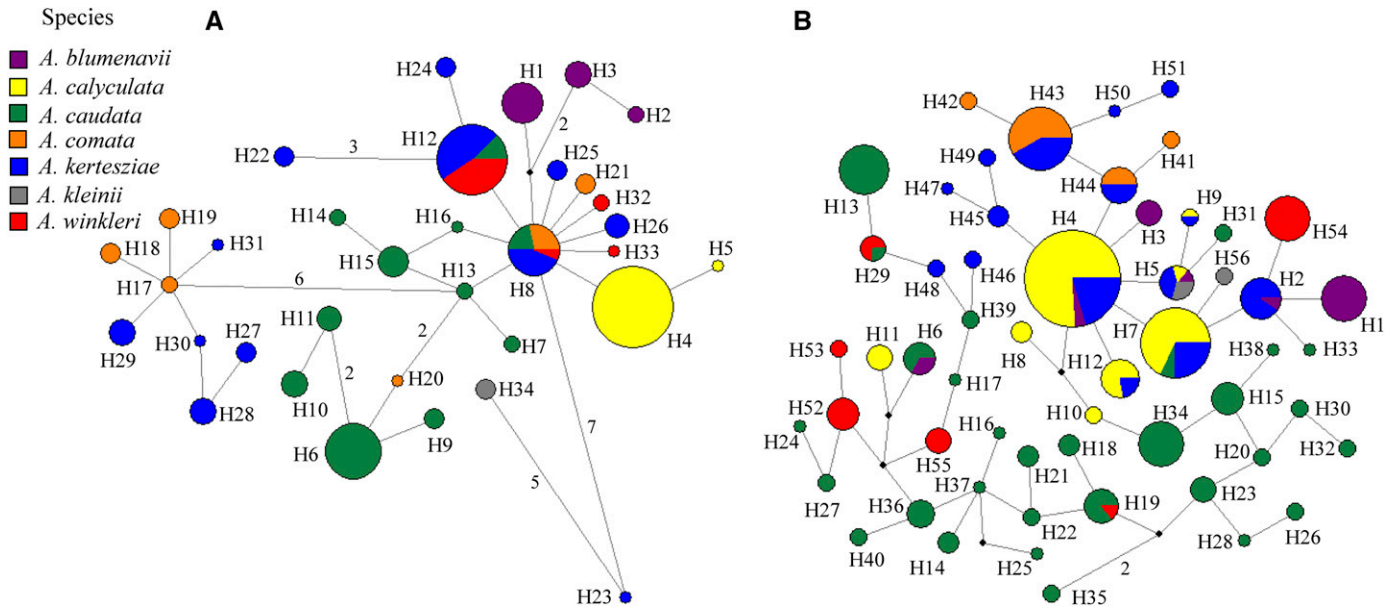

FIGURE 2 Median-joining network showing the genealogical relationship recovered for (A) 34 cpDNA and (B) 56 *phyC* haplotypes; each circle represents one haplotype, its diameter is proportional to its total frequency; more than one mutational step required to explain transitions among haplotypes are indicated by numbers along the network. Each color representing one of the seven yellow-flowered *Aechmea* subgenus *Ortgiesia* species as indicated by the key on the left.

genome is 25.26%. For both cpDNA and *phyC*, most of the genetic variation is due to intraspecific differences between populations (47.56% and 43.01%, respectively) (Table 3). Low to high genetic differentiation was detected with pairwise Φ_{ST} estimates at the population level with both data sets, varying from –0.018 to 1.00 for cpDNA and from –0.002 to 0.982 for *phyC*; most of the values were statistically significant (Appendix S2). The same pattern was observed at the species level. The pairwise Φ_{ST} estimates varied from 0.071 to 0.997 for cpDNA and from 0.063 to 0.951 for *phyC* (Table 4).

The Bayesian analysis of population structure (BAPS) identified $K = 6$ as the most likely number of genetic clusters for the cpDNA-based analysis, showing little agreement with species limits or geographical distribution (Fig. 3). As expected, no admixture was observed for the maternally inherited cpDNA. All species presented individuals in cluster I, with the exception of *A. calyculata* and *A. kleinii*. All *A. calyculata* individuals are part of cluster II, while several *A. caudata* individuals and one *A. comata* belong to cluster III. Some *A. caudata* and *A. kertesziae* individuals and all of *A. winkleri* belong to cluster IV, while most of *A. comata* and some individuals of *A. kertesziae* belong to cluster V. *Aechmea kleinii* and one individual of *A. kertesziae* are part of cluster VI (Fig. 3A). Further insights into population structure were obtained by plotting the six genetic clusters on the map (Fig. 3B). The plotting of the six genetic groups on the map revealed that most of the individuals of coastal *A. caudata* populations (AAG, AAP, AAM, AAA, and AAF) were strongly associated with cluster III, while individuals of inland populations (AAI and AAS) were associated with clusters I and VI. One individual of *A. comata* was found to belong to cluster III, together with *A. caudata* individuals from the coastal populations. The *A. comata* individual is the same that was found to carry the cpDNA haplotype H20, which, in the network analysis, was found to connect coastal and inland haplotypes of *A. caudata* (Fig. 2A). Individuals of the populations AOF (*A. comata*) and AKL (*A. kertesziae*) belonged to cluster V. Populations AOF and AKL were also differentiated from the other populations in the network analysis, where they formed the group of *A. comata* and *A. kertesziae* haplotypes that was more distantly related to the remaining haplotypes (Fig. 2A).

TABLE 3. AMOVA results among the species for the different markers.

Source of variation	Percentage of variation	*F* statistics	*P*-value
cpDNA			
Among species	34.84	$F_{CT} = 0.348$	<0.01
Among populations within species	47.56	$F_{ST} = 0.824$	<0.001
Within populations	17.60	$F_{SC} = 0.730$	<0.001
phyC			
Among species	25.26	$F_{CT} = 0.253$	<0.001
Among populations within species	31.72	$F_{ST} = 0.570$	<0.001
Within populations	43.01	$F_{SC} = 0.424$	<0.001

The BAPS analysis of *phyC* sequences revealed $K = 7$ genetic groups (Fig. 4A). The only admixture observed for *phyC* was in one of the 49 heterozygotes detected, an individual of *A. kertesziae*, which was assigned to both clusters II and VII. *Aechmea blumenavii* presented individuals in clusters I, II, and III. Most of the individuals of *A. calyculata* belonged to cluster II, but some were found in the clusters I, III, and IV. Individuals of *A. caudata* were found in all clusters, except VII. *Aechmea comata* belonged to cluster VII, while *A. kertesziae* individuals were found in clusters I, II, IV, V, and VII. Individuals of *A. kleinii* belonged to clusters I and II, while individuals of *A. winkleri* were found in clusters I, III, V, and VI (Fig. 4A). Plotting BAPS results on the map revealed no clear pattern (Fig. 4B). However, individuals of *A. comata* were grouped into cluster VII together with individuals of *A. kertesziae* from population AKL, in line with the results found with cpDNA (Fig. 3B).

Demographic analyses and time of divergence—No significant deviation from neutrality was detected for the cpDNA sequences for

TABLE 4. Pairwise genetic divergence for the yellow-flowered species of *Aechmea* subgenus *Ortgiesia* based on plastid (Φ_{ST}; below diagonal) and *phyC* data (Φ_{ST}; above diagonal).

Species	*A. blumenavii*	*A. calyculata*	*A. caudata*	*A. comata*	*A. kertesziae*	*A. kleinii*	*A. winkleri*
A. blumenavii		**0.399**	**0.288**	**0.704**	**0.218**	**0.194**	**0.261**
A. calyculata	**0.835**		**0.341**	**0.737**	**0.150**	**0.379**	**0.501**
A. caudata	**0.551**	**0.619**		**0.472**	**0.211**	**0.252**	**0.200**
A. comata	**0.486**	**0.658**	**0.406**		**0.395**	**0.714**	**0.636**
A. kertesziae	**0.350**	**0.384**	**0.328**	**0.071**		0.063	**0.302**
A. kleinii	**0.891**	**0.997**	**0.794**	**0.689**	**0.662**		**0.951**
A. winkleri	**0.643**	**0.873**	**0.471**	**0.435**	**0.141**	**0.251**	

Notes: Values in bold are statistically significant at $P < 0.05$.

any of the seven species based on either Tajima's *D* or Fu's F_S (Table 5). For *phyC* sequences in *A. caudata*, Fu's F_S was significantly negative. A negative F_S value indicates an excess of rare alleles, which may have been caused by purifying selection, population expansion, or selective sweeps (Fu, 1997; Nei and Kumar, 2000; Hahn et al., 2002). The BSP analyses mostly supported the findings of the neutrality tests, indicating that the effective population size of the analyzed species has remained constant and stable over time (data

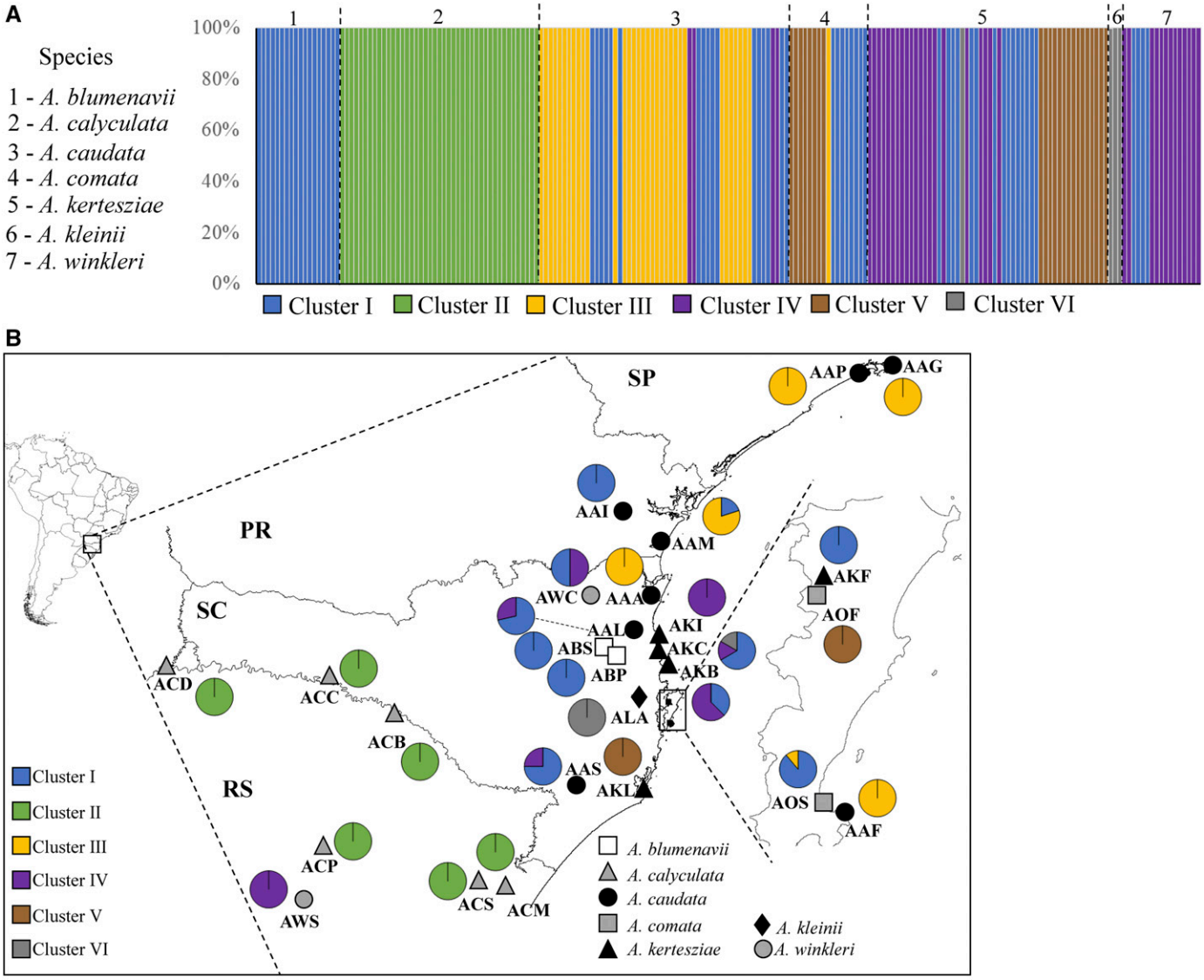

FIGURE 3 Population genetic structure based on cpDNA. (A) Bayesian admixture proportions inferred with BAPS for individuals of the seven yellow-flowered *Aechmea* subgenus *Ortgiesia* species for $K = 6$ groups model. Species are separated by dashed lines, and numbers correspond to the key on the right. (B) Distribution of the clusters recovered in the BAPS analysis. Population codes correspond to those in Table 1.

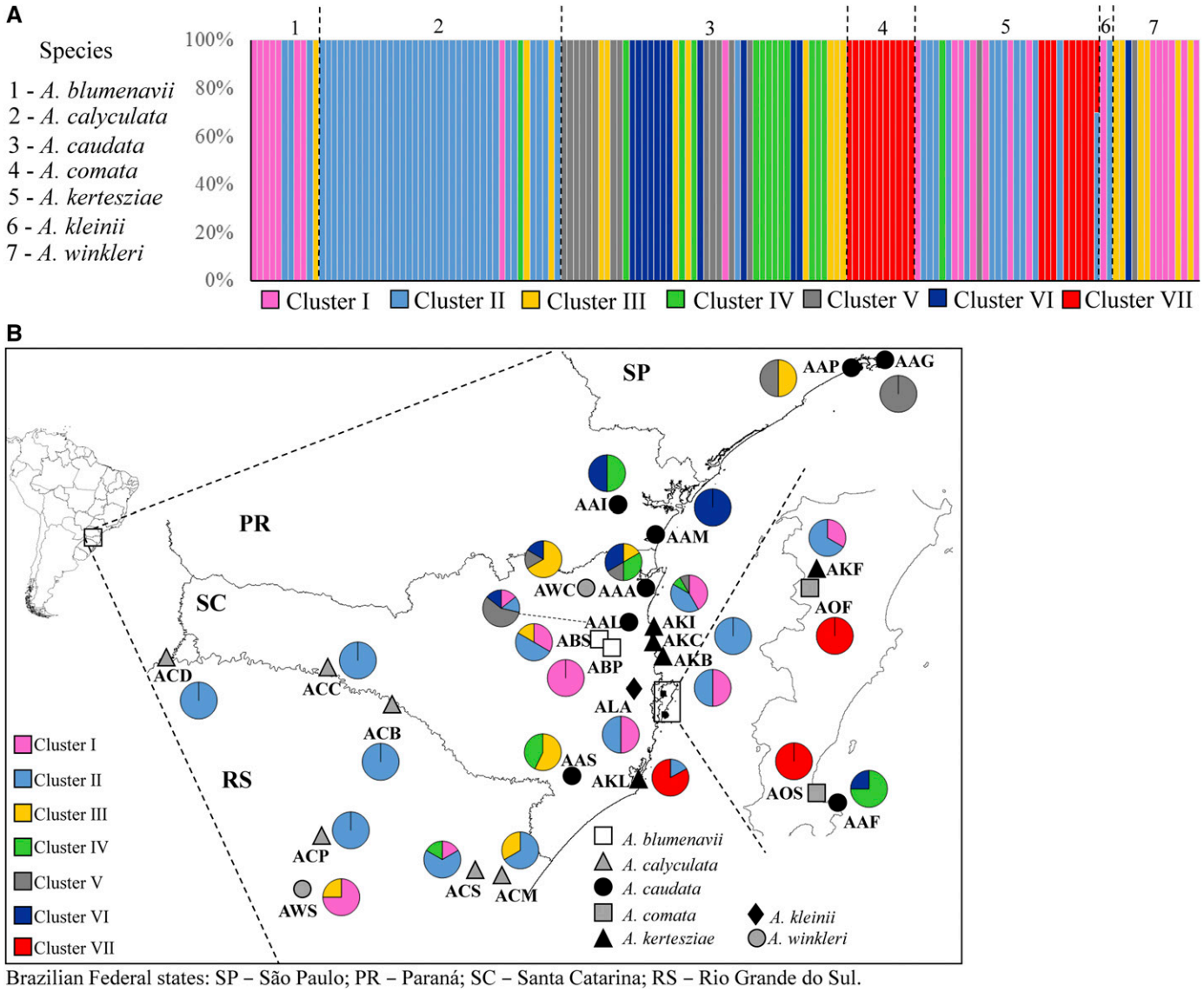

FIGURE 4 Population genetic structure based on *phyC*. (A) Bayesian admixture proportions inferred with BAPS for individuals of the seven-yellow flowered *Aechmea* subgenus *Ortgiesia* species for $K = 7$ groups model. Species are separated by dashed lines, and numbers correspond to the key on the right. (B) Distribution of the clusters recovered in the BAPS analysis. Population codes correspond to those in Table 1.

not shown). The one exception was *A. caudata*, for which *phyC* data indicated a recent bottleneck event. However, this result should be interpreted with caution given the size of the estimated confidence limits, which indicated no statistical significance (online Appendix S3).

The time of divergence of the cpDNA haplotypes of the seven *Aechmea* species was calculated to be around 4 Ma (95% highest posterior density: 2.50–6.33 Ma) (Fig. 5). Three main clades with low to high statistical support were observed in the phylogeny: clade A, comprising haplotypes 23 and 34, which were more distantly related to the others, but was not statistically supported; clade B, a group of eight haplotypes found in *A. comata* from AOF and *A. kertesziae* from AKL populations (H17–H19, H27–H31), which diverged from clade C around 3.2 Ma; and clade C, which comprised all remaining haplotypes. Clade C was further divided into two subclades, C1 and C2. Of these, only C1 was statistically supported. Clade C1 reunited most of the haplotypes from the seven yfAsO (15 haplotypes in total), while C2 was further subdivided, with one of the subclades grouping haplotypes from the coastal populations of *A. caudata* as well as the *A. comata* haplotype H20. Although the crown age of diversification is 4 Ma, most lineages inside the yfAsO probably started to diversify in the early Pleistocene at around 1.5 Ma (Fig. 5).

DISCUSSION

Genetic differentiation and species limits—The phylogenetic analysis of cpDNA haplotypes identified three main clades inside the yfAsO (Fig. 5). Clade A, albeit with low support (PP 0.4), comprised haplotype 23, which was found in a single *A. kertesziae* individual of the AKC population, and haplotype 34 of *A. kleinii*. These two haplotypes also formed a distinct and unique cluster in the BAPS

TABLE 5. Neutrality tests (Tajima's *D*, Fu's F_S) based on cpDNA and *phyC* for the seven yellow-flowered *Aechmea* subgenus *Ortgiesia* species.

	cpDNA		*phyC*	
Species	*D*	F_S	*D*	F_S
Aechmea blumenavii	0.000	2.543	0.616	0.286
Aechmea calyculata	−1.117	−1.510	−0.416	−2.357
Aechmea caudata	0.794	−0.681	0.034	−16.297*
Aechmea comata	1.233	2.064	0.738	−0.636
Aechmea kertesziae	−0.102	0.830	0.137	−4.137
Aechmea kleinii	0.000	—	2.080	2.719
Aechmea winkleri	−0.532	−0.916	1.512	2.104

Notes: —, index could not be calculated; *$P < 0.02$.

analysis of cpDNA sequences (cluster VI, Fig. 3A). *Aechmea kleinii* occurs in a very specific area at elevations above 1000 m a.s.l., where no other yfAsO have been found to date. For a long time, it was considered to belong to another subgenus of *Aechmea*, before being reclassified as part of the subgenus *Ortgiesia* in 1997 (Wendt, 1997). The genetic pattern observed here might therefore reflect the retention of ancestral polymorphisms. The close relationship between *A. kleinii* and *A. kertesziae* is in line with findings from our previous AFLP-based study (Goetze et al., 2016b). Pairwise Φ_{ST} estimates between most of *A. kertesziae* populations and *A. kleinii* were not statistically significant with *phyC* (Appendix S2). Since these two species occur in allopatry (Fig. 1), the results of the Φ_{ST} estimates were in line with retention of ancestral polymorphism.

Clade B included eight haplotypes (H17–H19, H27–H31), which were found in the AOF population of *A. comata* and the AKL population of *A. kertesziae* (Fig. 5). These haplotypes were also more distantly related to the remaining haplotypes in the network (Fig. 2A) and fell into cluster V in the BAPS analysis of the cpDNA (Fig. 3). Pairwise Φ_{ST} estimates between *A. comata* and *A. kertesziae* revealed the lowest value among pair of species, considering cpDNA (Φ_{ST} = 0.071, Table 4). The BAPS analysis of *phyC* sequences grouped all individuals of *A. comata* (including both AOF and AOS populations) and the AKL population of *A. kertesziae* together into cluster VII (Fig. 4). In the network analysis of *phyC*, *A. comata* only shared haplotypes with the AKL population of *A. kertesziae* (Fig. 2B). During our field trips, we did not find *A. comata* in the AKL locality, and a herbarium search did not find any records of this species in this area (*species*Link: http://www.splink.org.br). Nevertheless, our results point to a close relationship between *A. comata* and at least the AKL population of *A. kertesziae*. In this context, it is of interest that *A. kertesziae* occurs near the AOF locality, but *A. comata* seems to be more related to *A. kertesziae* from AKL than to the remaining *A. comata* individuals or to the geographically close AKF population of *A. kertesziae*. These results led us to hypothesize that the individuals grouped by clade B might be of hybrid origin. *Aechmea comata* and *A. kertesziae* are morphologically very similar, and it can be a challenge to distinguish between them. Any putative hybrids may be morphologically similar to one of the parental species, rather than showing a clear intermediate morphology, making their identification in the field difficult. Although the species used in this study were carefully identified, we cannot rule out that individuals identified as one species could actually be hybrids. For example, the *A. comata* identified in clade B could resemble *A. comata* morphologically, but be hybrids with *A. kertesziae*, which therefore clustered with this latter species in clade B. It is not uncommon to find plant hybrids that look like one of their parental species. An example of such similarity can be found in the genus *Vriesea* (Bromeliaceae), where most of the hybrids identified between *V. carinata* Wawra and *V. incurvata* Gaudich. by Bayesian analysis presented the morphology of one of their parental species (Zanella et al., 2016).

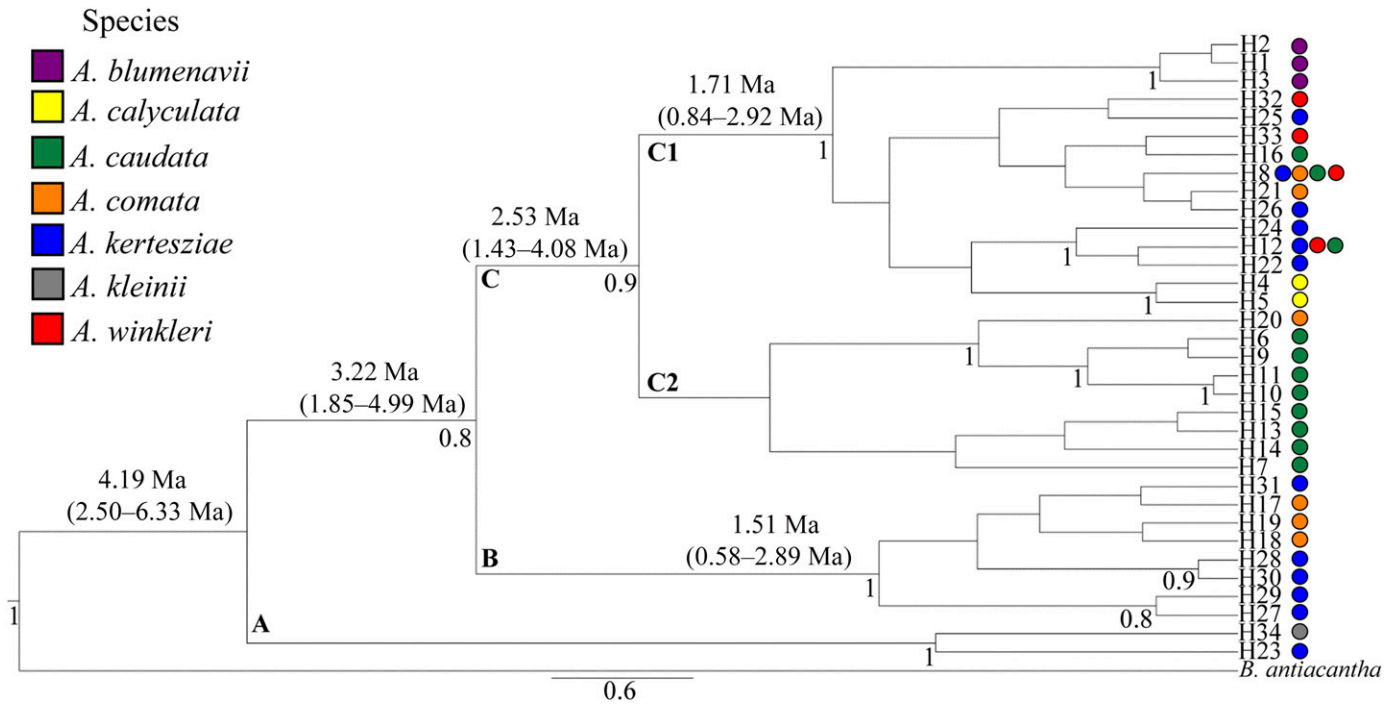

FIGURE 5 Bayesian phylogenetic tree of cpDNA haplotypes with posterior probabilities (>0.8) shown below the branches and ages indicated for selected nodes. The colored tips correspond to the species as indicated in the key on the left.

Aechmea comata and *A. kertesziae* blossom at the same time and share pollinators (Reitz, 1983; Lenzi et al., 2006; Dorneles et al., 2011; M. V. Büttow, unpublished results), which indicate that the reproductive barriers between them might be weak, leading to interspecific gene flow. The bumblebee *Bombus morio* is described as a pollinator for both *A. comata* and *A. kertesziae* (Lenzi et al., 2006; Dorneles et al., 2011; M. V. Büttow, unpublished results). The fly capacity of bumble bees is around 2500 m (Moure and Sakagami, 1962; Hagen et al., 2011). Although bumblebees do not fly long distances, it would be enough to ensure interspecific gene flow between *A. comata* and *A. kertesziae* as sympatric populations of these species are actually found in nature (Fig. 1). The potential hybrids identified here belong to distant populations (AOF and AKL), which may imply that *A. comata* and *A. kertesziae* may have shared refugia areas during climatic oscillations of the Pleistocene, where they might have hybridized. After climatic amelioration, these individuals colonized the areas where they are currently found. This hypothesis deserves further investigation.

All remaining haplotypes were grouped in clade C by the phylogenetic analysis. Lineages of all species could be found in this group, with the exception of *A. kleinii* (Fig. 5). Considering the recent origin of the yfAsO, this pattern may reflect the persistence of ancestral polymorphism within this group. Despite this, two species, *A. calyculata* and *A. blumenavii*, showed distinct and highly supported groups of haplotypes in the phylogenetic tree (Fig. 5). In the BAPS analysis of cpDNA sequences, *A. calyculata* was highly divergent, forming an exclusive genetic cluster (II, Fig. 3). Morphologically, this species is distinct from the remaining yfAsO species, with yellow sepals as well as petals, while sepals and petals are of different colors in all other species (Appendix S1). *Aechmea calyculata* is also the species whose geographical distribution extends farthest inland, into Argentina (Smith and Downs, 1979; Reitz, 1983; Goetze et al., 2016b). This distinct distribution pattern (Fig. 1), paired with specific aspects of its biology, as for example, the fact that hummingbirds are the main floral visitors (Favretto et al., 2010), may have genetically isolated *A. calyculata* from the remaining taxa of the group.

In the cpDNA network analysis, haplotype 20 was found to connect haplotypes of coastal *A. caudata* populations to those of populations farther inland (Fig. 2A). In the phylogenetic tree, H20 is on a sister branch to the coastal haplotypes of *A. caudata*; together, they form one of the C2 subclades (Fig. 5). This pattern is once again suggestive of a hybridization scenario. The individual carrying H20 was morphologically identified as *A. comata*, but both its plastid haplotype (Fig. 2A) and its behavior in the BAPS analysis (Fig. 3A) place it close to populations of *A. caudata*. Chloroplast inheritance is maternal in most angiosperms (Ennos, 1994), including other bromeliads (e.g., the genus *Fosterella*; Wagner et al., 2015). Assuming the same applies to *Aechmea,* a possible ancient hybridization event with chloroplast capture would imply pollination of *A. caudata* flowers by *A. comata* pollen, followed by recurrent backcrosses of the resulting hybrid with *A. comata*. The population where the putative hybrid originated, AOS, is geographically close to population AAF (*A. caudata*, Fig. 1). In some areas, individuals of *A. caudata* occur side by side with *A. comata*. These two species also blossom at the same time and share their main pollinators (Reitz, 1983; Lenzi et al., 2006; Dorneles et al., 2011; Kamke et al., 2011), thus enabling gene exchange. However, since only one individual with this pattern was identified, further studies are needed to confirm and clarify this event. As a first step, we are currently extending our Bayesian analyses to microsatellite data.

Despite the divergent lineages identified and discussed above, there was a lack of reciprocal monophyly between species (Figs. 2, 5). This pattern could be explained either by a recent origin paired with incomplete lineage sorting or by hybridization. In fact, our analyses support a recent origin for the yfAsO, between the late Pliocene and early Pleistocene, corroborating the results of previous studies (Schulte et al., 2009; Silvestro et al., 2014). However, our findings did also raise the possibility of hybridization between several yfAsO species. Our results do not show a strong geographical genetic structure; instead, we observed a diffuse and uniform pattern across space, consistent with incomplete lineage sorting. However, in addition to this, local hybridization events may also have contributed to the complex genetic patterns observed for the yfAsO species. Thus, the group's recent origin and the incomplete reproductive barriers between sympatric species indicate that the different species may not have had the time to accumulate sufficient genetic differences to establish firm boundaries between them. In other words, speciation may still be ongoing for the different members of this complex group. However, even with a lack of reciprocal monophyly among taxa and evidence of genetic differentiation for only three species with BAPS analyses, most of the seven yfAsO present morphological characters or isolated geographical occurrences that permit their identification. The exception though occurs between *A. comata* and *A. kertesziae*, which do not show clear morphological separation and occur in sympatry, deserving further investigation.

Distribution of genetic diversity—The genetic diversity observed in the seven species studied does not seem to be correlated with the extent of its geographical distribution. One example is the similarity in diversity levels found between *A. comata*, *A. caudata*, and *A. kertesziae*. While *A. comata* is an island species, *A. caudata* and *A. kertesziae* are widespread taxa (Table 2, Fig. 1). However, the high levels of genetic variation observed in *A. comata* could be indicative of hybridization. Furthermore, *A. blumenavii* and *A. winkleri*, two restricted species, presented higher levels of diversity than *A. calyculata*, a species with a wide geographical range (Tables 1, 2).

There was considerable variation in genetic diversity indices for each species, depending on whether they were based on plastid or nuclear DNA (Table 2). For example, the nuclear haplotype diversity for *A. calyculata* was 0.6597, while its cpDNA haplotype diversity was only 0.0465, one of the lowest indices within the group (Table 2). This incongruence between uni- and biparentally inherited markers may be attributed to differences in the effective population size, with cpDNA being more strongly affected by demographic changes and genetic drift (Ennos, 1994; Petit and Excoffier, 2009). It could also reflect the pronounced genetic structure observed in the AMOVA analysis of the cpDNA (Table 3), in line with previous reports for other bromeliads (Barbará et al., 2008; Palma-Silva et al., 2009, 2011; Paggi et al., 2010; Goetze et al., 2016a).

Our analyses provided insights into the distribution of genetic diversity in the yfAsO species, revealing that populations located in the north of Santa Catarina state harbor the greatest amount of haplotypes (Table 1). High levels of diversity were identified in populations AKL, AAL, and AWC for plastid DNA, and in populations AAA and AAL for the nuclear genome. These localities should therefore be considered potential areas of conservation value. Population AKL needs further investigation, since the high diversity observed could be indicative of the presence of interspecific hybrids. Although the localities mentioned above harbor greater levels of

genetic diversity, many other populations showed private haplotypes, including the AAI (*A. caudata*), AAS (*A. caudata*), and AKI (*A. kertesziae*) populations of Santa Catarina Island, as well as the three most differentiated species, *A. blumenavii*, *A. calyculata*, and *A. kleinii*. Therefore, most of the sampled localities in Santa Catarina, the ACS and ACM (*A. calyculata*) localities in Rio Grande do Sul, as well as AAI (*A. caudata*) in the state of Paraná, should be priorities for conservation. The geographical range between the latitudes of 26° and 27°S, which includes most of the populations analyzed here, is considered the center of species richness for the entire *Aechmea* subgenus *Ortgiesia* (Goetze et al., 2016b), corroborating its conservation value and highlighting its need for protection.

The yfAsO species are native to one of the most threatened biomes of the world, the Brazilian Atlantic rainforest (Myers et al., 2000; Ribeiro et al., 2009); they can consequently be considered in danger. Populations of this group of species are generally small, occurring in urban or agricultural areas. Many populations sampled by other researchers in the 1950s and 1960s could not be found anymore, suggesting a pattern of local extinction, which was confirmed by the occurrence, in some instances, of median vectors in the network analyses. Currently, only *A. kertesziae*, *A. kleinii*, and *A. winkleri* are included in the Brazilian Official List of Threatened Flora Species (MMA, 2014). We suggest that all seven yfAsO species should be part of the list, in particular considering their limited geographical range.

Diversification and demographic history—Our analyses pointed to a recent origin of the yfAsO in the late Pliocene or early Pleistocene, with most of the lineages diversifying during the early Pleistocene (Fig. 5). Neutrality tests and BSP analyses indicated no changes in effective population sizes for any of the seven species, indicating demographic stability (Table 5; Appendix S3). High levels of genetic diversity and private haplotypes were observed in most of the populations sampled in the present study (Table 1). These results may indicate that the origin of the yfAsO species may lie in the state of Santa Catarina, which is also considered the center of species richness of the entire subgenus (Goetze et al., 2016b). This state is geographically diverse, with many slopes and valleys, such as that of the Serra do Mar in the north and the Serra Geral farther south (Almeida and Carneiro, 1998; Frank et al., 2009). Forest-dependent species were probably able to inhabit slopes and valleys during the Pleistocene climatic oscillations, when, according to palynological studies, conditions were dryer and cooler than today, limiting forests to those areas (Behling, 2002; Behling et al., 2004; Behling and Pillar, 2007; Leonhardt and Lorscheitter, 2010). Studies of animal taxa of the southern Brazilian Atlantic rainforest suggest that species capable of occupying mountain habitats were less affected by the climatic oscillations of the Pleistocene due to their ability to tolerate cooler conditions (Thomé et al., 2010, 2014; Amaro et al., 2012). Species of the yfAsO are currently found in mountain areas (Table 1; Goetze et al., 2016b), which indicates that they can tolerate cooler conditions, although further studies are necessary to clarify this scenario.

Although the climatic oscillations of the Pleistocene have not strongly affected the genetic diversity of the species here investigated, some fragmentation of their geographical range may have occurred, as suggested by the strong genetic structure observed between populations of the same species, both for cpDNA (F_{ST} = 0.824) and for *phyC* (F_{ST} = 0.570) (Table 3). These high levels of genetic structure, paired with the evidence for demographic stability, indicate that the seven yfAsO species may have survived in multiple refugia during the climatic oscillations of the Pleistocene. This scenario also offers an explanation for the private haplotypes observed in some localities indicating long-term persistence of species and populations. Likewise, the strong substructure observed between coastal and inland populations of *A. caudata* in the cpDNA analysis (Fig. 3), and the identification of the most divergent species as *A. blumenavii*, *A. calyculata*, and *A. kleinii* (Figs. 2A, 3, 5), could also be explained by the multiple refugia hypothesis. This scenario has already been put forward in previous studies of taxa of the southern Brazilian Atlantic rainforest (Thomé et al., 2010; Amaro et al., 2012; Carnaval et al., 2014; Firkowski et al., 2016; Pulido-Santacruz et al., 2016; Turchetto-Zolet et al., 2016).

The disjunct pattern of distribution seen in *A. winkleri* may be a result of local extinctions between the two regions where it presently occurs. Lower levels of genetic diversity and no private haplotypes were found in the population of Rio Grande do Sul (Table 1). *Aechmea winkleri* was the last of the seven yfAsO species to be identified and described (Reitz, 1975), and outside the two regions sampled here, no records of it are registered in the herbaria used in our search (*species*Link: http://www.splink.org.br).

CONCLUSIONS

Our study suggests a complex scenario of diversification within the yellow-flowered *Aechmea* subgenus *Ortgiesia*. We identified several processes of evolutionary relevance to recently diversified closely related species resulting from adaptive radiation, including incomplete lineage sorting, as well as localized events of hybridization. These processes might explain why only three of the seven yfAsO showed some degree of genetic differentiation. The four remaining species showed less genetic structure, but their morphological and ecological characteristics generally permit their identification, albeit with exceptions, in particular for *A. comata* and *A. kertesziae*. Further studies are necessary to elucidate the role of hybridization within the yfAsO. The occurrence of hybridization within this group is highlighted for the first time in this study, and it may be one of the reasons for the taxonomic complexity of the yfAsO, mainly because the putative hybrids might resemble one of the parental species, making their identification in the field challenging. Finally, the results of our work are highly relevant to the conservation of the seven yfAsO species, as they highlight localities of conservation value, which would ensure the survival and maintain the evolutionary potential of the group. On the basis of the restricted geographical distribution of most yfAsO species, we also recommend that all be included in the Brazilian Official List of Threatened Flora Species.

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APPENDIX 1 **Herbarium vouchers of the populations and species sampled for this study.**

Species/Population code	Voucher (Herbarium)
Aechmea blumenavii	
ABP	*AL Gasper, A Korte, JT Cadorin, J Schmitt 2192* (FURB)
ABS	*JL Schmitt, JT Cadorin, AL Gasper 54* (FURB)
Aechmea calyculata	
ACD	*N Silveira 8461* (HAS)
ACC	*A Korte 6414* (FURB)
ACB	*J Spanholi s. n.* (ICN)
ACP	*E Musskopf s. n.* (HAVT)
ACS	*T Strehl 2070* (HAS)
ACM	*M Goetze s. n.* (ICN)
Aechmea caudata	
AAG	*RB Louzada 69* (SP)
AAP	*SE Martins, LM Versieux, AL Santos, GO Silva 921* (SP)
AAI	*R Kersten 734* (UPCB)
AAM	*J Sonehara, A Uhlmann, L Pena 34* (ALCB)
AAA	*S Dreveck, FE Carneiro 2066*(FURB)
AAL	*A Stival-Santos, E Legal, S Silveira 960* (FURB)
AAF	*LMS Costa, M Goetze s. n.* (ICN)
AAS	*T Strehl 1251* (HAS)
Aechmea comata	
AOF	*M Goetze s. n.* (ICN)
AOS	*LMS Costa, M Goetze s. n.* (ICN)
Aechmea kertesziae	
AKI	*M Goetze s. n.* (ICN)
AKC	*A Stival-Santos, E Legal, D Meyer, S Silveira 2721* (FURB)
AKB	*A Nuerberg, AS Mello 178* (CESJ)
AKF	*M Kaehler 54* (UPBC)
AKL	*M Goetze s. n.* (ICN)
Aechmea kleinii	
ALA	*M Goetze s. n.* (ICN)
Aechmea winkleri	
AWC	*M Goetze s. n.* (ICN)
AWS	*M Goetze s. n.* (ICN)

Notes: Population codes correspond to those in Table 1. ALCB, Herbarium Alexandre Leal Costa; CESJ, Herbarium Leopoldo Kriger; FURB, Herbarium Dr. Roberto Miguel Klein; HAS, Herbarium Alarich Rudolf Holger Schultz; HAVT, Herbarium Vale do Taquari; ICN, Herbarium of Instituto de Ciências Naturais; SP, Herbarium of Instituto de Botânica, São Paulo; UPBC, Herbarium Departamento de Botânica, Universidade Federal do Paraná.